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ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL: MODELLING AND EXPERIMENTAL DESIGN

GESTIMULEERDE BIOLOGISCHE FOSFAATVERWIJDERING: MODELBOUW EN EXPERIMENTEEL ONTWERP

door

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Table of Contents

List of Figures

List of Tables

List of abbreviations

Ac	Acetate
ACP	Amorphous calciumphosphate
ACS	acetyl-CoA synthetase
ADP	adenosinediphosphate
AK	acetate kinase
AMP	adenosinemonophosphate
ANOVA	Analysis of Variance
A/O	Anaerobic-Oxic
A^2/O	Anaerobic-Anoxic-Oxic
API	Analytical Profile Index
AS	AutoSampler
ASM	Activated Sludge Model
ATP	adenosinetriphosphate
BE	Bias Error
BO(Z)F	Bijzonder OnderzoeksFonds (*special research fund*)
BCFS	Biologische-Chemische Fosfaat-Stikstof verwijdering *(biological chemical phosphate nitrogen removal*)
CASS	Cyclic activated Sludge Systeme
CEC	Council of the European Commission
CEEP	Centre Européen d'Etudes des polyphosphates
cfu	colony forming units
CH	Carboxyhydrate
COD	Chemical Oxygen Demand
COST	(European) Cooperation in the field of Scientific and Technical research
DAPI	4'-6-diamidino-2-phenylindole
DCCDM'M'	Dicyclohexylcarbodiimide
DCP	Dicalciumphosphate, $Ca_2HPO_4.2H_2O$
DEBPR	Denitrifying Enhanced Biological Phosphorus Removal
also NDEBPR	Nitrification Denitrification Enhanced Biological Phosphorus Removal
DNA	DeoxyriboNucleic Acid
DO	Dissolved Oxygen Concentration
DPAOs	Denitrifying Polyphosphate Accumulating Organisms
EBPR	Enhanced Biological Phosphorus Removal
also BEPR	Biological Excess Phosphorus Removal
ED	Entner-Douderoff pathway
EEA	European Environmental Agency
EMP	Embden-Meyerhof-Parnas pathway
EPA	Environmental Protection Agency
FAD	Flavin Adenine Dinucleotide
FAME	Fatty Acid Methyl Ester
FID	Flame Ionisation Detector
FIM	Fisher Information Matrix
FISH	Fluorescence *In Situ* Hybridisation
FWO	Fonds voor Wetenschappelijk Onderzoek (*fund for scientific research*)
GAOs	Glycogen Accumulating Organisms
GC	Gas Chromatograph

HAP	Hydroxyapatite, $Ca_5(PO_4)_3OH$ or $Ca_{10}(PO_4)_6(OH)_2$ after Maurer (1996)
HCl	Hydrogen Chloride
HDP	Hydroxy Dicalciumphosphate, $Ca_2HPO_4(OH)_2$
HELCOM	Helsinki Convention
HRT	Hydrolic Retention Time
LMB	Laboratory for Microbiology
LPO	Lactic acid Producing Organism
MCRT	Mean Cell Retention Time
MgAP	Magnesiumapatite, $MgNH_4PO_4$
MIS	Microbial Identification Software
MLSS	Mixed Liquor Suspended Solids
also SS	Suspended Solids
MV	Methylvalerate
NAD(P)	Nicotinamide Adenine Dinucleotide Phosphate
ND	Nitrifying-Denitrifying Systems
NMR	Nuclear Magnetic Resonance
OED	Optimal Experimental Design
OCP	Octocalciumphosphate, $Ca_4H(PO_4)_3$
OSPARCOM	Oslo and Paris COnvention
PAOs	Polyphosphate Accumulating Organisms
p.e.	population equivalent
Phoredox	Phosphorus Reduction Oxidation
PHA	PolyHydroxyAlkanoates
PHB	Poly-β-HydroxyButyrate
PHV	Poly-β-HydroxyValerate
RNA	RiboNucleic Acid
SBR	Sequencing Batch Reactor
SCFAs	Short Chain Fatty Acids
also VFAs	Volatile Fatty Acids
also SCVFAs	Short Chain Volatile Fatty Acids
SCOPE	Scientific Committee on Phosphates in Europe
SIPHOR	Simulation of biological Phosphate Removal
SRB	Sulphate Reducing Bacteria
SRT	Sludge Retention Time
STOWA	Stichting Toegepast Onderzoek Waterbeheer (*institute for applied water management*)
STPP	Sodium TriPolyPhosphate
SVI	Sludge Volume Index
TCA	Tricarboxylic Acid
TCP	Tricalcalciumphosphate $Ca_3(PO_4)_2$
TKN	Total Kjeldahl Nitrogen
IWA	International Water Association
formerly IAWQ	International Association on Water Quality
formerly IAWPRC	International Association on Water Pollution Research and Control
formerly IAWPR	International Association on Water Pollution Research
UCT	University of Cape Town
VIP	Virginia Initiative Plant
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

The following list of symbols is mainly related to the articles published. For an extensive overview of the symbols used in the activated sludge models the reader is reffered to the different tables in Chapter one, paragraph 4

$C_{PHB,X}$	internal PHB content of the biomass	mg PHB/g sludge
C_{Ac}	acetate concentration in the mixed liquor	mg COD/ℓ
$C_{PO_4^{3-}}$	orthophosphate concentration in the mixed liquor	mg P/ℓ
f_{pp}	phosphate release to PHB accumulation ratio	mg P/mg PHB
k_{PHB}	rate constant for PHB accumulation	mg PHB/(g sludge . h)
k_{pp}	rate constant for PHB consumption	mg PHB/(g sludge . h)
k_X	rate constant for biomass synthesis	1/h
K_{Ac}	half saturation constant for acetate	mg COD/ℓ
K_{PP}	half saturation constant for internal polyphosphate	mg P/g sludge
n	number of measurements	
y	observed concentration of considered compound	
$\hat{y}$	model-based concentration of considered compound	
v	number of responses	
w_{ij}	$(ij)^{th}$ element of the response weighing matrix	
$X_{PP,X}$	internal polyphosphate content of the biomass	mg P/g sludge
X_{PAO}	amount of poly-P bacteria	g sludge/ℓ
β	acetate uptake to PHB accumulation ratio	mg COD/mg PHB

INTRODUCTION

Introduction

At the Department of Technical Chemistry and Environmental Technology, Ghent University research on modelling of Enhanced Biological Phosphorus Removal (EBPR) in wastewater treatment plants (WWTPs) was initiated already as early as 1986. This research was inspired by the ever increasing concern for protecting and preserving our environment. Disturbing news on increased eutrophication of the European aquatic environment and promising news from South African researchers concerning a possibility to treat wastewater in a biological, environmentally friendly way led to an increased number of international studies on the different aspects of EBPR.

From 1991 till 1994, the author was involved in a completely different research topic at the above mentioned department but fortunately had the opportunity to follow simultaneously the research progress on EBPR at the department. Promising results were gathered at the department, but no consistent operating conditions of the lab-scale installations could be obtained. However, international and national increased pressure urged for a reliable, cost-effective, environmentally friendly treatment system. Indeed, although the physico-chemical quality of the Flemish aquatic system did improve slightly over the period 1990 till 1992, progress was too slow to guarantee the implementation of national and international standards in time. It was shown that phosphorus concentrations still exceeded the imposed maximum limit values and, moreover, it was recognised that those limits values were even too high to prevent for eutrophication. Internationally, with the publication of the first Activated Sludge Model (ASM1) in 1987 by the Task group on Mathematical Modelling for Design and Operation of Activated Sludge Processes and with the publication of a kinetic model for Biological Phosphorus Removal in 1989 by the South African researchers, an increased number of research topics concerning the different aspects of EBPR has been initiated. Despite all these efforts, research results appeared to be incoherent and engineering firms, constructing wastewater treatment plants, and plant operators remained sceptic. In fact, the increased legislative pressure asked for a reliable well manageable process and process operators could not afford malfunction due to often unknown, thus non-controllable, external parameters.

In 1994 the department decided to re-activate the research on the EBPR process by initiating a Ph.D. study focussed on the modelling of it. From the observations made during the period '91-'94 it was obvious that the development of such a model asked for a close co-operation between national and international researchers dealing with the specific aspects of EBPR. Establishing a national and international network thus was a prerequisite for this research study. A direct collaboration between BIOMATH of the Ghent University (Faculty of Agricultural and Applied Biological Sciences), the Department of Biochemical Engineering of the Delft University of Technology (The Netherlands) and the Department for Microbiology of the Ghent University (Faculty of Sciences) was established.

The existing analysis techniques for the many components involved in EBPR proved to be unreliable and needed to be critically re-examined prior to any modelling study, in order to allow quantitative observation of the different processes involved. It was also concluded a new reactor concept was necessary to obtain the microbial population necessary to study the process. It was decided to model the overall anaerobic and aerobic process and to verify the results with the only at that time available "South-African model". A modelling study dedicated to the temperature influence on the EBPR process, aimed at the incorporation of temperature effects in the existing models in order to allow a single model to be applicable for installations both in cold climates as well as warm climates. To evaluate possible deterioration of the EBPR process due to different carbon sources, part of this Ph.D. study was further dedicated to the study of the growth of non-Phosphate Accumulating Organisms (non-PAOs), more particularly organisms performing anaerobic sulphate reduction on lactate rich feeds. In addition, to assess the applicability of existing models in real wastewater treatment plants it was felt that a study had to be performed on-site at a wastewater treatment plant in Belgium. Finally, this research study aimed at

providing a tool for an optimal planning of experiments to account for the different parameters involved in the processes without the necessity for an extensive measurement campaign.

The first chapter of this study gives an extensive overview of the gathered EBPR knowledge. Although the experimental research reported in this Ph.D. ceased mid 1998 and the author's work since then changed to a completely different topic, this literature review comprises the most relevant published articles up to mid 2000 (*Chapter 1: Literature review*). The second chapter in this study deals with the analysis technique for the internal storage product poly-β-hydroxybutyrate. This carbon storage compound is a central energy and carbon reserve for PAOs and its accurate analysis is a prerequisite for modelling of the EBPR process. Prior to this study, several analysis techniques existed but they were only used and validated for pure culture systems and mainly aimed at analysis of high concentrations of the internal storage component. The inherent characteristics of activated sludge systems, however, asked for proper selection and optimisation of the most adequate analysis technique. Setting up a consortium of researchers in different countries allowed to evaluate and validate the analysis technique for sludges originating from different plants and containing different amounts of the internal storage component. Participation in the European COST action 682 allowed regular meetings between the several partners involved. (*Chapter 2: Gas chromatographic analysis of PolyHydroxyButyrate in activated sludge: a Round-Robin test*). The third chapter deals with the evaluation of different lab-scale installations. Whereas initially the main focus at the department was to mimic true process operation at full-scale continuous installations using a lab-scale Phoredox installation, it was decided that for modelling purposes of specific aspects of the EBPR process, an enhanced culture was necessary. Through subsidies from the Ghent University BOF, a new reactor concept that allowed for selective growth of PAOs could be built and operated. (*Chapter 3: Stimulated phosphorus removal from wastewater: SBR versus Phoredox and UCT* also comprises the results presented in "Biological phosphorus removal using SBR technology: phenomenological aspects during start up period"). The fourth chapter in this study deals with modelling of the anaerobic-aerobic EBPR processes. For this research topic, a close collaboration was established with the Laboratory for Microbiology of Ghent University, to determine and follow the dominant microbial population. (*Chapter 4: Biological phosphorus removal: composition and microbial population and kinetic modelling*). The fifth chapter deals with the influence of carbon source and operational parameters on the EBPR process. This research focussed on possible deterioration of EBPR activity due to anaerobic sulphate reduction and concurrent growth of filamentous bacteria. *(Chapter 5: Enhanced Biological Phosphorus Removal: Competition and symbiosis between SRBs and PAOs on lactate/acetate feed)*. In the sixth chapter research focussed on the modelling of the effect of the external temperature parameter on the EBPR process and its overall influence on the efficiency of the process. The effect of temperature on the stoichiometric and kinetic process parameters in the anaerobic and aerobic phases was studied. (*Chapter 6: Temperature effects on Bio-P removal*). In the seventh chapter an extensive study on optimal experimental design is presented that provides practical information for future researchers to minimise experimental efforts for modelling and, thus, to increase the information content gained in the scope of their future studies. For this part of the research the already existing collaboration with the BIOMATH Department, Ghent University was further intensified. *(Chapter 7 : Optimal Experimental Design for the Calibration of Models of Phosphorus Removing Activated Sludge Systems)*. In the last chapter the theoretical knowledge on EBPR processes is confronted with data from a real wastewater treatment plant. The aim of this research was to provide a set of parameters applicable for the wastewater of a full-scale wastewater treatment plant that needed upgrading to include biological phosphorus removal. A pilot scale EBPR installation located on-site and receiving the wastewater of the full-scale installation was sampled to investigate the process and to determine the EBPR kinetic process parameters. This research was performed at the Department of Biochemical Engineering of the Delft University of Technology (The Netherlands) for and with the help of Aquafin NV (Aartselaar, Belgium). *(Chapter 8: Model based upgrading for nutrient removal: modelling study - efficiently calibrating ASM2d)*.

CHAPTER 1

Literature review

Chapter 1

Literature review

1. INTRODUCTION

This chapter is dedicated to an overview of the different aspects involved in biological phosphorus removal, ranging from why to eliminate phosphorus to the final application of enhanced biological phosphorus removal in full scale plants. The chapter should be an aid to anyone starting with research concerning biological phosphorus removal but should also provide guidance to anyone applying the system and being confronted with practical problems. Finally this chapter aims to be a tool to get easy access to the state-of-the-art literature available mid 2000.

Although the experimental research reported in this Ph.D. ceased mid 1998 and the author's work since then changed to a completely different topic, this literature reviews aims to comprise the most relevant published articles up to mid 2000. Whereas these articles are used in this introduction chapter, earlier own published results in the following chapters do not comprise the latest publications. If required in the following sections, reference will be made to the newly published results in annex form.

From the moment this research was initiated at the Laboratory of Technical Chemistry and Environmental Technology in 1987 until today, a vast number of articles has been published by numerous authors originating from a variety of disciplines. During this period many hypotheses have been proposed and later eventually recalled. Often, different authors use different terminologies, different experimental conditions and different ways of presenting their experimental results mathematically. For anyone starting a study on biological phosphorus removal, these research papers can thus be confusing. This chapter is meant to guide interested people through these discussions and provide the latest conclusions or statements.

2. THE PRESENCE OF PHOSPHORUS IN THE ECO-SYSTEM

2.1. *NATURAL CONDITIONS*

Because phosphates are non-volatile, their occurrence is limited to soil and aquatic environments. In Figure 1 the water-based phosphate cycle is presented. In aquatic environments phosphorus only exists in the +5 form, limiting naturally occurring phosphorus to salts and esters of phosphoric acid (Reddy, 1998).

Phosphorus is a macro-nutrient that is necessary to all living cells. It is a limiting nutrient with regard to growth of algae and plants in lakes. Bacteria and many other organisms use orthophos phates to create organic phosphates and build solid-phase structures. As such, it is an important component of adenosine triphosphate (ATP), nucleic acids (DNA and RNA) and phospholipids in cell membranes. In both prokaryotes and eucaryotes phosphorus can be stored in intracellular volutin granules as polyphosphates.

Anaerobic activity in the bottom accounts for release of orthophosphates to the standing water, with subsequent consumption by bacteria and algae (Reddy, 1998).

Whereas above it was stated that phosphates are non-volatile, volatile phosphorus products such as PH_3 or P_2H_4 may move into the atmosphere and become oxidised and airborne as aerosol droplets. As such, microbial processes have been shown to generate volatile phosphorus compounds in sewage sludges, faeces, landfill, compost heaps and coastal sediments. Moreover, when accounting for an overall phosphorus cycle, dust from combustion of coal and forest fires and from erosion of phosphorus containing soils needs to be accounted for. The atmospheric phosphorus deposition is estimated at 0.04-2 kg P/ha/yr (Brunner and Bachofen, 1998).

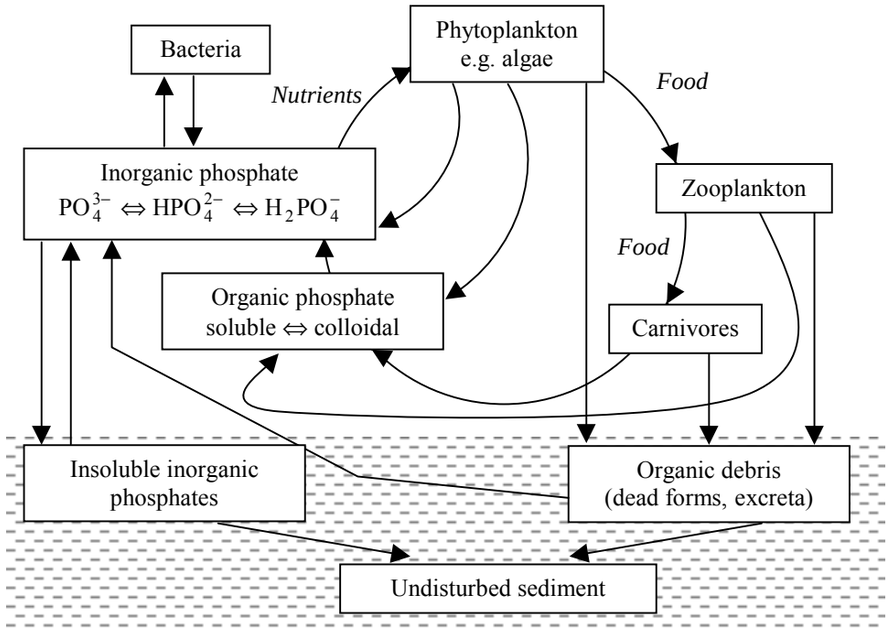

Figure 1 The water-based phosphate cycle (Reddy, 1998)

Pollution-related phosphorus compounds are orthophosphates, organic phosphorus and polyphosphates. The average concentration of total phosphorus (inorganic and organic forms) in wastewater is in the range of 5-20 mg P/ℓ (Bitton, 1994) and usually below 25 mg P/ℓ (SCOPE, 1998).

2.2. *EUTROPHICATION*

In natural conditions the phosphorus concentration in water is balanced, i.e. the accessible mass of this constituent is close to the requirements of the ecological system. However, if the input of phosphorus to waters is higher than can be assimilated by a population of living organisms, the problem of excess phosphorus content occurs. Etymologically, eutrophication means high concentrations of plant nutrients, but nowadays the word is often used to refer to the adverse response of an ecosystem to access nutrients (Harper, 1991). The UK Environment Agency defines eutrophication as "*The enrichment of waters by inorganic plant nutrients which results in the stimulation of an array of symptomatic changes. These include the increased production of algae and/or other aquatic plants, affecting the quality of the water and disturbing the balance of organisms present within. Such changes may be undesirable and interfere with water uses.*" (Environment Agency, 1998).

Nitrogen and phosphorus are often the limiting factors for biological growth in natural waters. However, cyanobacteria are capable of fixing molecular nitrogen, thus eliminating the requirement for ammonia or nitrate for their growth. In addition, they obtain metabolic energy by photosynthesis and use CO_2 as a source of carbon (Schlegel, 1993). In practice, growth prevention thus only needs a lowering of phosphate availability (Lee *et al.,* 1978). Experiments with large water reservoirs have shown that no eutrophication

occurs when the phosphorus concentration is reduced to 8-10 µg P/ℓ, even when the nitrogen concentration amounts to 4-5 mg N/ℓ (Clasen, 1979).

According to a survey of rivers across Europe (EEA, 1998) only 10% of the 1000 monitoring stations reported mean total phosphorus concentrations below 50 µg P/ℓ. In Table 1 a summary is given of the types of problems found in different EU Member States. Since all Member States are continuously trying to improve their water quality by restrictions on discharge to surface water and by the implementation of improved wastewater purification techniques, this table is subject to continuous changes and should be regarded at in that respect.

Table 1 Extent of eutrophication in EU Member States (SCOPE, 1998)

Member State	Extent of Eutrophication
Austria	*Some localised eutrophication.*
Belgium	*Widespread eutrophication in rivers, canals, lakes and coastal waters. Most of the surface waters in Flanders are classified as eutrophic.*
Denmark	*Extensive eutrophication of rivers and lakes.*
Finland	*Eutrophication limited to a few localised areas.*
France	*Eutrophication localised to particular river basins, lakes and reservoirs, especially the Loire and Meuse.*
Germany	*Serious eutrophication, especially lakes in southern Germany, the Baltic Sea and Schleswig-Holstein.*
Greece	*Highly localised eutrophication, but important.*
Ireland	*Highly localised eutrophication, but important.*
Italy	*Serious eutrophication problem affecting lakes, rivers and reservoirs. Discharge to the Adriatic Sea causes acute problems.*
Luxembourg	*Significant eutrophication.*
Netherlands	*Widespread eutrophication in most surface water bodies.*
Portugal	*Eutrophication rarely a problem.*
Spain	*Eutrophication is localised, but an increasing problem.*
Sweden	*Eutrophication is localised in the south, but generally of decreasing significance.*
United Kingdom	*Eutrophication localised, but of high significance in a number of rivers, canals, lakes and reservoirs.*

2.3. *PHOSPHORUS SOURCES*

The phosphorus load to surface waters is mainly due to discharge of wastewater and fertilisation of the soil. In 1991 agriculture was responsible for 56% of the phosphorus load, whereas households account for 22%, industry for 16% and traffic for 6% of the net nitrogen and phosphorus load to the environment in Flanders. For these calculations it was taken into account that the share of nitrogen and phosphorus is not equal, i.e. 1 kg of phosphorus contributes as much as 10 kg of nitrogen. According to this ratio nitrogen and phosphorus contribute to eutrophication of surface water (Geypens *et al.*, 1994). A survey in Europe indicated that point sources, e.g. from wastewater treatment plants, industry and some agricultural activities, account for more than half of the phosphates discharged in Europe (SCOPE, 1998). According to Morse *et al.* (1993) 23% originates from human origin, 11% from detergents, 32% from livestock, 17% from fertilisers, 7% from industry and 10% from background sources. In general it was found that over the North Sea catchment area, roughly 50% of the total phosphorus discharge is originating from sewage, somewhat more than a third from agriculture and the remainder from industrial and background sources (EEA, 1999).

The phosphorus surplus due to top-dressing in Belgium raises up to 24,000 ton P or 49% of the total phosphorus supply (Geypens *et al.*, 1994). 1998 results presented by van Starkenburg and Rijs, indicate that in the Netherlands 18,000 ton P per year comes from domestic wastewater while only 4,200 ton P per year comes from industrial discharges, i.e. 75% comes from domestic wastewaters. Similar calculations for Krakow (Poland) showed as much as 85% of the total phosphorus load originating from households (Rybicki, 1997). Polyphosphates added to drinking water is an important source, but its contributions varies with the utility. Polyphosphates typically are used in water systems to protect distribution pipes from corrosion (Reddy, 1998).

In general much caution is needed when interpreting the reported values of nutrient discharges. Clearer summary information is required, especially indicating how data are obtained and how they are calculated within the different countries.

As for the phosphorus load originating from agriculture, it is known that ortho-phosphate attaches strongly to soil particles. When high doses of fertilisers are used, phosphorus will build up in the upper layers and finally wash out into the groundwater. Moreover, when the phosphorus limit has been reached in the upper ground layers, phosphorus breakthrough to the lower levels occurs. In case of high groundwater level the phosphorus concentration in the soil water can increase with concomitant breakthrough to the surface waters.

Sewage contains phosphates from human sources, about 2 g P/capita/day, detergents, food waste, food additives and other products (SCOPE, 1998). According to Reddy (1998), phosphorus excreted by humans has been estimated at 0.5-2.7 g P/capita/day, with an annual mean of 1.6 g P/capita/day. In 1991, the phosphorus load in Belgium from households was mainly due to phosphorus containing detergents (Geypens *et al.*, 1994). According to estimates made for the UK (CEEP, 1998b), an average of 1.2 g P/capita/day enters urban sewage systems from the diet, with a further 1.3-1.8 g P/capita/day from other household activities, including contributions from household detergents. According to the authors, it would seem unlikely that domestic discharge of phosphates into sewage will ever fall far below 2g/capita/day in developed countries such as those of the European Union. According to Reddy (1998) an investigation conducted by Jenkins and Harmanowicz (1991) revealed that the contribution of soap and detergents industry has an estimated contribution of 0.3 g P/capita/day.

Synthetic detergents, introduced in 1947, contain a so-called builder, sodium tripolyphosphate (STPP), with as main role to create optimum water conditions for the surfactants. According to an Australian study, these polyphosphates are hydrolysed to ortho-phosphate (mainly due to high temperature and pH in washing machines, partly by the presence of multivalent cations Ca^{2+}, Fe^{2+} and Mg^{2+} in the wastewater) prior to reaching the wastewater treatment plants (Jolley *et al.,* 1998). Meanwhile, the detergent industry came up with new detergent builders, i.e. organic nitriloacetic acid (NTA) and synthetic zeolites and since then the contributions of households to the phosphorus load of wastewater treatment plants has substantially decreased. However, these new builders require the addition of chemicals to compensate for the performance short-comings exhibited by the alternatives (CEEP, internetsite 1998b). In 1994, the Netherlands Organisation for Applied Scientific Research (TNO), Institute of Environmental Sciences, published the results of research showing that the use of phosphate-free detergents gives no improvement in surface water quality, and leads to eutrophication that is in many cases worse than that experienced when phosphate-based detergents are used. The main reason for this is that the alternative non-phosphate detergent ingredients were found to be so toxic that they kill the vital zooplankton that feeds on algae and phytoplankton in naturally balanced freshwater systems (CEEP, internetsite 1998b).

The approximate concentrations of various phosphorus forms in wastewater have been estimated as ortho-phosphate (5 mg P/ℓ), tripolyphosphate (3 mg P/ℓ), pyrophosphate (1 mg P/ℓ) and organic phosphates (1 mg P/ℓ) (Jenkins and Tandoi, 1991). For the US a survey indicated a decrease of the average orthophosphate concentration from 6 mg P/ℓ to 3 mg P/ℓ due to the ban of phosphate detergents which

went into effect in the 1980's. The total phosphorus reduction ranged from 15 to 50%, with an average of 32%, with almost all of the reduction to be attributed to the ortho-phosphate reduction (Reddy, 1998).

2.4. *EVOLUTION*

The number of heavily polluted rivers has been reduced significantly, mainly as a result of improved wastewater treatment (industries as well as households). The proportion of heavily polluted rivers in Western Europe has fallen from around 25% in 1972-80 to around 5% in 1992-98. These reductions have been most pronounced in the most polluted rivers. Similar improvements have been achieved in lakes. Over the past 15 years, many northern European countries have achieved 60-80% reduction in phosphorus discharges from urban wastewater treatment plants. Full implementation of the urban wastewater Directive 91/271 should further reduce phosphorus discharges from sewage by 21% across Europe in the near future (EEA, 1999). In general it has to be stressed that the water quality in many cases is still poor and below that of good ecological status (Table 1).

Construction and upgrading of sewage works in Accession Countries would result in considerable reduction in pollutant discharges. The application of the urban wastewater Directive 91/271 in these countries would reduce phosphorus and nitrogen loading to the Baltic and Black Seas by 15-30%.

Although the yearly accumulated amount of phosphate is decreasing and the phosphate removal facilities are increasing, there is still a net accumulation of 18 Gg/yr in the Netherlands (Smolders, 1995).

2.5. *POLICIES TO CONTROL PHOSPHATE POLLUTION*

Phosphorus constraints on industrial and sewage discharge to prevent eutrophication differ from country to country. Even within Europe different policies, i.e. legislation, conventions, etc, exist. With the publication of the urban wastewater Directive 91/271 (CEC, 1991), Member States are enforced to designate "sensitive" and "less sensitive" areas. An area is to be classified sensitive (SCOPE, 1998):

- either when the receiving water is eutrophic or which in the near future may become eutrophic if protective action is not taken.
- or if the water is used for drinking water abstraction.
- or if the water requires a more stringent than secondary treatment in order to meet other EU Directives.

Table 2 Technical compliance deadlines for collection and treatment requirements from Directive 91/271 (SCOPE, 1998)

	12/1998	12/2000	12/2005
sewerage collection	> 10,000 p.e in sensitive areas	> 10,000 p.e in normal and less sensitive areas	> 2,000 p.e in all areas
primary or secondary treatment		> 15,000 p.e in less sensitive areas	> 10,000 p.e in less sensitive areas
secondary treatment		> 15,000 p.e in normal areas	> 2,000 p.e in normal and less sensitive areas
more advanced treatment	> 10,000 p.e in sensitive areas		
If the sewerage is collected in agglomerations < 2,000 p.e., appropriate treatment should be applied			

In Table 2 the technical compliance deadlines for collection and treatment requirements enforced by the EC Directive 91/271 are presented. Although the first compliance date has already past as today, it has been left in the table since not all Member States have fulfilled the requirements yet. Indeed, even some

members, i.e. Italy and Greece, have failed the designation of sensitive areas (CEC, 1998). The report furthermore indicates that at the moment of its survey, more than 40000 sewage works are in operation across the EU. Of those built before 1992, some 30% is estimated in need of upgrading. Overall, according to the same report, around 40000 sewage works will need building or upgrading.

Member States are basically free to choose among five approaches of how to apply "more stringent treatment" as mentioned in Table 2. The basic four options are presented in Table 3. The fifth option states that the mentioned requirements need not apply in sensitive areas for which it can be shown that the minimum percentage of reduction of the overall load entering all urban waste water treatment plants in that area is at least 75% for total phosphorus and nitrogen.

Table 3 Requirements for discharge from urban wastewater treatment plants to sensitive areas (CEC, 1991)

Parameters	Concentration		Minimum percentage of reduction
Total phosphorus	*Option 1*		*Option 3*
	10,000 - 100,000 p.e.	2mg P/ℓ	80%
	> 100,000 p.e.	1mg P/ℓ	
Total Nitrogen	*Option 2*		*Option 4*
	10,000 - 100,000 p.e.	15mg N/ℓ	70 - 80%
	> 100,000 p.e.	10mg P/ℓ	

According to Reddy (1998) in the US, the effluent limits are not uniform. The limits vary, as in Europe, based on the receiving body and its water use, water quality and relative pollutant loads to each water body. Regional water quality criteria for nutrients may also be established by state and local agencies.

Already as early as 1956, uniform effluent standards for the whole of South Africa were introduced under the Water Act. As for phosphorus, a standard of 1 mg P/ℓ was advised by the Department of Water Affairs in certain areas from 1985 on (Alexander *et al.*, 1994)

Beside the Directive 91/271/EEC, international conventions are established to protect the marine environment.

- As a result of Dutch implementation of agreements between Rhine and North Sea countries (OSPARCOM), Dutch phosphorus discharge limits are more stringent than those in the rest of Europe, as they are based on ten-day moving average concentrations, rather than annual averages. As such an overall phosphorus removal level of 75% is required in The Netherlands. (CEEP, 1998b)
- The Helsinki convention (HELCOM) to protect the Baltic Sea, is special in its kind that it includes both EU Member States that have already taken extensive to reduce nutrients (Sweden) and Eastern European countries which have poor levels of wastewater treatment, especially concerning phosphorus (SCOPE, 1998).
- The Barcelona Convention to protect the Mediterranean Sea has only little impact of phosphorus discharge limits (SCOPE, 1998).

It is up to any country to imply its own discharge limitations, as long as they are conform the Directive 91/271/EEC. It is out of the scope of this study to list discharge limitations for the different Member States.

As stated above, it is up to the individual countries to determine areas, but moreover also to determine how the discharge limits will be met. The Directive 91/271/EEC does not indicate which techniques have to be used to meet the standards.

To ensure an enhanced protection and improvement of the aquatic environment, the EU Water Framework Directive, adopted September 2000 which is supposed to come into force autumn 2000 after final publication, introduces the "use of a global ecosystem approach at the catchment level". The new Directive provides that EU levels of protection established by existing EU environmental and water quality legislations will be at least maintained, but that in addition an overall catchment approach has to be adopted. Other sources of phosphorus, in particular agriculture, will be focussed (SCOPE, 2000). Within the new Water Framework Directive, for the first time the role of the European Parliament is enforced (Bloech, 2000). It is to be noted that the definition of "good status" takes 40 pages in the new Directive (Bloech, 2000).

When taking into account the options formulated in Table 3, it becomes clear that any biological process used to achieve the effluent standards and/or the removal ratio, should be manageable very well, i.e. the underlying processes should be understood thoroughly to avoid any malfunctioning to meet the standards. The process should also be operable under very stable conditions. As for the Enhanced Biological Phosphorus Removal (EBPR) process, stricter regulations have led a vast number of research institutes, researchers, plant operators to study the process. Since the development of the process by Barnard (1976), and even more since the introduction of activated sludge wastewater models, research papers increased enormously with positive effect on for the application of EBPR to achieve the standards laid down in the Directive 91/271/EEC.

3. PHOSPHORUS REMOVAL AND/OR RECOVERY PROCESSES

Since gaseous forms of phosphorus are limited, phosphorus must be converted to a particulate (solid) form and removed as such from the wastewater (cf. nitrogen components can be removed as N_2). Orthophosphate, the most abundant phosphorus species (2.3), is a reactive species in chemical reactions and can be consumed in biological growth. Organic phosphorus can be converted to both orthophosphate and polyphosphate. Polyphosphate possibly reacts with metal salts and can be used for biological growth as well (Reddy, 1998).

This paragraph provides an overview of phosphorus removal processes, i.e. natural, chemical and biological processes, with a special emphasis on biological phosphorus removal processes. Attention is also focussed on the sustainability of the process, i.e. to create an optimal phosphorus cycle to preserve the natural resources.

3.1. *NATURAL P-REMOVAL OCCURRING IN ACTIVATED SLUDGE TREATMENT PLANTS*

Primary treatment of wastewaters removes 5-10% of the phosphorus which is associated with particulate matter (SCOPE, 1998), up to 15% according to Bitton (1994). Conventional biological treatment removes up to 10-25% of phosphorus according to standard wastewater treatment handbooks (Metcalf and Eddy, 1991). According to an overview published by the scientific committee on phosphates in Europe (SCOPE, 1998), this secondary treatment due to bacterial metabolic action, is responsible for up to 20-40% of phosphate removal.

3.1.1. Phosphorus assimilation

According to Riding *et al.* (1979) the stoichiometric composition of micro-organisms and the composition of the wastewater is such that 20 to 30% of inlet phosphorus is removed by normal assimilation, i.e. stoichiometric coupling to microbial growth. From the Activated Sludge Model No2 (ASM2) on (see 4.2), the phosphorus content of the micro-organisms involved in wastewater treatment is taken into account for modelling purposes. A weight value of 2% phosphorus (g P/ g COD) is proposed in the these

models (Henze *et al.*, 1995). A phosphorus content of MLSS on a dry solids basis of 1.5 to 3% was reported in conventional activated sludge (Tetreault *et al.*, 1986). According to Reddy (1998), 1.5 to 2.5 wt% phosphorus (based on VSS) is necessary for bacteria for nutritional requirements. Arvin (1983) proposed values ranging between 1 and 2.5% phosphorus for phosphorus assimilation.

3.1.2. Natural, chemical bulk precipitation

Phosphorus removal in sludge may also be the result of natural simultaneous precipitation, if cations such as Ca, Fe, Al, Mg and Zn are present. Up to 6 g P/m³ can be removed taking into account average wastewater compositions (Table 4), which suggests that natural chemical precipitation plays an important role in total phosphorus removal (Arvin, 1985). According to Arvin (1983) it is normally assumed that the solid phase is an amorphous or poorly crystalline calciumphosphate such as tricalciumphosphate (TCP, $Ca_3(PO_4)_2$) or hydroxyapatite (HAP, $Ca_5(PO_4)_3OH$). Amorphous calciumphosphate (ACP, ~$Ca_9(PO_4)_3$) octacalciumphosphate (OCP, $Ca_4H(PO_4)_3$) and dicalciumphosphate (DCP, $CaHPO_4.2H_2O$) are expected to be precursors for the biological apatites. According to the same author, it has been shown that the Ca/P-ratio of the precipitates in biological systems may be as high as 2.23, whereas for HAP this ratio equals 1.67. This observation indicates that simple calcium phosphates probably never form.

Precipitation with Ca is stimulated when pH > 7.5 and is favoured when roughly 50 mg Ca/ℓ is available (Arvin, 1983, 1985). The presence of high Fe- (Brett, 1997) and high F-concentrations (Arvin, 1983) is thought to stimulate the chemical bulk precipitation. The process of chemical bulk precipitation is inhibited when high concentration of Mg are presented, i.e. for Mg/Ca-molar ratios > 0.45. Magnesium stabilises the ACP form and inhibits nucleation and crystallisation of apatites (Arvin, 1983; Brett, 1997). According to Arvin (1983) other precipitation inhibitors are pyrophosphates (added as antiscalant in cooling towers) and bicarbonate. Moreover, magnesium enhances the effects of these inhibitors (Arvin, 1983).

Small amounts of soluble phosphate may also be removed by adsorption to coal particles and other minerals (Arvin, 1983).

Table 4 Potential removal of phosphates by natural, chemical bulk precipitation (after Arvin, 1985)

Cation	Conc. in wastewater [mg/ℓ]	Removal [mg/ℓ]	Cation/P molar ratio	Phosphorus removal [mmol]
Ca	30 – 100	5	1.5	0.08
Mg	5 – 30	2	1.5	0.05
Fe	0.5 – 3.0	1	1	0.02
Al	0.1 – 2.0	0.7	1	0.03
Zn	0.1 – 2.0	0.8	0.5	0.01
			Total	0.19 mmol (6 mg P/ℓ)

3.1.3. Biologically induced phosphate precipitation

It has been observed that phosphate precipitation was induced in wastewater treatment plants due to the microbial reactions occurring in these reactors. Three main processes were distinguished and will be elaborated on in the following paragraphs:

- induced phosphate precipitation due to microbial activity in the aerobic phase
- induced phosphate precipitation caused by denitrifying conditions in biofilms
- induced phosphate precipitation due to the elevated orthophosphate concentrations in EBPR processes

Because of the very tight link between biological activity and calciumphosphate formation, the term "biologically induced/mediated precipitation" or accelerated bulk precipitation is used.

Precipitation of phosphate and its subsequent removal from wastewater is mediated by microbial activity in the aeration tank of the activated sludge process. At the head of a plug flow aeration tank, microbial activity leads to a low pH, which solubilises phosphate compounds. At the end of the tank, a biologically mediated increase in pH leads to phosphate precipitation and incorporation into the sludge (Menar and Jenkins, 1970). Accordingly, Lan (1983) indicated the correlation between alkalinity and phosphorus removal, providing evidence for a calciumphosphate precipitation due to pH increase in the aerobic phase.

Biologically mediated phosphate precipitation also occurs inside denitrifying biofilms. Since denitrification produces alkalinity, denitrifier activity leads to an increase in pH inside the biofilm and subsequent precipitation of calcium phosphate (Arvin and Kristensen, 1983).

Arvin and Kristensen (1985) stated that the higher the phosphorus concentration in the system, the faster the precipitation rate. Thus, precipitation of phosphorus can also be induced by the increase in phosphate concentration that results from the release of phosphorus from the polyphosphate pool under anaerobic conditions. According to the model developed by Maurer *et al.* (1999) precipitation only occurs at relatively high phosphorus concentrations, conditions which are typical for the anaerobic release of phosphorus in EBPR systems (see 3.4). As such, "good" EBPR favours biologically induced phosphate precipitation. Using inactivated sludge (to prevent release of internally stored polyphosphate) containing relatively high concentrations of dissolved calcium (≈ 60 mg/ℓ) and phosphorus (≈ 30 mg P/ℓ), showed a pH-sensitive and partly reversible precipitation of calciumphosphates at pH values below 8.0. The high phosphorus concentration can also induce nucleation of a solid phosphorus phase where supersaturation is normally too small for nucleation to occur. In order to obtain a net phosphorus removal, the pH, the calcium activity and other factors have to be such that precipitated phosphorus does not redissolve under the aerobic conditions. The mechanism is presented in Figure 2. Arvin and Kristensen (1985) observed a ratio of 1 mg Ca/mg P (0.8 mol Ca/ mol P) when precipitation occurred. They also observed partial concurrent precipitation of magnesium, i.e. 0.4 mol Mg/mol P.

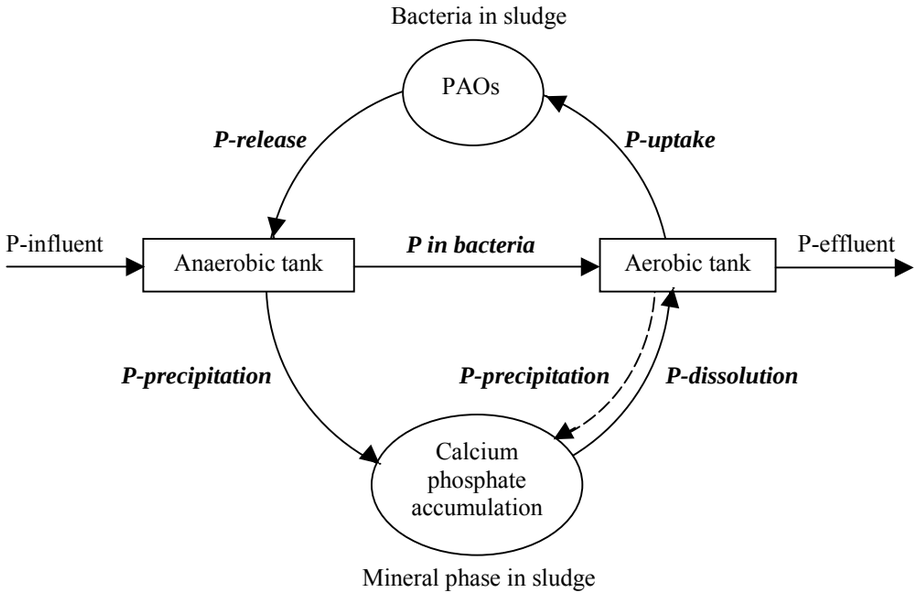

Figure 2 Phosphate accumulation in the mineral phase mediated by the EBPR process (Arvin, 1983)

3.2. *CHEMICAL P-REMOVAL PROCESSES*

This process falls under the category of tertiary chemical phosphorus removal processes and is designed to achieve 95% removal efficiencies (SCOPE, 1998).

Chemical phosphorus removal can be achieved by adding iron and aluminium salts or lime ($Ca(OH)_2$) to wastewater. Commercially available aluminium and iron salts are alum ($Al_2(SO_4)_3.nH_2O$), ferric chloride, ferric sulphate, ferric sulphate and waste pickle liquor (ferrous sulphate and ferrous chloride) from the steel industry (Metcalf and Eddy, 1991; Brett, 1997). These are generally added in excess to compete with natural alkalinity (Bitton, 1994). Aluminium chlorohydrate and polyaluminium chloride (PAC) have also been investigated as possible precipitants. Polymers are used in conjunction with alum and lie as flocculant aids (Brett, 1997).

To achieve a low level of dissolved phosphorus compounds, the metal ions should be present in excess relative to the phosphorus ion concentration. The EPA Design Manual (U.S. EPA, 1976) suggests that 20w% excess of aluminium ions is necessary if 80% reduction of phosphorus is desired while 100% excess is recommended for 95% phosphorus removal. The exact dosage is determined by on-site testing and varies with the specific characteristics of the wastewater.

Lime, which previously was one of the main chemicals used for phosphorus removal is nowadays less frequently used because of increased production of sludge as well as the operational and maintenance problems associated with its use (U.S. EPA, 1987; Metcalf and Eddy, 1991). For cost-effective operation, a thermal lime regeneration unit is required.

In the activated sludge wastewater treatment process, addition of chemical coagulants can occur at various points (see below). Each of these locations will have its advantages and disadvantages, e.g. when alum is used as coagulant, some organic compounds compete with bicarbonate and phosphate species for the aluminium ions (Omoike and van Loon, 1999). Since polyphosphates and organic phosphates are less easily removed by chemical precipitation than orthophosphates, adding aluminium or iron salts after secondary treatment, i.e. when polyphosphates and organic phosphates have been transformed to orthophosphates, usually results in the best removal efficiency (Metcalf and Eddy, 1991).

In general, with chemical precipitation 70 to 95% phosphorus removal can be obtained (Johansson, 1994; Brett, 1997) and effluent concentrations below 0.3 mg P-tot/ℓ can be achieved, depending on the operating conditions. The major disadvantages of chemical P-removal are that it creates large amounts of sludge, the cost of the precipitants and the negative ecological effect of the concentration of aluminium- and iron salts in the effluent (Johansson, 1994).

3.2.1. Direct precipitation

This process needs no further biological removal steps following it and is especially applied when highly effective phosphorus removal is necessary.

The precipitant is applied before the primary settling tank (Figure 3). The concept is usually applied when the receiving water body can deal with relatively high concentrations of carbonaceous matter and nitrogen compounds but where euthropication needs to be controlled by preventing phosphorus loads to the receiver. In the year 1977 138 of 760 Swedish treatment plants used this method and achieved 0.7 g P/m³ average effluent concentration (Rybicki, 1997).

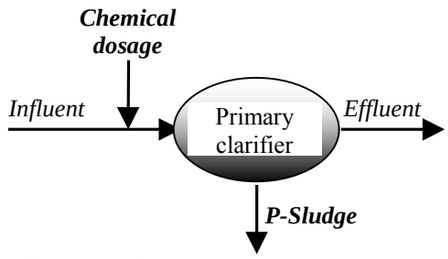

Figure 3 Direct precipitation

3.2.2. Pre-precipitation

Pre-precipitation is preceding further biological treatment. The addition of salts is followed by rapid mixing, flocculation and (primary) sedimentation (Figure 4).

Anionic polymers are sometimes added before flocculation to enhance solids separation. Strong base is also added between addition of ferrous iron and a polymer to counteract the depression of pH. Careful operation can lead to final concentrations as low as 0.1 g P/m³ (Rybicki, 1997).

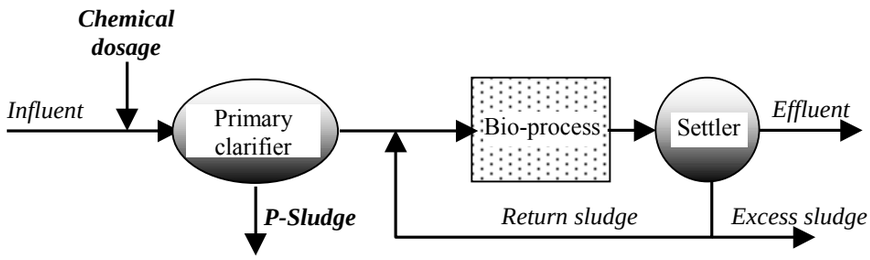

Figure 4 Chemical P-removal using pre-precipitation

Besides chemical phosphorus removal, 70-90% reduction in BOD and SS entering the secondary treatment stage can be achieved, compared wit a 10-40% reduction for unaided settling (Brett, 1997). As stated above, only orthophosphates are removed with this configuration. According to Metcalf and Eddy (1991) disadvantages to this configuration are the possible requirement for addition of polymers for flocculation and the poor dewatering capacity of the sludge. The increased sludge production has the disadvantages of handling problems, but attributes to the increased production of digester bio-gas (Brett, 1997).

3.2.3. Simultaneous precipitation

In this configuration, precipitating salts are added to the inlet of the aeration basin, directly to the basin or to the effluent from the biological treatment before the secondary clarifier (Figure 5). This allows the choice of a proper dosage point to create optimal conditions for coagulation and flocculation (Rybicki, 1997). According to Metcalf and Eddy (1991) addition of polymers to enhance flocculation is not generally not necessary. However, the disadvantage of this method is that velocity gradients and turbulence levels for rapid mixing and flocculation processes can be far from desired conditions (Rybicki, 1997). Moreover, Seyfried *et al.* (1988) showed that the application of ferrous sulphate is an inhibiting factor for *Nitrosomonas*, which gives a problem when simultaneous nitrogen removal is aimed for. Alum did not create any problem while ferric and ferrous chlorides caused activation of *Nitrosomonas*. Resulting effluent concentrations are generally below 1.0 g P/m³ (U.S. EPA, 1987). For higher efficiency simultaneous precipitation can be combined with post-precipitation (see below). According to Brett (1997) care needs to be taken in the choice of chemicals when applying this system along with EBPR. Ferric sulphate appears to inhibit the polyphosphate hydrolysis and thus reduces EBPR.

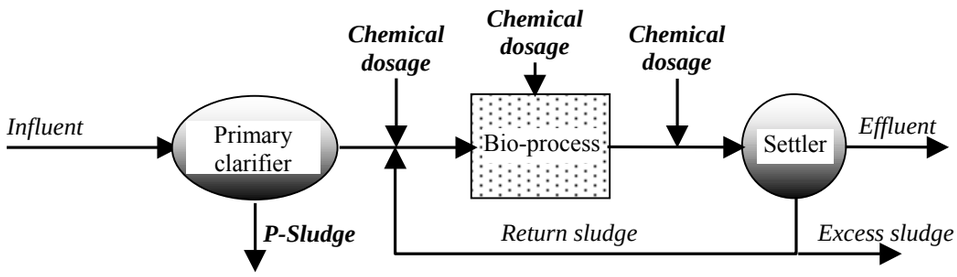

Figure 5 Chemical P-removal using simultaneous precipitation

3.2.4. Post-precipitation

Strictly this term is used to describe the addition of chemicals after secondary clarification to the inflow of tertiary flocculation and sedimentation facilities (Rybicki, 1997; Brett, 1997) (Figure 6). As such the return sludge to the biological treatment does not contain any chemicals added. However, the term is also used to describe the addition of chemicals to the effluent from the secondary biological reactor prior to the secondary sedimentation unit.

In Sweden, an early country to require extensive phosphate removal (SCOPE, 1998), post-precipitation was the most common phosphorus removal process and was applied in 87% of the treatment plants in 1980. The average concentration in the effluent was 0.53 g P/m³ (Rybicky, 1997). Most systems in Sweden are still chemical plants and those with biological systems (see 3.4) have combined chemical treatment (SCOPE, 1998)

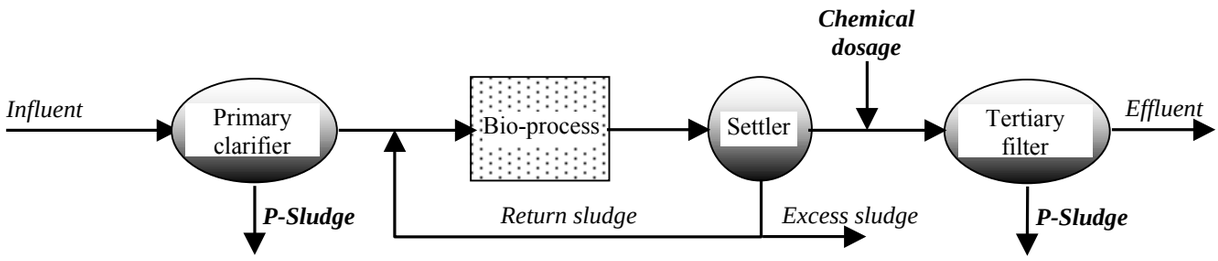

Figure 6 Chemical P-removal using post-precipitation

3.3. *PHYSICO-CHEMICAL P-REMOVAL PROCESSES*

Other less widespread treatment options for removal of phosphorus include adsorption to activated alumina, ion exchange, electrochemical methods and deep-bed filtration (Meganck and Faup, 1988). In the scope of this study no further attention is addressed to these treatment techniques because they are only applied to a much lesser extent.

3.4. *MICRO-ORGANISM MEDIATED, ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL (EBPR)*

Early surveys on biological phosphorus removal in activated sludge plants were made as early as the 1940's by Sawyer (1944), Rudolfs (1947) and in 1955 by Greenburg *et al.*. However, the best known indication of biological phosphorus removal in a wastewater treatment process was published by Srinath *et al.* (1959) from India. Inhibition of the process by toxic substances and the oxygen necessity, were early indications that the phosphorus uptake was a biological process.

The observed high P-removal was initially referred to as "Luxury uptake" (Levin and Shapiro, 1965). In the initial phases of development of biological phosphorus removal some researchers credited this phosphorus removal in activated sludge systems to chemical precipitation (see 3.1.3) and adsorption on the biological sludge (Menar and Jenkins, 1970; Wiechers and van Vuuren, 1979; Simpkins, 1979). Convery (1970) even recommended to avoid the design of WWTPs based on phosphorus removal by luxury uptake and/or physical phenomena. However, by the 1970's several wastewater treatment plants reported total phosphorus removal of 85 to 95%, with the phosphorus content of the waste sludge between 2 and 7% on a dry weight basis (Reddy, 1998). This high phosphorus content of the biomass, higher than for nutritional requirements, clearly indicates the role of biological phosphorus.

Initially, little attention was paid to the reactions occurring in the anaerobic phase and aerobic uptake was thought to occur because of stress caused to the organisms due to the different stages the system was operated in. As such, nearly all attention was initially focussed on aerobic reactions and optimisation of

that phase. This had its implications on microbial research, where emphasis was put on micro-organisms capable of high aerobic phosphorus uptake (see 3.4.4). Considerably more insight has now been gained in the metabolisms underlying the EBPR process (3.4.3).

Currently the existence of pure biological phosphorus is proven, but - depending on concentration levels of other constituents - partial chemical precipitation and/or adsorption processes can occur simultaneously (see 3.1).

The most important disadvantage of EBPR concerns the reversible nature of biological phosphate storage. Thus, the organisms can breakdown the internal phosphorus content and release it again to the environment. Careful handling of the sludge is necessary (3.4.6). Sludge retention times in the settler should be limited and the oxygen supply to the aerobic phase should provide sufficient oxygen also to the outlet of the basin to prevent anaerobic conditions occurring in the secondary clarifier (Reddy, 1998). Release of phosphorus can, however, be turned to advantage when coupled with chemical phosphorus recovery processes (3.6). The important advantages of EBPR are low sludge production and the fertiliser value of the sludge. Difficulties in assuring stable and reliable operation unfortunately still are reported.

Literature reviews on EBPR have been presented by Barnard (1976), Toerien *et al.* (1990), Wentzel *et al.* (1991a), Jenkins and Tandoi (1991), Rybicki (1997), van Loosdrecht *et al.* (1997a) and Mino *et al.* (1998).

3.4.1. The mechanisms underlying EBPR

Several mechanisms have been proposed to explain the enhanced uptake of phosphorus by micro-organisms in wastewater. It has been shown that for biological phosphorus removal to occur in wastewater treatment plants, biomass first needs to pass through an oxygen and nitrate free phase, i.e. an anaerobic phase, before entering a phase where an electron acceptor is present, i.e. an anoxic phase where nitrate is present or an aerobic phase where oxygen is present (References see sections below). The oxygen and nitrate free phase can be achieved in a separate reactor, the first section of a plug flow reactor or part of a sequencing batch reactor cycle. Figure 7 presents the concentration profiles of the mean measurable components for EBPR operated under anaerobic-aerobic conditions.

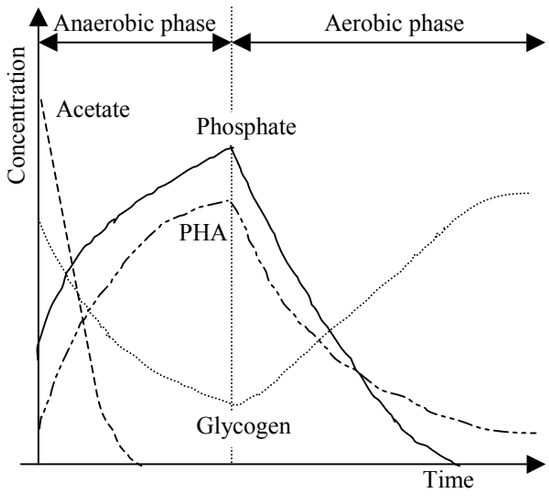

Figure 7 Schematic representation of concentration profiles for EBPR under anaerobic-aerobic conditions

When the wastewater enters the anaerobic phase, specialised organisms, called poly-phosphate accumulating bacteria (PAOs) accumulate carbon sources as an internal polymer called PolyHydroxyAlkanoates (PHAs). The main forms of these PHAs are Poly-beta-HydroxyButyrate (PHB) and Poly-beta-HydroxyValerate (PHV). The energy to store this polymer is obtained from breakdown of glycogen and hydrolysis of an energy rich internal phosphorus chain called poly-Phosphate (poly-P). Since poly-P is broken down to ortho-phosphate for energy supply, the phosphate concentration in the anaerobic phase increases. The anaerobic phase needs to be followed by an oxygen or nitrate rich phase, i.e. an anoxic phase (anoxic P-removal) or an aerobic phase (aerobic P-removal). During this phase the stored PHB is consumed, generating energy for growth, for uptake of ortho-phosphate from the liquid phase and generating energy and carbon for replenishment of the glycogen and poly-P pools.

Under these conditions the ortho-phosphate concentration thus decreases. Most importantly, since the amount of biomass containing large amounts of poly-P - PAOs are able to store up to 10% of their dry weight (Yeoman, 1988a) - is increasing under these conditions, a net phosphorus removal occurs with the wasted sludge.

The anaerobic phase was believed to provide a unique, positive environment for the PAOs, enabling them to reserve the necessary amount of carbon to themselves without having to compete with other micro-organisms (Matsuo *et al.,* 1992). Later research provided evidence that other, so-called Glycogen Accumulating Organisms (GAOs) are able to store carbon sources as well (see 3.4.2.2) and can compete with PAOs in such systems.

It has been assumed that the availability of Short Chain Fatty Acids (SCFAs, also referred to as fermentation products with as main constituent acetate) is a prerequisite for EBPR. In the absence of these components, fermentation of readily biodegradable carbon sources under anaerobic conditions is necessary. Later research results also indicated good phosphorus removal with direct utilisation of readily biodegradable material (see 3.4.2.1).

The intracellular *poly-P pool* in PAOs is divided into two sub-fractions, i.e. the intracellular "volutin" granules and a fraction located in the periplasmic space and/or loosely bounded to the cell membrane (Streichen and Schön, 1991). Because they were first described in *Spirillum volutans* and because they bring about characteristics changes in the pigmentation of certain dyes, the terminology "volutin granules" has been introduced (Schlegel, 1993). Experimentally poly-P granules (e.g. volutin granules) can be easily observed under bright-field or phase-contrast microscopy (Meganck and Faup, 1988), or by staining with many basic dyes such as toluidine blue (poly-P granules become reddish violet) (Brock and Madigan, 1991) or by staining with high 4'-6-diamidino-2-phenylindole (DAPI) concentrations (Kawaharasaki *et al.*, 1999). Internally the polyphosphate molecule is linked to magnesium and potassium. The composition of polyphosphates, based on measured release of phosphorus, magnesium and potassium, is generally presented according the stoichiometric formula $Mg_{1/3}K_{1/3}P$ (Comeau *et al.*, 1987b; Smolders, 1995). Arvin and Kristensen (1985) mentioned that 0.23 mol poly-P was neutralised by K^+, 0.64 mol poly-P was neutralised by Mg^{2+} and 0.13 mol poly-P was neutralised by Ca^{2+}. Ky *et al.* (2000) proposed an internal stoichiometric relationship between magnesium and phosphorus of 0.2 mol Mg/mol P in their model.

Inoue *et al.* (1996) indicated that *PHA* granules in EBPR processes are composed of random co-polymers with different monomer compositions. The physical properties of these polyesters vary considerably and many PHAs have plastic-like consistencies. With the electron microscope the position of the PHA granules can often be seen as light areas that do not scatter electrons, surrounded by a nonunit membrane. PHA granules have an affinity for fat soluble dyes such as Sudan black (Brock and Madigan, 1991).

Glycogen is a starch like polymer of glucose sub-units. Glycogen granules are usually smaller than PHB granules, and can only be seen by electron microscopy. However, the presence of glycogen in a cell can be detected in the light microscope because the cell appears as red-brown when treated with dilute iodine, due to a glycogen-iodine reaction (Brock and Madigan, 1991).

Research to explain for the different phenomena occurring inside the cells, led to more insight in the internal structure and the internal coupling of the different storage polymers. Fleit (1995) reported on research by Anderson and Dawes (1990) and Reusch and Sadoff (1988) who stated that the PHA molecule is a linear polymer arranged in a helix which forms a cylinder, spanning through the cell membrane providing a lipophilic channel (coat) into which the membrane bound fraction of the poly-P chain is twisted from inside, much like a screw. The poly-P helix is stabilised by Ca^{2+} cations within the complex core chain and to the outer PHA shell, stabilising and binding the two chains together.

3.4.1.1. ***Aerobic P-removal (EBPR)***

In the early days of EBPR research, nearly all, if not all, attention was focussed on the aerobic processes. A link between anaerobic processes and aerobic processes was not recognised. The aerobic phosphorus, then called "overplus" or "luxury", uptake (Levin and Shapiro, 1965) was supposed to result from stress conditions due to the dynamic feeding of the activated sludge plants. It is now recognised that the aerobic processes, use of PHA, replenishment of glycogen and uptake of phosphorus, are linked with the anaerobic processes.

3.4.1.2. ***Anoxic P-removal (DEBPR or EBPR with denitrifying PAOs, DPAOs)***

Initially, it was believed that the micro-organisms responsible for EBPR lacked the ability to denitrify and, hence could only grow and accumulate phosphate under aerobic conditions. This idea was initially supported by the observations that nitrate entering the anaerobic phase adversely affected EBPR, that a lower rate of denitrification occurred compared to systems with non-PAO heterotrophs organisms and that generally a net phosphorus release occurred rather than an uptake under anoxic conditions (Barker and Dold, 1996). Later interpretations of earlier results and investigations, however, proved that at least a fraction of the PAOs can accumulate phosphate under anoxic conditions (Malnou *et al.*, 1984; Hascoet and Florentz, 1985; Comeau *et al.*, 1986; Gerber *et al.*, 1987; Vlekke *et al.*, 1988; Kern-Jespersen and Henze, 1993; Kuba *et al.*, 1993, 1994; Jørgensen and Pauli, 1995; Barker and Dold, 1996).

Vlekke *et al.*(1988) probably first proved the denitrifying capacity of PAOs by operating Sequencing Batch Reactors (SBRs, see 3.4.5.3) using an anaerobic-anoxic sequence instead of an anaerobic-aerobic sequence. Complete phosphorus removal could be obtained. Later on Kuba *et al.* (1993) performed similar experiments with the same observations. Evidence is obtained that nitrate probably is not as efficient as oxygen for phosphorus removal since more stored carbon (PHB) is utilised for a given amount of phosphorus removed (Kuba *et al.*, 1993; Filipe and Daigger, 1999). The observed phosphorus uptake rate is significantly lower for anoxic uptake than for aerobic uptake, i.e. 10 and 70 mg P/g VSS.h respectively. Earlier observations indicating a slower rate of anoxic phosphorus uptake compared to aerobic uptake were reported by Osborn and Nicholls (1978), using sludge originating from an anaerobic-aerobic pilot plant and subjecting this sludge during batch tests to aerobic or anoxic conditions. Since the sludge was not continuously subjected to anaerobic-anoxic conditions, and because contradictory results continued to be obtained by several authors (Wentzel 1989a, 1991b; Hascoet and Florentz, 1985; Iwema and Meunier, 1985) controversy regarding the existence of DPAOs continued. The experiments performed by Vlekke *et al.* (1988) and by Kuba *et al.* (1993), however, did provide sufficient evidence and phosphorus removal under anoxic conditions is now generally accepted. Later on, Kuba *et al.* (1996a) used their observations to propose a model for DPAOs (see 4.3.5.3).

The use of nitrate rather than oxygen in PAO sludges, is advantageous for several reasons. The supply of organic substrates in wastewater, needed for both biological phosphorus and nitrogen removal, is usually limited. With DEBPR, the same organics can be used for nitrate and phosphorus removal. This double use of carbon source will result in reduced sludge production, and the use of nitrate as electron acceptor for at least a portion of the phosphate uptake will reduce aeration demand (Kuba *et al.*, 1996b; Copp and Dold, 1998). Moreover, it makes aeration only necessary for nitrification (Kuba *et al.*, 1996b). Additionally, Kuba *et al.* (1993) observed a clear difference in growth yield between anaerobic-aerobic sludges and anaerobic-anoxic ones, i.e. 0.35 and 0.25 mg SS/mg COD respectively. Using a two-sludge system, i.e. DPAOs and nitrifiers completely separated in two sludges with recirculation of the nitrified supernatant from the nitrifying stage to the anoxic stage, Kuba *et al.* (1996b) observed the required COD to be up to 50% less than for conventional aerobic phosphorus and nitrogen removing systems. Oxygen requirements and sludge production decreased to about 30 and 50%, respectively. Kuba *et al.* (1993) also observed a better SVI value, indicating better sludge settleability compared to anaerobic-aerobic systems.

Experimental evidence indicates that two different populations of PAOs exist in EBPR systems (Kern-Jespersen and Henze, 1993): PAOs that can only use oxygen as a terminal electron acceptor and DPAOs that can use both oxygen and nitrate as terminal electron acceptors. Filipe and Daigger (1999) stated the DPAOs have a competitive disadvantage when competing with PAOs, because of a lower thermodynamic efficiency of anoxic growth compared to aerobic growth.

Kuba *et al.* (1994), Chuang *et al.* (1996) and Filipe and Daigger (1999) provided evidence that DPAOs can use both internally stored PHA and external substrate for denitrification. When external substrate is used, phosphorus release is observed whereas phosphorus uptake is expected when internally stored PHA is degraded. Both mechanisms occur concurrently. According to Chuang *et al.* (1996), the kinetic competition observed is determined by the poly-P content of the micro-organisms. With an increased poly-P content, an increased specific phosphorus release rate is observed.

3.4.2. Factors influencing the EPBR process

In this section, external factors influencing the EBPR process will be discussed. Whereas initial research mainly focussed on the aerobic processes it became gradually clear that good phosphorus removal activity can only be obtained when anaerobically micro-organisms are subjected to conditions favouring storage of sufficient carbon sources, to be utilised under aerobic conditions with simultaneous uptake of ortho-phosphate.

This section comprises external factors that directly influence the anaerobic process and thus indirectly the overall process. First, attention will be focussed on the carbon sources utilised by the responsible micro-organisms, and how these carbon sources influence the phosphorus release, the formation of PHAs and the influence on the overall EBPR performance. These paragraphs are followed by a paragraph on possible proliferation of non-PAOs under anaerobic conditions and their influence on the EBPR process. This section follows the carbon sources, since the latter will reveal predominantly responsible for proliferation of non-PAOs competing for the same substrate as PAOs. Other factors mentioned in literature that can influence the EBPR performance are external pH, SRT, presence of nitrite and temperature. These factors will be discussed in separate sections.

3.4.2.1. ***Short Chains Fatty Acids (SCFAs) and non-SCFAs as carbon sources***

On an average basis the COD to phosphorus ratio should be at least 35, or the BOD to phosphorus ratio should be at least 20 to achieve good phosphorus removal (SCOPE, 1998). Randall *et al.* (1992) more explicitly states that a ratio of BOD_5 to total phosphorus of 20:1 or greater is needed to reliably achieve an effluent with a total phosphorus concentration of 1.0 mg P/ℓ or less, when gravity sedimentation is used as the last treatment step. For the total COD to total phosphorus ratio, Randall *et al.* (1992) state that ratios of 45 or greater are necessary. According to Janssen (1999) 1 g of phosphorus can be removed when 10 g of readily biodegradable carbon source is available. According to Reddy (1998) 50 mg COD/mg P removed is a conservative number for North American municipal wastewater, and is recommended for design purposes. In their work, Ekama and Marais (1984a) suggested 50 to 59 mg COD/mg P is necessary to remove phosphorus efficiently from South African wastewaters. For higher ratios, the authors stated that it is very likely removals down to 0.5 mg P/ℓ can be achieved. However, most important for good phosphorus removal to occur is the kind of carbon source anaerobically available. In general carbon sources are subdivided in so-called short chain fatty acids (SCFAs) and non-SCFAs.

In initial studies concerning EBPR authors continuously refer to good EBPR activity in connection with carbon sources belonging to the group of short chain, low molecular monocarboxylic acids (C_1-C_6) also called short chain fatty acids (SCFAs) or volatile fatty acids (VFAs). Fuhs and Chen (1975), Potgieter and Evans (1983), Malnou (1984), Ekama *et al.* (1984b), Arvin and Kristensen (1985) and Comeau *et al.*

(1987b) all report a more important phosphorus release when acetate or propionate are used instead of other substrates. According to Reddy (1998) an accepted rule of thumb is that the readily available organic matter concentration, which corresponds to the group of SCFAs, within the initial anaerobic zone must be more than 25 mg COD/ℓ to accomplish significant EBPR. Increases will increase the phosphorus release and the organic storage in the anaerobic zone, up to some optimum VFA-to-phosphorus ratio. Conversely, studies by Randall and Chapin (1995, 1997) show that high concentrations of acetic acid (greater than 400 mg COD/ℓ), also can cause failure of EBPR processes.

The group of non-SCFAs studied in relation to EBPR performance mainly comprises the oxocarboxylic acid pyruvate, hydroxy fatty acids such as lactic acid and malic acid, di-acids such as oxalic acid, malonic acid, succinic acid and maleic acid, monosaccharides such as glucose and disaccharides such as lactose and sucrose. Starch, a glycogen-like polymer, is also relatively often tested for its EBPR activity. These components are considered because of their appearance in wastewater, their production during fermentation or glucose metabolism (e.g. pyruvate).

Depending on the carbon sources different anaerobic release rates/amounts and PHA uptake rates and compositions can be encountered. In the following sections these relations are discussed.

3.4.2.1.1. *Different carbon sources and their influence on phosphorus release*

- *SCFAs as carbon sources*.

When *formate* is supplied as carbon source Comeau *et. al.* (1987b) reported fast phosphorus release. In several cases, Arvin and Kristensen (1985) observed nearly no or only limited uptake of formate. However, despite this, phosphorus release was significant in some cases with ortho-phosphate release concentrations up to 40 mg PO_4-P/ℓ for an initial concentration of 250 mg COD-formate/ℓ which was not utilised under anaerobic conditions. Using sludge acclimatised to glucose feed and subjecting this to formate, inconsistent results were obtained (Randall *et al.*, 1994). According to Reddy (1998) polymerisation of one carbon components is thermodynamically unfavourable for the bacteria.

Since the discovery of EBPR, *acetate* has been the carbon source to which most attention has been attributed because of its positive influence on the process. Moreover, fermentation processes preceding or active in the anaerobic phase will provide large amounts of acetate. In Table 5 phosphate release to acetate-uptake ratios experimentally observed by different authors are presented. The calculated ratio of 0.71 mg PO_4-P/mg COD reported by Abu-Ghararah and Randall (1991) overestimated their measured ratio (Table 5). No explanation could be provided by the authors. The pH influence observed and theoretically elaborated by Smolders *et al.* (1994a) provides a possible explanation for the observation made by Abu-Ghararah and Randall (1991).

Table 5 Phosphate release/acetate uptake ratios (after Smolders, 1995)

Authors	ratio (mg PO_4-P/mg COD-acetate)
Wentzel *et al.* (1986)	0.23
Arun *et al.* (1988)	0.20 – 0.38
Mino *et al.* (1987)	0.38
Wentzel *et al.* (1988)	0.50 – 0.55
Arvin and Kristensen (1985)*	(0.60 – 0.72)* 0.68
Comeau *et al.* (1987b)	0.68 – 0.73
Kunst *et al.* (1991)	0.30
Abu-Ghararah and Randall (1991)	0.37
Smolders (1994a)	ratio is pH dependent (see 3.4.2.3)
Henselmann *et al.* (2000)	ratio is glycogen and poly-P dependent

* These results mentioned in Smolders (1995) relate to experiments not having reached full steady state.

Rustrian *et al.* (1997) using pure cultures of different *Acinetobacter* strains and Satoh *et al.* (1996) using sludge from a full scale WWTP, found that the amount of VFA consumed is highest when acetic acid (and propionate, see below) is used as carbon sources. They also observed a significant decrease in carbohydrate (glycogen) concentration along with acetate consumption. Comeau *et al.* (1987b) reported immediate fast phosphorus release when using acetate as substrate. Using acetate as carbon source, the highest amount of phosphorus was released according to Abu-Ghararah and Randall (1991) (see Table 5 and Table 6).

As mentioned already in the previous paragraph, Satoh *et al.* (1996) reported high phosphorus release when using *propionate* as carbon source. The carbohydrate (glycogen) concentration in the sludge, however, only decreased slightly. Using pure cultures of different *Acinetobacter* strains, Rustrian *et al.* (1997) observed the least amount of VFA consumed when using propionic acid. Arvin and Kristensen (1985) reported the same molar ratio of phosphorus release to propionic acid uptake as to acetate uptake, i.e. 1.5 mol P/mol propionic acid (0.41 mg PO_4-P/mg COD-propionate). Using propionic acid as carbon source revealed the lowest phosphorus uptake according to Abu-Ghararah and Randall (1991) (Table 6).

When *butyrate* was supplied as carbon source, Rustrian *et al.* (1997) observed the amount consumed to be in between the acetate and propionate amounts consumed. Comeau *et al.* (1987b) reported immediate fast phosphorus release when using butyric acid. *Branched chain isomers of butyrate* yielded small but significant improvements in phosphorus removal compared to their linear counterparts.

Comeau *et al.* (1987b) also reported immediate fast phosphorus release using *valerate* as carbon source.

In Table 6 an overview is presented from ratios of phosphorus released and different SCFAs utilised as observed by Abu-Ghararah and Randall (1991). Taking into account the molar ratios it is observed that the phosphorus release to SCFA ratio increases with increasing molecular weight of the SCFA except for acetic acid and the fact that isoforms stimulated more release than the non-branched forms. Taking into account the weight ratios, it observed the greatest amount of phosphorus released was obtained using acetate followed by isovaleric acid.

Table 6 Ratio phosphorus released and SCFAs utilised (Abu-Ghararah and Randall, 1991)

Carbon source	weight ratios of P-release to COD-VFA utilised [mg P/mg COD]	molar ratios of P-release to COD-VFA utilised [mol P/mol COD]
Acetic acid	0.37	0.77
Propionic acid	0.12	0.44
Butyric acid	0.15	0.78
Isobutyric acid	0.16	0.80
Valeric acid	0.19	1.72
Isovaleric acid	0.25	2.31

- *Non-SCFAs as carbon source*

Where information is available attention is focussed on the question whether fermentation of the non-SCFAs occurs, or whether this carbon source is directly used by the PAOs. Direct uptake of non-SCFAs by other organisms, causing possible risks for breakdown of EBPR activities is addressed in a separate section (3.4.2.2).

Satoh *et al.* (1996) reported *pyruvate* (and succinate and lactate, see below) to disappear faster from the supernatant (about 5 minutes) than acetate, propionate and malate, although a lower net phosphorus release was observed compared to acetate or propionate. It was observed that pyruvate was initially stored in a form other than PHA (not mentioned which), without phosphorus release. The accumulated material was then gradually converted to PHA with concomitant phosphorus release. Without really having

conclusive experimental evidence, they assumed no fermentation occurred and both internal storage and transformation to PHA was performed by PAOs. Possible adsorption of the carbon source to the sludge was not discussed.

Using *lactate* Comeau *et al.* (1987b) reported immediate fast phosphorus release. Satoh *et al.* (1996) reported lactate to disappear faster from the supernatant than acetate, propionate and malate, although a lower net phosphorus release was observed compared to acetate or propionate. As for pyruvate, the authors observed a fast storage in a form other than PHA, followed by a phase with phosphorus release and concomitant PHA formation. Jeon and Park (2000) observed lactate production by fermentative bacteria with concurrent uptake of this produced lactate and phosphorus release by PAOs.

According to Satoh *et al.* (1996) the uptake of *malate* was slowest with the lowest amount of phosphorus released (comparable to pyruvate) and of PHA formed. Malate also disappeared slower from the supernatant than succinate, pyruvate and lactate.

Succinate disappeared faster from the supernatant than acetate, propionate and malate according to Satoh *et al.* (1996). As for pyruvate and lactate, the authors observed a fast storage in a form other than PHA, followed by a phase with phosphorus release and concomitant PHA formation.

With the discovery of the EBPR process (Levin and Shapiro, 1965), it was initially thought *glucose* was the most important carbon source (Nicholls and Osborn, 1979). Although, in 1975 Fuhs and Chen postulated an anaerobic phase was necessary for fermentation processes to produce SCFA, they also postulated the fermentation products were consumed aerobically, i.e. no link was made between anaerobic and aerobic phosphorus related processes. With the basic background of the EBPR process becoming more and more clear from the 1980's on and the indication of a link between the anaerobic and aerobic processes, attention became more focussed on anaerobic usage of the SCFA sources. However, since the studies by Fukase *et al.* (1985) and by Cech and Hartman (1993) indicating possible deterioration of EBPR activity using glucose as a carbon source, more and more authors try to find experimental evidence for the fate of glucose in EBPR systems. Regarding the relation between anaerobic glucose uptake and phosphorus release, only little information is encountered in literature. Using glucose feed Arvin and Kristensen (1985) and Fukase *et al*. (1982) observed a time delay effect, i.e. P-release is somewhat delayed relative to the glucose uptake. Mostly authors report on the overall EBPR performance (3.4.2.1.3) and the storage components (3.4.2.1.2) linked with glucose uptake.

Whether or not glucose uptake is coupled with fermentation is still unclear. Nakamura and Dazai (1989) and Carucci *et al.* (1997) reported on release of lactic acid in anaerobic batch tests on a glucose and peptone feed, indicating fermentation took place. Jeon and Park (2000) observed that EPBR with glucose supply was accomplished at least by two kinds of bacterial populations, a lactic acid producing organism (LPO) and a polyphosphate accumulating organism (PAO). Jeon *et al.* (2000) described the morphological characteristics of the sludge present in these systems. Sudiana *et al.* (1997) observed small amounts of acetate and propionate in the bulk solution when using glucose as carbon source in batch experiments. Wentzel *et al.* (1991a) stated that direct uptake of glucose by PAOs is not possible. Randall *et al.* (1994) on the contrary observed rapid transport and storage into the cells before fermentation. The EBPR activity deteriorated at certain occasions, attributed by the authors to growth of GAOs outcompeting the PAO population.

According to Carucci *et al.* (1999), fermentation should be considered an alternative way for heterotrophs other than PAOs or GAOs to obtain the necessary energy for the storage of organic substrates (as glycogen or other storage products) and possibly for growth itself. Fermentative micro-organisms can thus compete for soluble organic compounds in the anaerobic reactor of nutrient removal plants. This additional competition could also explain the high variability often observed in the performance of biological P removal in real plants (Morales *et al.*, 1991; Harremoes, 1998). Whereas it is accepted in general that PAOs use SCFAs (anaerobically produced by fermentative bacteria), Carucci *et al.* (1999)

stated that this implicitly means that the fermentative metabolism is completely useless for micro-organisms performing it, while it gives an advantage to their competitors, the PAO heterotrophs, which are able to use the catabolic products (SCFAs). Such altruism is unlikely to occur in the microbial world. In ASM2d (Henze *et al.*, 1995), growth of fermentative bacteria indeed is not accounted. But, the authors used this simplification because the growth of low yielding fermentative bacteria has only a minor impact on the overall process.

The state-of-the-art clearly shows that anaerobic metabolic processes due to non-PAO heterotrophs should include not only growth but also storage (Carucci *et al.*, 1999). Whereas fermentation is generally neglected in anaerobic-anoxic-aerobic processes because the typical fermentative metabolism has a very low yield, usually in the range 0.1-0.2 (Pavlostathis and Giraldo-Gomez, 1991), storage is a high yield mechanism. For a given amount of fermentable substrate, the fraction converted into fermented products (useful to PAOs) will decrease while the fraction converted into useful products for non-PAOs will increase (Carucci *et al.*, 1991, 94).

Using *starch*, a glycogen like polymer, as feed for an SBR system, Randall *et al.* (1994) observed no EBPR activity. Marais *et al.* (1983) even reported the direct use of *sewage substrate* as carbon source.

To summarise the last paragraphs on non-SCFAs, it can be concluded that whereas initially it was generally accepted that PAOs are unable to directly utilise carbon sources other than SCFAs under anaerobic conditions, experimental evidence is such that PAOs appears to have mechanisms to directly use non-SCFAs without prior fermentation by heterotrophic bacteria. Much controversy still exists concerning the impact of these carbon sources on the overall phosphorus removal capacity (Liu *et al.*, 1996b; Mino *et al.*, 1998).

3.4.2.1.2. *Different carbon sources and their influence on storage components*

- *PHA composition*

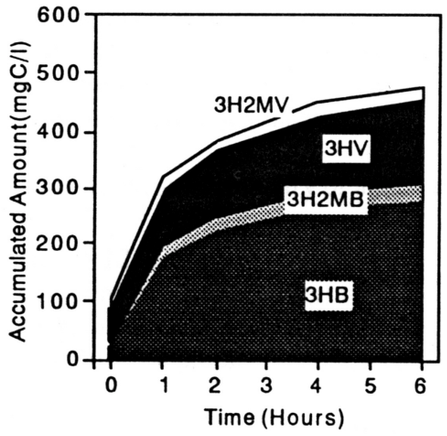

Figure 8 PHA composition for acetate uptake (Satoh et al., 1994)

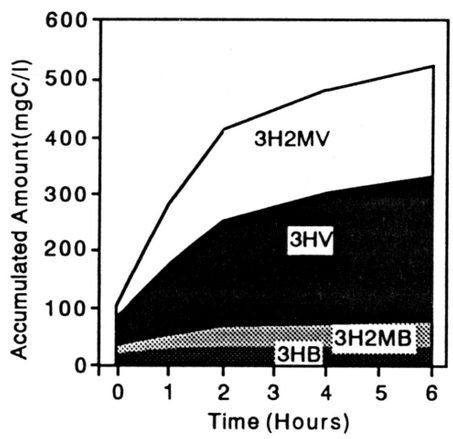

Figure 9 PHA composition for propionate uptake (Satoh et al. 1994)

Intracellular carbon storage other than PHB was first reported by Comeau *et al.* (1987b), indicating the existence of co-polymers composed of 3HB and 3HV (3-Hydroxy-Valerate). In 1992 Matsuo *et al.* added 3H2MV (3-Hydroxy-2-MethylValerate) and 2H2MB (2-Hydroxy-2-MethylButyrate) to the list of possible co-polymers. Using sludge acclimated to synthetic feed, Satoh *et al.* (1992) identified that the sink of carbon in anaerobic uptake of acetate and propionate was PHA composed of 3HB, 3HV, 3H2MB and 3H2MV (Figure 8 and Figure 9). From anaerobic experiments performed by Satoh *et al.* (1996) using sludge from a pilot-scale UCT-installation, it was shown that acetate feed will mainly result in intracellular storage of PHB, whereas a smaller fraction of PHV was formed Figure 10.

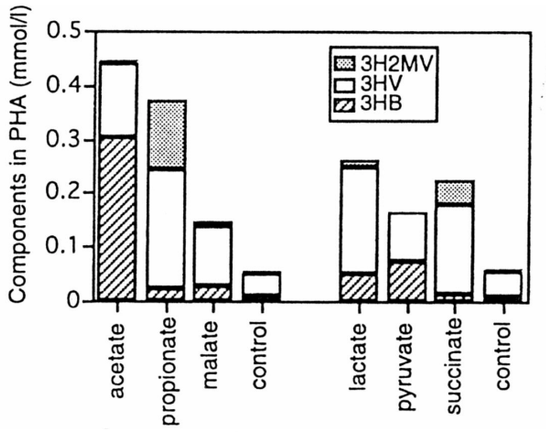

Figure 10 Content of accumulated PHA within 3 hours after injection of different substrates (Satoh et al., 1996)

Using acetate as a sole carbon source, Smolders *et al.* (1994) also observed mainly production of PHB. The amount of PHV formed amounted to only 10% of the PHB formed. Using the same carbon source, Murnleitner *et al.* (1997) consider 20% PHV and 80% PHB as an average value. Lemos *et al.* (1998) reported on the production of a copolymer of HB and HV, with the HB units being dominant, when using acetate as a sole carbon source. Using propionate as sole carbon source, PHV and PH2MV were formed (Satoh *et al.,* 1992; Lemos *et al.*, 1998). Using lactic acid, malic acid or succinate, mainly PHV is accumulated.

Using butyrate, Lemos *et al.* (1998) reported HB units to be produced to a higher extent, but the total amount of polymer formed was much less compared to acetate or propionate feed. Using pure lactic and malic acid, a small fraction of PHB is formed, whereas a small fraction of PH2MV is formed using succinate alone (Satoh *et al.*, 1996). Louie *et al.* (2000) observed both PHB and PHV accumulation when acetate was used as carbon source. A PHV to PHB ratio of 0.6-0.75 of was observed. Feeding pyruvate combined with acetate resulted in accumulation of PHB and PHV in approximately a 1:1 ratio. Citrate addition did not result in PHA. Succinate or succinate combined with acetate resulted in a 2-fold higher accumulation of PHV than PHB.

The yield of polymer produced per carbon consumed was found to diminish from acetate (0.97 C/C), to propionate (0.61 C/C), to butyrate (0.21 C/C). Using a mixture of acetate, propionate and butyrate and increasing the carbon concentration, but maintaining the relative concentration of each substrate, propionate was primarily consumed, thus PHA was enriched with HV units.

Liu *et al.* (1996b) experimentally proved that non-PAOs are also able to utilise acetate, propionate, butyrate, valerate, pyruvate, lactate (most key intermediates in the TCA cycle) and gluconate under anaerobic conditions to form PHAs, just like PAOs do. The energy necessary to store these compounds is obtained from breakdown of glycogen, aerobically obtained from PHA (see further GAOs, 3.4.2.2).

Sudiana *et al.* (1997) observed no difference in total PHA content between glucose and acetate fed systems showing good EBPR performance. However, no information is provided regarding the PHA fractionation. Liu (1998) observed that glucose uptake was coupled to a decrease in the sludge's carbohydrate (glycogen) content with probable conversion to non-carbohydrates as PHA. No important PHB profile was recorded, but since PHB was the only component measured, the authors assumed that probably other PHAs were involved. Carucci *et al.* (1995), however, reported PHAs were not detected during the anaerobic release of ortho-phosphate in an anaerobic/aerobic sequencing batch reactor when glucose and peptone were supplied as mixed carbon substrate. Glucose decrease in these systems was not connected to the release of ortho-phosphate, but overall good phosphorus removal was observed. Jeon and Park (2000) observed that the amount of PHAs synthesised was less than the amount of glucose added since glucose was converted to other storage compounds by the LPO.

- *Total carbohydrate content*

Studies considering the deterioration of EBPR activity are closely linked with the presence of glucose under anaerobic conditions. For these systems much controversy still exists regarding the fate of total carbohydrates, i.e. with main emphasis on glycogen. To study these systems, deterioration was often

induced by feeding the systems with a very low phosphorus to carbon ratio (Liu *et al.*, 1994, 1996b; Sudiana *et al.*, 1997). The next paragraph should therefore be interpreted carefully when evaluating the results mentioned in literature.

Liu (1998) observed good phosphorus removal in systems fed with glucose when the sludge carbohydrate content was low (9-10% CH/MLSS). Sudiana *et al.* (1997) observed a clear increase in total carbohydrate content when using acetate and glucose to low phosphorus sludges.

Jeon and Park (2000) observed concurrent rapid glucose disappearance and glycogen accumulation instead of glycogen decrease, as reported by other authors. Results showed that the EPBR with glucose supply was accomplished at least by two kinds of bacterial populations, a lactic acid producing organism (LPO) and a polyphosphate accumulating organism (PAO) (Jeon *et al.*, 2000).

3.4.2.1.3. *Different carbon sources and their influence on EBPR performance*

It is noticed that little information is available on the overall observed influence of different carbon sources on the EBPR activity.

For their research Satoh *et al.* (1996) performed release experiments subjecting activated sludge from the end of the aerobic zone of a WWTP treating real sewage to anaerobic-aerobic conditions with different carbon nutrients as feed. As mentioned above, acetate, lactate, pyruvate, malate, succinate and fermented sewage were used as carbon source. The experiments only comprised one cycle, i.e. the sludge was not exposed to longer periods of cyclic aerobic-anaerobic conditions. Complete phosphorus removal was obtained. However, it has to be stated that in the control experiment too, in which no substrate was added, a small anaerobic phosphorus release and full aerobic phosphorus removal were observed.

Randall *et al.* (1994), performing batch tests using sludge acclimatised to glucose feed, experimentally observed increased phosphorus removal when C_1-C_5 carboxylic acids, except propionate, were used as feed stock. Acetate and isovalerate showed the best results in terms of phosphorus removal. Formate gave inconsistent results. Valerate and the dicarboxylic acid succinate also improved phosphorus removal by amounts approaching those of acetate and isovalerate. Branched chain isomers of valerate and butyrate yielded small but significant improvements in phosphorus removal compared to their linear counterparts. Lactate and pyruvate did not affect EBPR (Randall *et al.,* 1994). These results differ from observation by Abu-Ghararah and Randall (1991), using sludge from a UCT pilot plant fed with domestic sewage. They experimentally verified the amounts of COD required per mg phosphorus removed. Acetic acid had to be dosed in the least amount to obtain the highest phosphorus removal capacity, followed by isovaleric acid and propionic (Table 7).

Table 7 Ratio of COD utilised per mg of phosphorus removed (Abu-Ghararah and Randall, 1991)

Carbon source	ratio of mg COD-VFA utilised to mg P removed
Acetic acid	18.8
Isovaleric acid	23.5
Propionic acid	31.5
Isobutyric acid	36.1
Butyric acid	39
Valeric acid	94

<u>*Glucose*</u>, alone or in a mixture with acetate promoted good EBPR activity (Fukase *et al.*, 1985; Matsuo and Miya, 1987; Appeldoorn *et al.*, 1992; Carucci *et al.*, 1995; Sudiana *et al.*, 1997; Liu, 1998; Jeon and Park, 2000). Nakamura *et al.* (1991) reported anaerobic phosphate release and glucose consumption using strain NM-1. Overall good phosphorus removal was observed.

3.4.2.2. ***Deterioration of EBPR through predominance by micro-organisms other than PAOs?***

Sometimes anaerobic-aerobic operation does not yield EBPR activity for reasons yet unclear (Matsuo *et al.*, 1982; Fukase *et al.*, 1985; Cech and Hartman, 1990,1993). Cech and Hartman (1993) and Satoh *et al.* (1994) reported so-called G-bacteria to predominate in the system after deterioration of EBPR activity when glucose was used as carbon source. Once glucose was removed from the medium, the EBPR activity slowly recovered. According to Jeon and Park (2000) glucose in the feed does not necessarily induce a deterioration of the EBPR activity. On the contrary, a symbiosis then occurs between non-PAOs and PAOs. Glucose is partially accumulated as glycogen and partially metabolised to lactic acid, by so-called LPOs. PAOs then slowly convert the lactic acid produced by the LPOs into PHAs with hydrolysis of poly-P. The authors stated LPOs can compete with other acidogenic bacteria since they rapidly accumulate glucose as glycogen before other acidogenic bacteria can ferment glucose to produce SCFAs.

In general it became clear that besides PAOs, other micro-organisms showed the ability to store not only non-SCFAs but SCFAs as well under anaerobic conditions without concurrent phosphorus release, thus creating a potential competition for PAOs (Cech and Hartman, 1993; Liu *et al.*, 1996b, Sudiana *et al.*, 1997). In feed stocks providing both acetate and glucose, GAOs could become predominant, storing acetate faster than PAOs by utilising glucose as energy source (Liu *et al.*, 1996b; Sudiana *et al.*, 1997). In the absence of glucose, PAOs outcompete GAOs for acetate as the acetate uptake rate of PAOs is higher than that of GAOs (Liu *et al.*, 1996b). When acetate is present in the anaerobic phase in excess of the requirements for PAOs, both GAOs and PAOs can be present in the system (Manga *et al.*, 2000).

Liu *et al.* (1996b) promoted growth of non-PAOs by feeding the system with low phosphorus/carbon ratio wastewaters (2w%). They experimentally observed anaerobic uptake of acetate, propionate, butyrate, valerate, pyruvate, lactate and gluconate with concurrent storage of PHA. Feeding glucose, fructose, maltose, sucrose, trehalose and raffinose to the system, concurrent storage of glycogen and PHA was observed. The energy and reducing equivalents required for the carbon uptake and storage are thought to be generated from the glycolysis of either cellular glycogen or a sugar taken up, indicating non-PAOs were involved.

Using sludge from a system showing deterioration of EBPR activity, Satoh *et al.* (1994) indicated uptake of acetate and propionate with concurrent storage of PHA without polyphosphate breakdown.

During long term experiments performed by Randall *et al.* (1994) occasional breakdown of the phosphorus removal capacity occurred, once with partial recovery and on another occasion without later recovery of the EBPR. They assumed GAOs competed with PAOs.

Since GAOs seem always to be present, but are suppressed as a minority in good functioning EBPR processes (Cech and Hartman, 1993; Matsuo, 1994) it can be stated that GAOs may possibly function as a scavenger for soluble COD, and may become the dominating population in deteriorated EBPR processes (Liu *et al.*, 1996b). Indeed, as the GAOs are able to utilise different mechanisms for anaerobic uptake of substrates they will be in a favourable position whenever the internal poly-P reserve of PAOs is depleted due to external unfavourable conditions (nitrate inhibition, unfavourable growth conditions, or unknown factors).

Satoh *et al.* (1994) postulated that if the influent contains a low content of amino acids or proteins, the growth of PAOs might become slower and the GAOs might become dominant. If amino acids are not present in the influent, micro-organisms have to produce them from inorganic nitrogen and carboxylic acids from the TCA cycle. It is assumed that the carboxylic acids in the TCA cycle are supplied from PHA. Once this PHA reserve is depleted, micro-organisms have to use glycogen as the raw carbonaceous material. Enzymes available in GAOs but rather less in PAOs are then expected to supplement the TCA carboxylic acids from glycogen.

3.4.2.3. ***External pH-influence***

Studies performed by Tracy and Flammino (1985) have shown that EBPR mechanisms do not function at less than pH 5.4. According to Reddy (1998) studies have not been performed to evaluate the effect of high pH-values on the EBPR process. However, the author stated that it is known that the EBPR mechanism can operate in the pH-range 8.5 tot 9, but, chemical precipitation also becomes important.

In the following paragraphs the influence of the external pH on the anaerobic and the aerobic reactions and on the overall EBPR process is elaborated. However, in literature not very many reports can be found describing the influence of pH on the EBPR process, limiting the number of references used.

3.4.2.3.1. *External pH influence on the anaerobic reactions*

Smolders *et al*. (1994a) observed a relationship between the amounts of orthophosphate released and of acetate taken up under anaerobic conditions at the one hand and the pH at which the experiments were carried out at the other. Since PAOs use poly-P breakdown and thus phosphate release to obtain energy for acetate uptake and PHA formation, it was initially thought the ratio should be constant. However, the transport of acetate over the cell membrane consumes energy and the amount of energy necessary is pH dependent (Smolders, 1994a). Fleit (1995) and Filipe and Daigger (1998), however, disagree with this statement and put forward that acetate is taken up in the undissociated form and that uptake proceeds without energy consumption. According to Bond *et al.* (1999) polyphosphate is broken down to maintain a constant internal pH. However, this means that they can no longer explain for the pH dependency of the orthophosphate release to acetate uptake ratio.

According to Smolders *et al.* (1994a) the acetate uptake rate showed no pH dependency (7.5 C-mmol/ℓ/h or 41 mg C/g VSS/h), whereas the phosphorus release rate increased with increasing pH. From Figure 11 and taking into account a value of 2.2 g VSS/ℓ a phosphorus release rate ranging from 4 to 7.3 P-mmol/ℓ/h is observed (124 up to 227 mg P/g VSS/h).

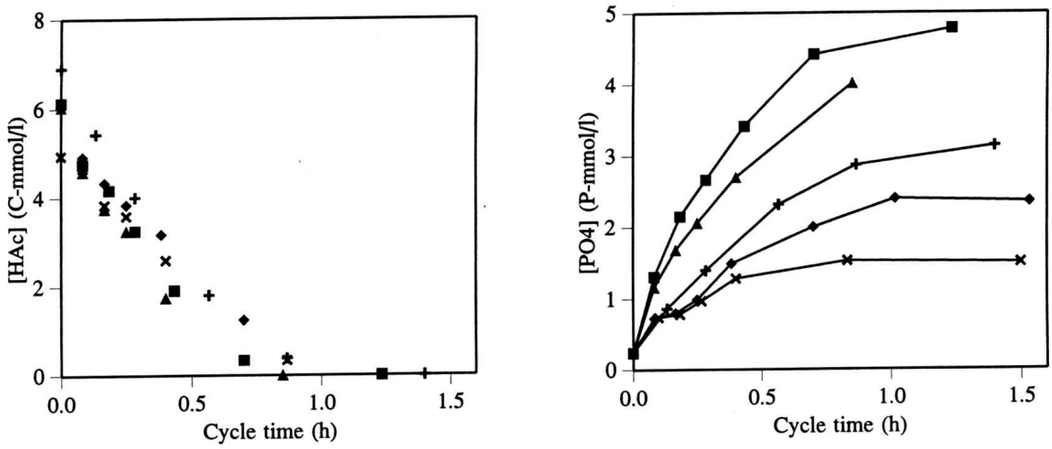

Figure 11 Acetate uptake and phosphorus release at different pH values.
Average initial acetate concentration 6 C-mmol/ℓ, MLSS 3.2 g/ℓ, VSS 2.2 g/ℓ
pH 5.8 (X), pH 6.4 (♦), pH 7 (+), pH 7.8 (▲), pH 8.2(■) (From Smolders, 1995)

In contradiction with Smolders *et al.* (1994a), Liu *et al.* (1996a) also observed dependency of the acetate uptake rate. Below a pH of 5 no acetate uptake was observed, in the pH range from 5 to 6.5 the acetate uptake rate increased linearly from 0 to about 50 mg C/g VSS/h. In the pH range between 6.5 and 8 no pH dependency was observed, in accordance with Smolders *et al*. (1994a). Above pH 8 the acetate uptake rate started to decrease. As for the ortho-phosphate release rate significantly lower values than Smolders *et al.* (1994a) were observed, i.e. in the pH range from 5.0 till 6.5 the ortho-phosphate release rate ranged

from 20 to about 50 mg P/g VSS/h, above 6.5 this value continuously increased. From pH 8.5 on a decrease in ortho-phosphate release rate was observed (Figure 12).

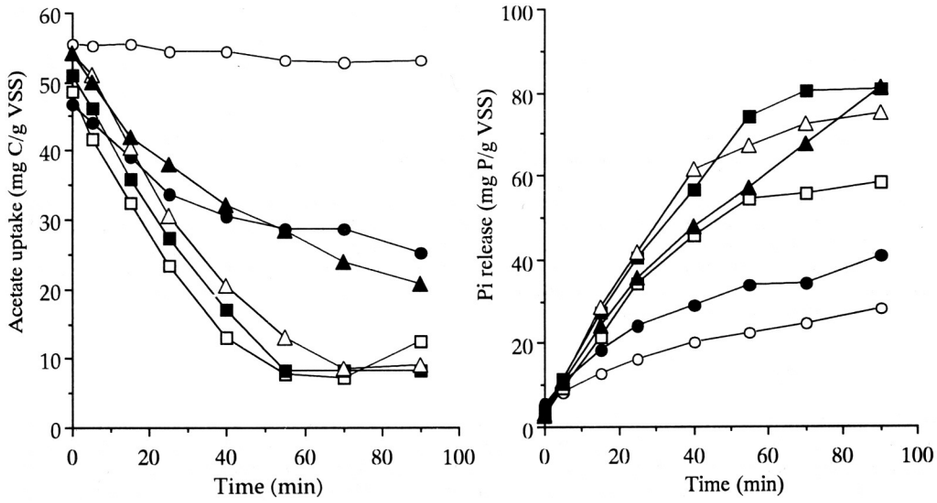

Figure 12 Acetate uptake and phosphorus release at different pH values. Initial phosphorus content of the sludge 12 w%., pH 5.0 (o), pH 5.7 (•), pH 6.5 ( ), pH 7.1 (■), pH 7.8 (△), pH 8.6(▲) Liu et al. (1996a)

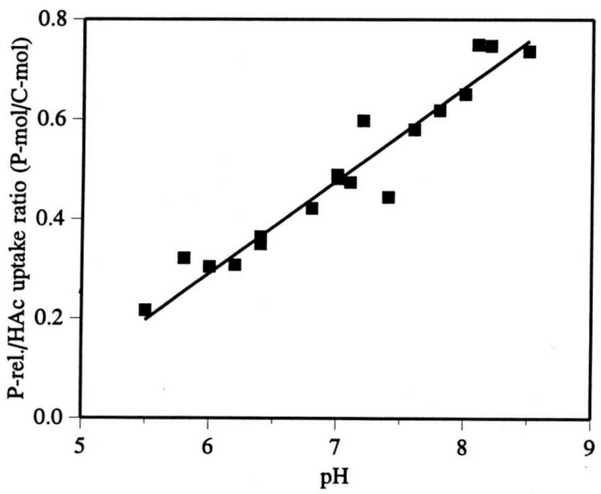

Figure 13 P-release/acetate uptake ratio (α_{tot}) as a function of pH (Smolders, 1995)

In a pH range of 5.5 to 8.5, Smolders *et al.* (1994a) observed the phosphorus release to acetate uptake ratio to vary between 0.25 and 0.75 P-mol/C-mol (Figure 13) (0.24 to 0.73 mg P/mg COD-acetate). This ratio is further denoted as α_{tot} (= 0.25 + α_1 with the notation used by Smolders *et al.* 1994a). Liu *et al.* (1996a) observed the ratio to be about 1 in the pH range between 5.0 and 6.5 and a proportional increase of this ratio with pH up to a ratio of 1.75 for the pH range between 6.5 and 8.5. The ratio observed was dependent on the initial phosphorus content of the cells, i.e. above mentioned values were reported for sludge initially containing 12w% P, whereas lower values were reported for sludge initially containing only 8 w% P (ratios of 0.75 and 1.5 respectively). According to Smolders *et al.* (1994a) at low pH (5.5) no energy is required for the uptake of acetate, thus the energy is solely used for the conversion of internal acetate to Acetyl-CoA.

The observed pH-dependency allowed Smolders *et al.* (1994a) to distinguish between the metabolic pathways presented in literature (see 3.4.3.1).

It is stressed that the experimental evidence gained by Smolders *et al.* (1994a) and by Liu *et al.* (1996a) were obtained from batch experiments, i.e. sludge was exposed to different pH conditions only for short periods. A long term influence of pH on the anaerobic orthophosphate release to acetate uptake ratio was not studied.

3.4.2.3.2. *External pH influence on the aerobic reactions*

No report is known on pH influence on the kinetics or stoichiometry of the EBPR process under aerobic conditions.

3.4.2.3.3. *Overall influence of the external pH on the EBPR performance*

Whereas Smolders (1995) only studied the pH influence under anaerobic conditions, Smolders *et al.* (1994) stated that at low pH possibly less energy needs to be spent in the aerobic phase for ortho-phosphate uptake than at high pH since anaerobically less ortho-phosphate was released and thus less ortho-phosphate had to be taken up again aerobically. According to the same authors the remaining energy could be used for other purposes, i.e. an effect on the biomass yield is predicted.

Few articles are encountered in the literature where pH influence is taken into account. These articles mainly focus on the anaerobic processes and do not consider the overall effect. Further research is necessary to elucidate the overall effects of pH on EBPR. These experiments need to take into account not only short term influence, but long term influences of pH on the overall EBPR process as well.

3.4.2.4. ***Impact of excessive aeration***

Brdjanovic *et al.* (1998b) demonstrated that phosphorus uptake stops due to a gradual depletion of PHB in an over-aerated process. If organic substrate is introduced to the system, phosphorus release is immediately at its maximum rate. However, the released phosphorus cannot be taken up fully again because the PHB content limits the uptake rate. The authors assumed this phenomena can be an explanation for observed deterioration of EBPR activity after heavy rainfall (Fukase *et al.*, 1985) or weekends. It is hypothesised that in these cases excessive aeration took place at some of those plants due to inadequate control of the aeration system during weekends and rainfall periods.

3.4.2.5. ***Effect of the Sludge Retention Time (SRT)***

In general the EBPR process is not very sensitive to the SRT and it has been shown in practice that good phosphorus removal is possible at SRTs ranging from 3 to 68 days (Reddy, 1998). Wentzel (1989a) showed that, although EBPR is possible at SRTs of less than 3 days, the process is not as stable and the effluent is not clear. Smolders (1995) experimentally observed that the biomass yield does not depend very much on the SRT, mainly because the maintenance value for PAOs is relatively low in comparison with normal heterotrophic growth. The lower decay rate of the PAOs compared to non-PAOs, means that at longer SRTs a proportionally larger part of the active biomass will consist of PAOs. Consequently, also the phosphorus content of the biomass increase with an increase in SRT (Wentzel *et al*., 1989a; Chuang *et al.*, 1996)

For non-PAOs an increased SRT is linked only with an increased biomass concentration, i.e. the minimum SRT is directly linked with the maximum growth rate of the micro-organisms ($SRT_{min} = 1/\mu_{max}$). However for PAOs, i.e. when storage polymers are involved, Smolders (1995) and Brdjanovic (1998a) found experimental proof for a more complex link between SRT and EBPR operation. Changing SRT resulted in content changes of the internal storage components with possible deterioration of the EBPR process. Smolders (1995) indicated that for SRTs below 3 days the polyphosphate content becomes too low to allow anaerobic acetate uptake. As such the EBPR process was halted although sufficient external acetate was available.

Brdjanovic (1998a) determined a minimal aerobic SRT based on the necessary aerobic time to convert the anaerobically stored PHB. As for other micro-organisms, it was shown that a decreased SRT resulted in lower biomass concentration. As such, using the same acetate load to the system, the anaerobic PHB content of the biomass increased with decreasing SRT. The anaerobic and aerobic polyphosphate content of the biomass decreased with decreasing SRT and aerobically the glycogen content increased with decreasing SRT. According to Smolders (1995), the glycogen content needs to follow the PHB behaviour,

i.e. when an increased anaerobic PHB content occurs, the aerobically replenished glycogen content needs to be high enough to allow aerobic provision of reducing equivalents for the production of PHB. Although it can be stated that also polyphosphate should follow the PHB behaviour, since polyphosphate serves as energy pool for acetate transport, experimental evidence was found that the polyphosphate content was not able to follow this expected pattern (Smolders, 1995). Polyphosphate limitations linked to decreased SRT thus limited the EBPR performance.

Apart from a minimal SRT necessary to allow anaerobic acetate uptake, no influence of SRT on the overall EBPR performance, i.e. on the phosphorus removal capacity, is reported.

3.4.2.6. ***Impact of the anaerobic hydrolic retention time (HRT)***

Because the uptake of VFAs in the anaerobic phase is rapid, the size (nominal HRT) of this phase tends to be less significant than the operating SRT (the SRT in the anaerobic phase) when there is an excess of VFAs in the process influent relative to the available phosphorus (high TCOD:TP ratio) (Reddy, 1998).

According to Randall (1992) the change in EBPR performance with change in anaerobic HRT is relatively small when the TCOD:TP ratio varied between 42 and 68 (i.e. phosphorus limiting conditions). For COD-limiting conditions, i.e. for TCOD:TP values ranging between 20 and 40 days, changes in anaerobic HRT between 0.5 and 2.7 hours, induced increased EBPR performance.

3.4.2.7. ***Nitrate effect***

3.4.2.7.1. *Decrease of EBPR performance*

With the discovery of the EBPR process in the 1970's (Barnard, 1976), it became very soon apparent that the anaerobic phase had to be absolutely anaerobic. The presence of nitrate in this phase caused the suppression of anaerobic phosphorus release and a decrease in the treatment efficiency (Mc Laren and Wood, 1976; Rensink, 1981). For a system achieving 88% phosphorus removal, Malnou *et al.* (1984) observed a decrease in EBPR efficiency to only 70% when nitrate was added to the anaerobic reactor.

Nitrate entering the anaerobic phase of a treatment plant can be caused first by nitrate present in the influent and secondly by nitrate present in the recycle streams from the aerobic phase (due to nitrification). For the latter case, processes can be designed to prevent high nitrate concentrations to the anaerobic phase (3.4.5.2). Since the adverse affect of nitrate intrusion in the anaerobic phase is thought to be caused by the competition for carbon source between the denitrifiers and the PAOs (Barker and Dold, 1996), higher COD amounts need to be supplied to the anaerobic phase when nitrate is present in the influent. Using different COD loads to the anaerobic reactor at a constant nitrate influent concentration of 50 mg $N\text{-}NO_3/\ell$, Hascoet *et al.* (1985) observed a net phosphorus release when the COD concentration was as high as 300 mg/ℓ, i.e. COD/TKN = 6. To prevent bad EBPR performance Randall *et al.* (1992) suggested the use of additional carbon sources in order to increase the nitrogen removal when COD/TKN $\leq$ 9. Carucci *et al.* (1995) reported phosphorus removal efficiency only to be 61.6% when the COD/TKN ratio was 9.3. Increasing the COD/TKN ration to 16.1 by removing ammonium from the influent increased the phosphorus removal capacity to 93.5%.

Several ways to increase the COD/TKN ratio, and thus to improve EBPR efficiency, are encountered in literature. Bortone *et al.* (1992) supplemented glucose and methanol for piggery waste Pitman *et al.* (1983) applied primary sludge and supernatant from the thickener for municipal wastewater but was not successful due to operating problems. Choi *et al.* (1996) reported on the favourable addition of night soil, after 3 months storage, to increase not only the COD amount, but directly the SCFA concentration. Addition was applied to both low strength municipal waste and piggery wastes with COD/TKN ratios of 6 and 2.2 respectively. Addition of acetate should be a very good carbon source but with a serious increase in cost (Iwema and Meunier, 1985; Kristensen *et al.*, 1992).

3.4.2.7.2. *Proliferation of denitrifying PAOs (DPAOs)*

When the reactor configuration is such that nitrate, formed during nitrification, is prevented from entering the anaerobic phase, and when denitrifying PAOs are present in the anoxic phase, less carbon is necessary to have concomitant phosphorus and nitrogen removal (see 3.4.1.2). As such, the adverse effect of nitrate on EBPR activity can be limited to a negative effect only when nitrate is present in the influent or when nitrate formed in the treatment plant is not prevented from entering the anaerobic phase.

3.4.2.8. ***Nitrite effect***

From batch experiments with sludge from a pilot scale sequencing batch reactor Meinhold *et al.* (1999) found that the presence of nitrite in the anoxic phase influences the overall DEBPR capacity. This influence was dependent on the nitrite concentration. The experiments showed that nitrite is not detrimental to anoxic phosphate uptake at low concentration levels, i.e. up to 4 or 5 mg NO_2-N/ℓ, and can even serve as an electron acceptor for anoxic phosphate uptake. Higher nitrite levels might lead to inhibition of phosphate uptake. Depending on sludge conditions, the critical nitrite concentration above which nitrite inhibition of phosphate uptake occurs, lies in the range between 5 and 8 mg NO_2-N/ℓ for the experimental conditions in their study. Nitrite, nitrate and a mixture of both resulted in the same performance with regard to anoxic phosphate uptake rates.

3.4.2.9. ***Temperature influence***

Especially when looking at EBPR efficiency at different temperatures, results from literature show contrasting observations. Where at first it would seem logical to find higher efficiencies at elevated temperatures (20-37°C) (Yeoman *et al.*, 1988a; McClintock *et al.*, 1993; Converti *et al.*, 1995; Jones *et al.*, 1996 and Scheer, 1994), different authors report on improved efficiencies at lower temperatures (5-15°C) (Sell *et al.*, 1981; Kang *et al.*, 1985; Krichten *et al.*, 1985; Barnard *et al.*, 1985; Vinconneau *et al.*, 1985 and Florentz *et al.*, 1987). According to Helmer and Kunst (1997) a drop in temperature from 15°C to 10 and then to 5°C had no significant influence on the efficiency of the EBPR capacity. From full-scale SBR results (Marklund and Morling, 1994) a sharp decrease in EBPR efficiency was observed when the wastewater temperature dropped below 4.5-5°C.

When the kinetics of the EBPR process are being studied, more consistency is observed in the literature. In the temperature range from 5°C to around 30°C, increased P-release and/or P-uptake rates with increased temperatures are being reported by Shapiro *et al.* (1967), Boughton *et al.* (1971), Spatzierer *et al.* (1985), Mamais and Jenkins (1992) and Brdjanovic *et al.* (1997). Helmer and Kunst (1997), however, reported a higher specific P-uptake at 5°C than at 10°C. Declining phosphate release and uptake rates were observed at temperatures of 35°C and higher, with a significant inhibition at 42.5°C and above, while no phosphate release or uptake was observed at 45°C, indicating that at this temperature the phosphate removing bacteria were probably dead (Jones and Stephenson, 1996).

Regarding the stoichiometry of the anaerobic process, it was observed that this stoichiometry appears to be insensitive towards temperature changes whereas some effects on the aerobic stoichiometry were observed (Brdjanovic *et al.*, 1997).

3.4.3. Metabolism, metabolic pathways and metabolic reactions

The different processes occurring under anaerobic and aerobic conditions, require energy. This energy, required for the maintenance of the bacterial population and for the synthesis of cell compounds, is obtained via the cell-*metabolism*, i.e. by the ordered transformation of substances in the cell. The energy sources are the nutrients which are obtained from the environment. These nutrients are transformed in the cell by a series of successive enzymatic reactions via specific *metabolic pathways*. The metabolic

pathways have two functions: (i) to provide precursors for cell components and (ii) to provide energy for synthetic and other energy-requiring processes (Schlegel, 1993).

Energy-requiring processes in the cell are made possible by participation of adenosinetriphosphate (ATP). ATP is the chemical form in which energy, obtained by photosynthesis, respiration or fermentation, can be utilised by the cell. ATP is the universal transfer agent of chemical energy between energy-yielding and energy-requiring reactions. The pyrophosphate bonds between the phosphate groups are "energy rich", that is, they have a high group-transfer potential (Schlegel, 1993). Hydrolysis of ATP and adenosinediphosphate (ADP), with water as a hydrolysing agent, releases large amounts of energy (Scheme 1).

$$ATP + H_2O \rightarrow ADP + P_i + H^+ + \text{energy} \quad \Delta G° = -31{,}0 \text{ kJ/mol}$$
$$ADP + H_2O \rightarrow AMP + P_i + H^+ + \text{energy} \quad \Delta G° = -31{,}8 \text{ kJ/mol}$$

Scheme 1 Energy release from hydrolysis of ATP and ADP (Schlegel, 1993)

ATP is also used to convert compounds to a reactive state. The following ATP hydrolysis reactions are important for this study (Scheme 2) :

$$\text{Glucose} + ATP \rightarrow \text{glucose-6-phosphate} + ADP$$
$$\text{Fatty acids} + ATP \rightarrow \text{fatty-acyl-AMP} + \text{diphosphate}$$

Scheme 2 ATP hydrolysis for activation of intermediary compounds (Schlegel, 1993)

Regeneration of ATP is possible via three processes, namely photosynthetic phosphorylation (not relevant for this study), oxidative or respiratory chain phosphorylation and substrate level phosphorylation (e.g. fermentation). Although few exceptions exist, in most phosphorylation processes, ADP serves as the phosphate acceptor. AMP must first be converted to ADP, with the participation of ATP (AMP + ATP $\leftrightarrow$ 2 ADP) before it can by phosphorylated (Schlegel, 1993).

The overall transformation of substances by the cell, i.e. cell metabolism, leading from simple nutrients such as glucose, non-SCFAs, or even aromatic compounds to *de novo* synthesis of cell material can be simplified by dividing this process into three major phases: (i) breakdown or *catabolism* of nutrients into smaller fragments, (ii) formation of building blocks and (iii) production of polymeric macromolecules (nucleic acids, proteins, reserve materials, cell wall constituents) which make up the composition of the cells. The two last phases of biosynthesis of cell material, are collected in the term *anabolism*.

In the following sections the metabolic pathways of relevance for this study are explained in more detail. Because of the importance of competition between PAOs and GAOs, both their central metabolism is explained.

3.4.3.1. *Cell internal metabolic pathways for PAOs in the anaerobic phase*

With the development of metabolic models most attention has been focussed on the most cited carbon source that is used by PAOs, i.e. acetate. As for the deterioration of EBPR activity, most attention has been focussed on glucose as possible carbon source. Therefore, in the following sections, most emphasis is put on possible metabolic pathways involved in acetate and glucose utilisation. Where information available, other carbon source are elaborated on.

3.4.3.1.1. *SCFAs as carbon source*

- *Acetate/acetic acid transport across the cell membrane*

Abu-Ghararah and Randall (1991) considered acetate uptake to be an active transport in which the acetate anion is transported across the cell membrane using 0.5 mol ATP. Smolders *et al.* (1994a) considered that α_1 mol ATP is necessary for active transport of acetate, depending on the external pH (α_1 = 0 - 0.5).

However, according to later papers transport of acetate has never been observed in bacteria, making passive diffusion of acetic acid and in general all SCFAs, across the cell membrane the preferred mechanism (Fleit, 1995). From experimental evidence, Bond *et al.* (1999) believe to have found proof for the passive diffusion of acetic acid. For metabolic modelling of the anaerobic processes initial models relied on the active transport whereas later models now account for passive diffusion (Filipe and Daigger, 1998; Hesselmann *et al.*, 2000). No conclusive answer is formulated, yet. It is expected more research will elucidate on the fate of carbon transport across the cell membrane. A mechanism explicitly taking into account chemical equilibrium and combined passive and active acetate uptake should be considered as well.

For general microbiological processes, i.e. not attributing specific attention on EBPR processes, Schlegel (1993) elaborated on 4 possible secondary transport mechanisms (i.e. for transport of cellular metabolites which are driven by electrochemical potential gradients) through the cytoplasmic membrane. Passive diffusion is accounted for when substances are not part of the normal intracellular environment. Not considered so far in EBPR processes, but maybe of importance, is facilitated transport, mediated by a substrate-specific permease, whereby a substance is transported along its concentration gradient. Schlegel (1993) provided no information as to which specific substrates are transported across the cell membrane by means of facilitated transport. For active transport, Schlegel (1993) indicated the dependence of this system on energy. When metabolic energy is available, substrates can be accumulated inside the cell against a concentration gradient. Energy can be provided by proton potential (i.e. maintaining a potential by constantly pumping out protons and other ions as Na^{+}), by ATP or by phosphoenolpyruvate. Besides active transport, group translocation exists, whereby the difference lays in the final cell internal product released. For active transport the molecule released inside the cell is identical to the one taken up, whereas for group translocation the molecule is modified during transport.

- *Activation to acyl-CoA*

The conversion of acetate to acetyl-CoA requires 1 mol ATP per mol acetate (Smolders *et al.*, 1994a); Abu-Ghararah and Randall, 1991). Hesselmann *et al.* (2000), however, provided experimental evidence that acetate activation is performed by acetyl-CoA synthetase (ACS) consuming 2 mol of ATP per mol of acetate. According to the authors, the combined action of acetate kinase (AK) and phosphotransacetylase (ATP), generally applied in EBPR modelling, cf. Smolders *et al.* (1994a), is not likely to happen since acetate activation by AK is only encountered in environments with high acetate concentrations according to Jetten *et al.* (1989). Acetate activation in EBPR processes received only minor attention in the past and researchers adopted (too easily) existing models. The research by Hesselmann *et al.* (2000) adds a new discussion topic and opens probably new research arrays. Future research will hopefully deliver new insights to confirm Hesselmann's or older theories.

Activation of propionate to propionyl-CoA is considered in literature but not elaborated on.

- *Conversion of acyl-CoA to PHA*

According to current research, it is believed that with acetate as sole carbon source mainly storage of PHAs occurs with mainly HB units and partially HV units (see 3.4.2.1). Since PHA is a reduced polymer, its synthesis from acetyl-CoA requires reducing power. For a decade, a fierce controversy existed on the two possible biochemical models that were present in literature to explain for the source of the reducing power, i.e. the (adapted) Mino-model and the Comeau-Wentzel model.

Meanwhile, more evidence is becoming available that both models are partially valid, but that a combination of both (Pereira et al., 1996) or even a completely different metabolism (Hesselmann et al., 2000) is in better agreement with biochemical laws and explains better the observed anaerobic phenomena. Thus, although many researchers have devoted enormous energy to elucidate the "true"

anaerobic metabolic processes for conversion of acetyl-CoA to PHB/PHV, still no generally accepted model exists. Apparently, all models contain valuable information, and newer suggestions often partially comprise these historical models. Therefore, older models, yet rejected, are also explained below. The evolution within these models also has to be seen in the light of evolving analysis techniques. As such, initially only PHB was considered as possible PHA source, whereas later models also try to explain on the produced co-polymer with HB, HV, HMB and HMV units.

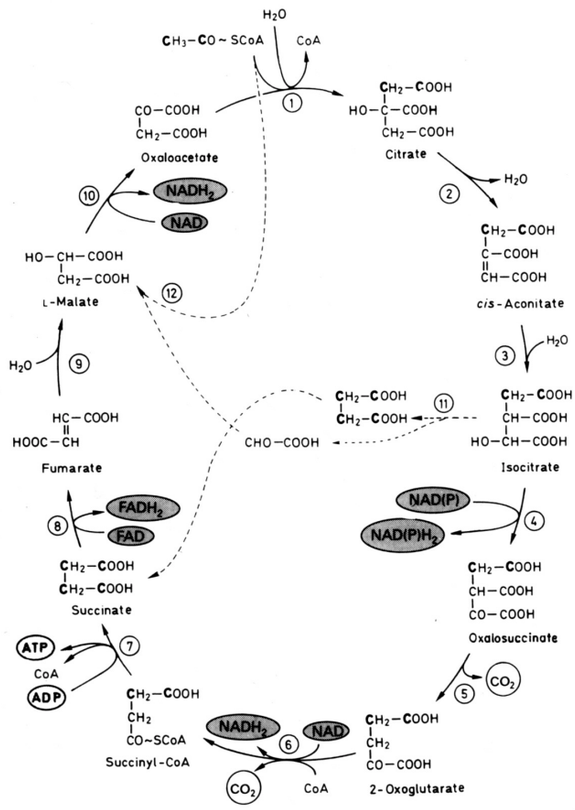

Figure 14 TriCarboxylic Acid (TCA) pathway (Schlegel, 1993).

- *<u>Comeau-Wentzel model</u> (Matsuo, 1985; Comeau et al., 1986; Wentzel et al. 1986)*

In the Comeau-Wentzel model, assimilation of acetate and partial oxidation of acetyl-CoA through the TriCarboxylic Acid (TCA) cycle (Figure 14) is assumed to produce the required reducing power (Figure 15). Florentz and Hartemann (1982) and Lötter (1985) verified the TCA functioning under anaerobic conditions and observed activity of the TCA cycle enzymes under anaerobic conditions. However, Mino *et al.* (1987) and Arun (1988) did not agree with the Comeau-Wentzel model, stating that the TCA cycle can not be operative during anaerobic conditions, one of the problems being the formation of $FADH_2$, a component that cannot be used in PHB synthesis and can only be regenerated with an external electron acceptor (nitrate or oxygen). Smolders *et al.* (1994a) assumed that $FADH_2$ is converted to $NADH_2$, the necessary ATP for conversion being obtained from ATP produced in the TCA cycle (see reaction below). Currently, the sole functioning of the TCA cycle has been completely abandoned, but parts of the TCA cycle apparently remain necessary to account for the observed phenomena (see below).

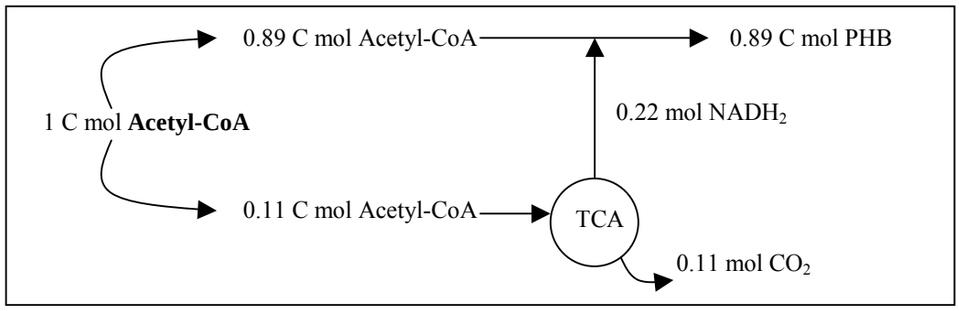

Figure 15 Carbon and reducing power flows for the Comeau-Wentzel model (Filipe and Daigger, 1998)

The overall $NADH_2$ production in the TCA-cycle can be written as (Smolders *et al.*, 1994a):

$$\text{Acetyl-CoA} + 0.5\ \text{ATP} + H_2O \rightarrow 2\ NADH_2 + CO_2$$

- *The (adapted) Mino-model* (Mino and Matsuo, 1984; Mino *et al.*, 1987; Arun *et al.*, 1988, Wentzel *et al.,* 1991; Smolders *et al.,* 1994a)

In the original Mino-model the reducing power was considered to be derived from degradation of intracellularly stored glycogen (catabolism) to generate glucose that is directed through the Embden-Meyerhof-Parnas (EMP) pathway (glycolysis) to acetyl-CoA (and production of PHB) as well as partial production of CO_2 (Figure 16). After observations made by Juni (1978), Wentzel *et al.* (1991) suggested not the EMP but the Entner-Doudoroff (ED) pathway is used for glucose degradation (Figure 17). The modification is based on observations on *Acinetobacter*. Since it is currently thought this micro-organism is not the (sole) responsible one for EBPR activity, the experimental proof for the proposed modification should be given considerable doubt. However, it is thought conclusive evidence for the functioning of the ED-pathway and not the EMP-pathway, is to be found in the fact that with the ED-pathway $NAD(P)H_2$ is formed instead of $NADH_2$, the former indicated in EBPR sludges (van Loosdrecht, personal communication). According to Schlegel (1993) the different pathways show considerable differences in their yields of ATP, $NADH_2$ and $NAD(P)H_2$. The EMP-pathway yields 2 mole ATP and 2 mole $NADH_2$ whereas the ED-pathway yields only 1 mole ATP and 1 mole of $NAD(P)H_2$ per mole glucose converted to pyruvate.

CO_2 and PHB measurements during anaerobic batch tests, proved that 0.17 C-mol CO_2/C-mol acetate and 1.33 C-mol PHB/C-mol acetate were formed (Smolders *et al.,* 1994a), indicating the Mino model is preferred over the Comeau-Wentzel which only predicts 0.89 C-mol PHB/C-mol acetate. Accounting for the energy necessary for acetate transport over the cell membrane in their metabolic model, Smolders *et al.* (1994a) found theoretical proof for the observed pH dependent anaerobic phosphorus release. The experimental variation, i.e. 0.25 to 0.75 P-mol/C-mol, could best be explained using the Mino-model and varying the necessary poly-P breakdown with the pH value. From experimental observations at a pH of 5.5, i.e. when α_1 is supposed to be equal to zero, Filipe and Daigger (1998) observed a P-release to acetate uptake ratio of 0.33, indicating the adapted Mino-model should be preferred. Currently, some of the assumptions made by Smolders *et al.* (1994a) (e.g. active acetate transport across the cell membrane) are doubted. Further research needs to clarify this. Moreover, the parameter α_1 should be considered as an overall parameter for ATP cell membrane related reactions and not only related to active acetate transport. Therefore, the statement as would α_1 be zero at a pH of 5.5 is probably not valid.

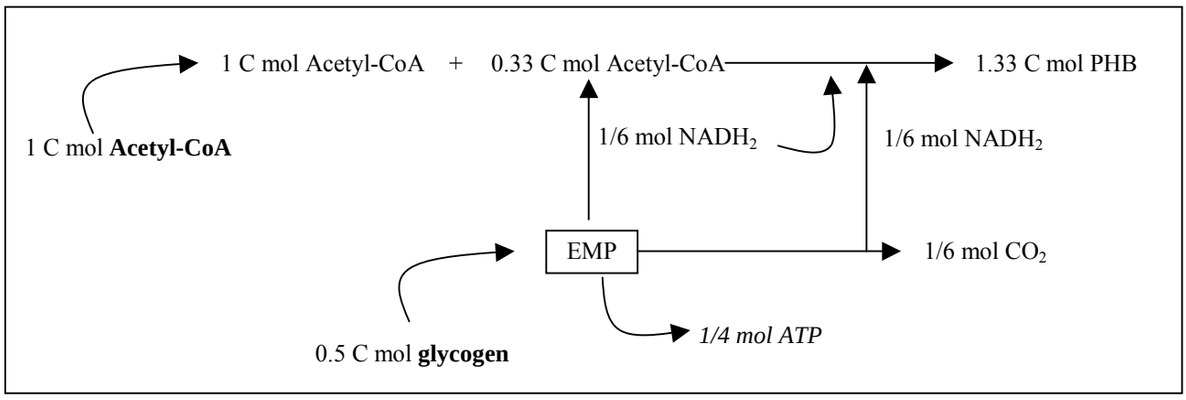

Figure 16 Glycolysis pathway (Mino model) (after Filipe and Daigger, 1998) (=Embden-Meyerhof-Parnas)

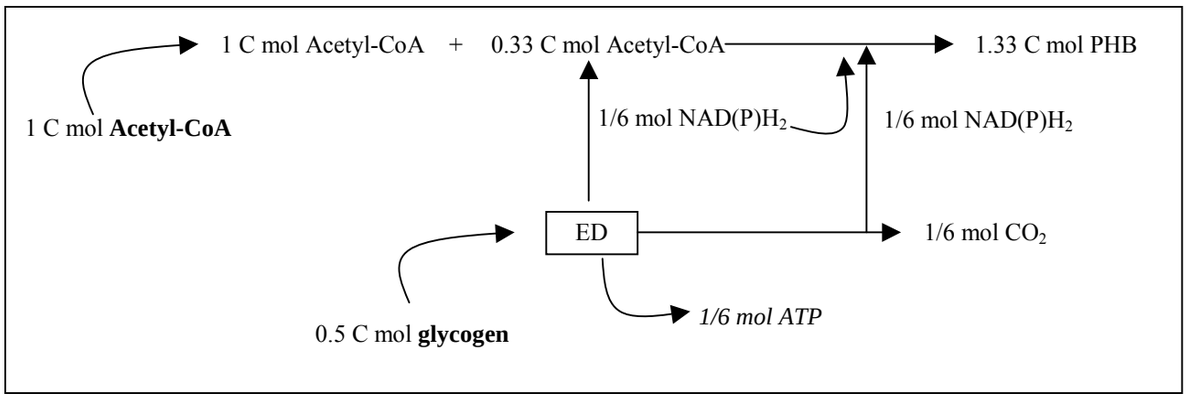

Figure 17 Glycolysis pathway (adapted Mino model) (after Filipe and Daigger, 1998) (=Entner-Doudoroff)

The overall reaction for glycogen degradation and PHB production in the EMP-glycolysis pathway can be written as (Smolders *et al.*, 1994a):

$$\text{Glycogen} + 1/6\ H_2O \rightarrow 2/3\ \text{PHB} + 1/3\ CO_2 + 0.5\ NADH_2 + 0.5\ \text{ATP} \qquad \text{C-mol based}$$

$$\text{Glycogen} + H_2O \rightarrow \text{PHB} + 2\ CO_2 + 3\ NADH_2 + 3\ \text{ATP} \qquad \text{Product based}$$

Comparing the above reactions with Figure 16, it is observed that Smolders *et al.* (1994a) and Filipe and Daigger (1998) account for different amounts of $NADH_2$ produced in the glycolysis pathway.

Application of *in situ* and solid state ^{13}C-nuclear magnetic resonance (^{13}C-NMR) provide unequivocal evidence for the activity of the anaerobic glycolytic pathway in EBPR processes (Bordacs and Chiesa, 1989; Satoh *et al.*, 1992; Pereira *et al.,* 1996; Maurer *et al.*, 1997; Louie *et al.*, 2000). Assuming the (adapted)Mino-model is the valid one means that PAOs possess a complete glucose degradation and synthesis metabolism without necessitating external glucose. Indeed, the glycogen content is replenished aerobically from PHA stored during the anaerobic phase (see further).

- <u>*Combined TCA-glycolysis model*</u> (Pereira *et al.*, 1996; Maurer *et al.*, 1997; Louie *et al.*, 2000)

Pereira *et al.* (1996) provided experimental evidence that acetate anaerobically taken up is converted to PHA, which, in the subsequent aerobic phase, is converted to glycogen, which further supplies carbon for PHA formation and CO_2 production in the next anaerobic phase. Because of the importance of these experiments as an undeniable proof for the activity of the anaerobic glycolytic pathway in EBPR processes, the results obtained by Pereira *et al.* (1996) are presented in Figure 18, Figure 19 and Figure 20. In Figure 18 the flux from of the label from acetate to P(HB/HV) is observed. In Figure 19 the conversion of P(HB/HV) into glycogen in the subsequent aerobic stage is proven. Finally, in Figure 20 it is shown that glycogen provides not only reducing equivalents but also carbon to replenish anaerobically the PHA content. For this experiment, unlabelled acetate was used as carbon source to distinguish clearly between direct use of acetate for PHB formation and the use of glycogen-carbon for PHB and PHV production.

Pereira *et al.* (1996) acknowledge an inability to balance the redox chemistry if the glycolytic pathway is used alone and proposed at least partial functioning of the TCA cycle supplying a minor part (30%) of the reducing power for PHA formation. Henceforth, the Comeau-Wentzel model has some credit too. The metabolic model proposed by Pereira *et al.* (1996) is presented in Figure 21. The problem that $FADH_2$ is produced in the TCA cycle and can not be used for PHB production is not accounted for. Using metabolic inhibitors to prevent specific steps in the TCA cycle combined with ^{13}C-NMR allowed Louie *et al.* (2000) to find experimental proof for a biochemical model that incorporates elements of the TCA cycle and of the glyoxylate pathway. Also for this model (Figure 22), the problem that $FADH_2$ is produced in the TCA cycle and can not be used for PHB production is not accounted for.

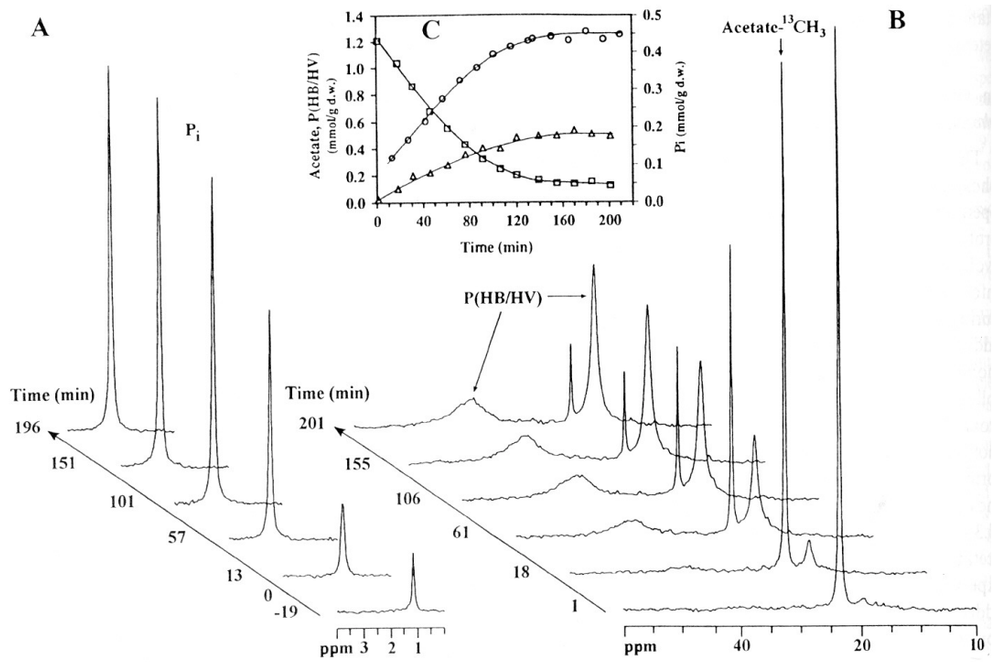

Figure 18 Time course of phosphate release, acetate consumption and P(HB/HV) formation by activated sludge under anaerobic conditions as monitored by in vivo ^{13}P-NMR (spectra A) and ^{13}C-NMR (spectra B), respectively. Inset C provides the corresponding data. The cell suspension was supplied with [2-^{13}C]acetate (i.e. ^{13}C label on the methyl group of acetate) at time zero. (P (o), Ac ( ), P(HB/HV) (△)) (Pereira et al., 1996)

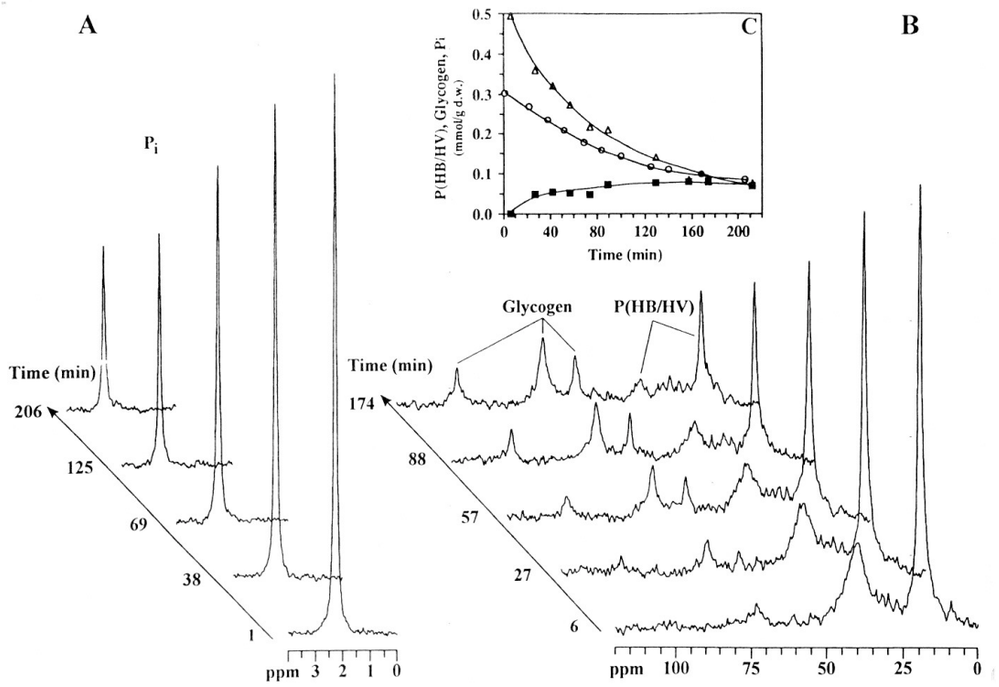

Figure 19 Time course for the aerobic uptake of phosphate (A) and degradation of P(HB/HV) (B), by activated sludge under aerobic conditions, as monitored by in vivo ^{13}P-NMR and ^{13}C-NMR, respectively. Inset C provides the corresponding data. Following the experiment shown in Figure 18, oxygen was provided at time zero. (P (o), P(HB/HV) (△), glycogen (■)) (Pereira et al., 1996)

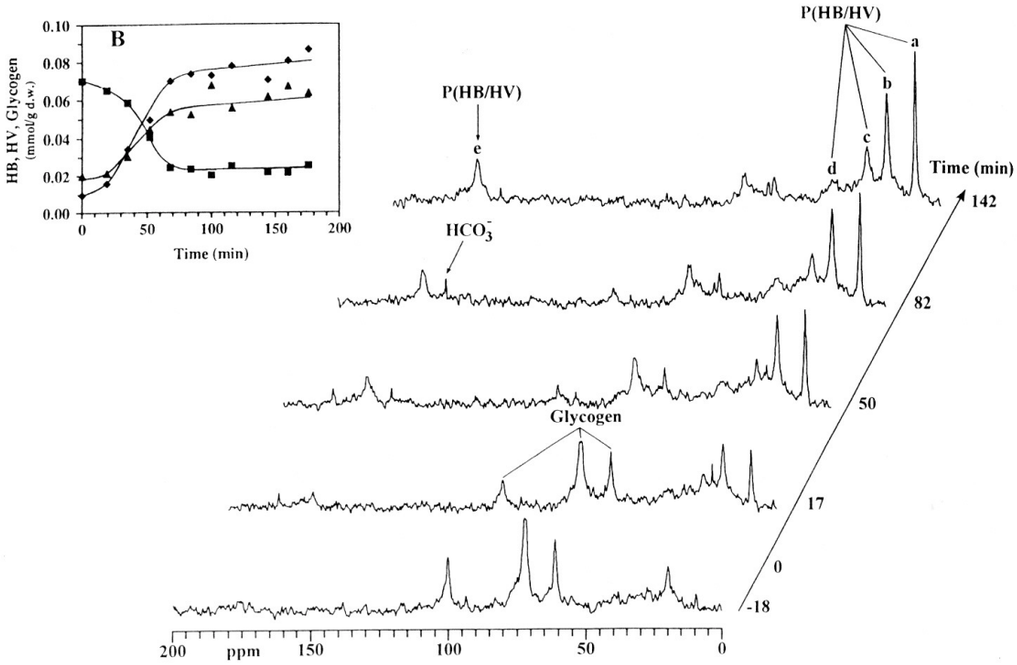

Figure 20 Time course for glycogen consumption and biosynthesis of P(HB/HV), by activated sludge, as monitored by in vivo ^{13}C-NMR. Inset C provides the corresponding data. Following the experiment illustrated in Figure 19 the cell suspension was supplied with unlabelled acetate at time zero (Glycogen (■), HB units (▲), HV units(♦)) (Pereira et al., 1996)

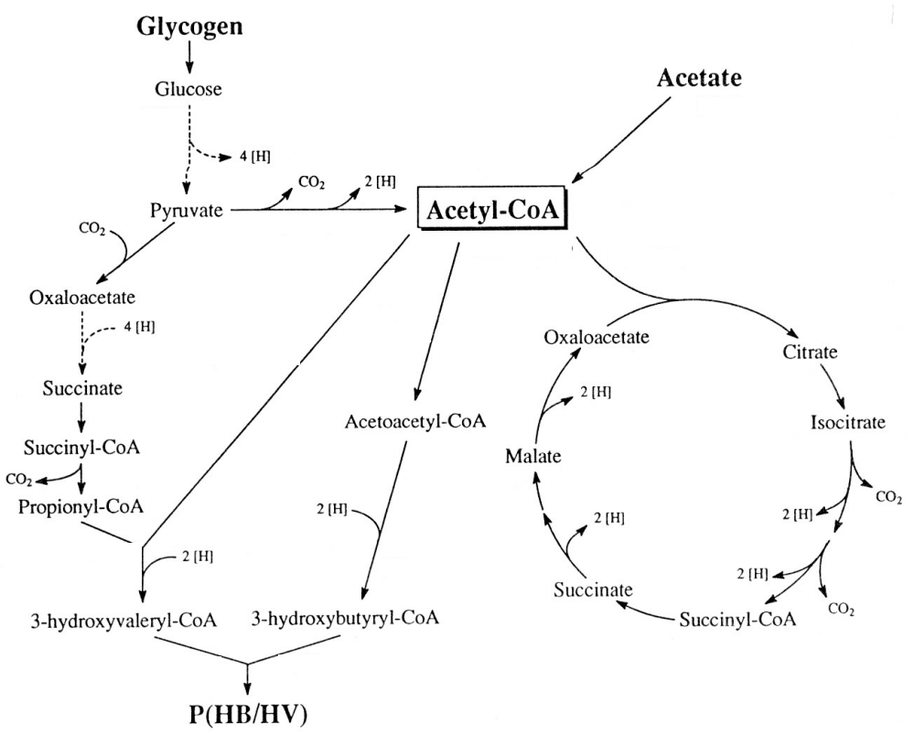

Figure 21 Metabolic model presented for conversion of acetate and glycogen to P(HB/HV) by activated sludge under anaerobic conditions (Pereira et al., 1996)

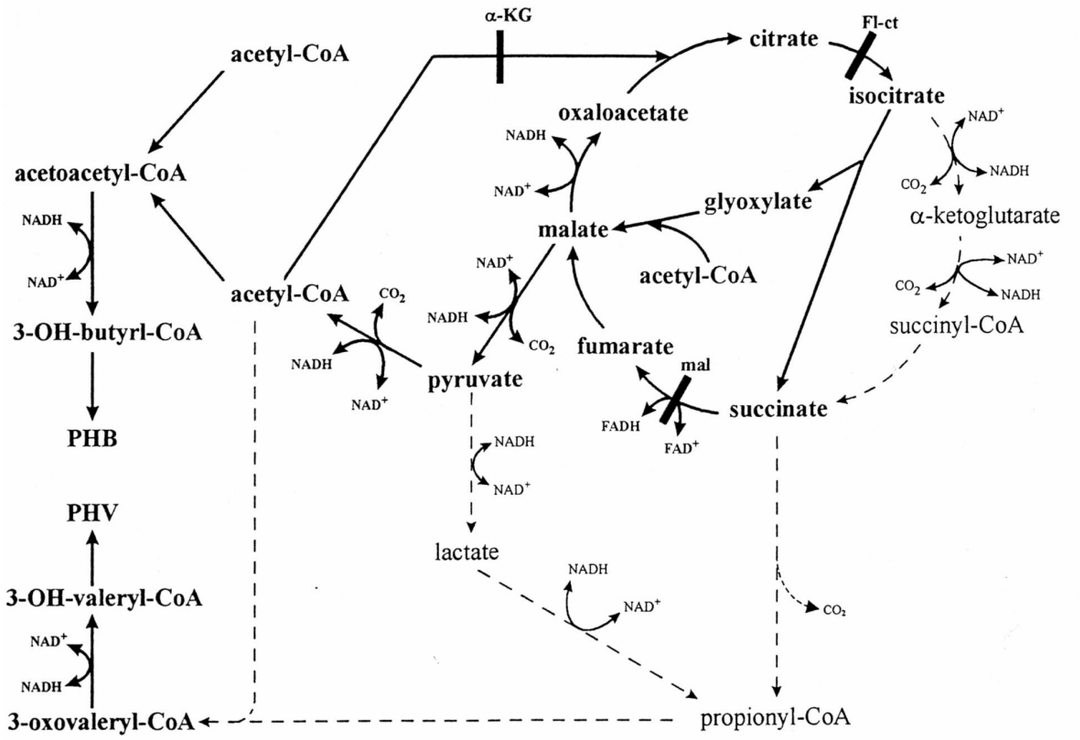

Figure 22 Biochemical model for the conversion of metabolites to PHA by EBPR sludge (Louie et al., 2000) ▬ *points of inhibitors*

- <u>*The Hesselmann model*</u> (Hesselmann *et al.*, 2000)

To account for the problems specified for the operation of the TCA cycle, Hesselmann *et al.* (2000) presented a modified succinate-propionate-pathway, whereby the succinate dehydrogenase of the TCA cycle is deactivated if $FADH_2$ cannot be re-oxidised as is the case in PHA production in EBPR processes Figure 23. In this model succinate is formed out of fumarate instead of the reverse reaction in the TCA cycle. This reversed reaction needs reducing equivalents as $NADH_2$ and produces ATP. As such, production of $FADH_2$ is not occurring anymore. The model thus accounts for the observations that:

- enzymes from the TCA cycle are functioning (the direction of the reaction is not important)
- production of HV units for the PHA co-polymer through functioning of the glycolysis pathway
- $FADH_2$ should not be produced

The proposed model is only recent and further research will be necessary to test the validity of the model.

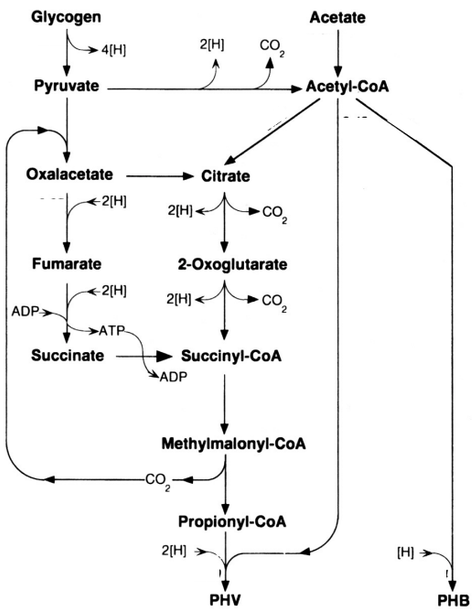

Figure 23 Combined operation of enzymes from the TCA cycle and the modified succinate-propionate-pathway (Hesselmann et al., 2000).

To round off the discussion concerning possible pathways it is stressed that prior paragraphs only dealt with acetate as a carbon source for the PAOs. However, in EBPR processes, the PAO sludges have to be

ready for the uptake of various kinds of reduced or oxidised organic substrates in the anaerobic phase without disturbing the redox balance in the cell. The function of stored glycogen to maintain the redox balance, therefore, appears to be essential (Satoh *et al.*, 1992). As the discussion on acetate already revealed vivid and no conclusive answers are yet formulated, it is beyond the scope of this work to elaborate in great extend on possible pathways for other carbon sources. Limited information is provided in 3.4.3.1.2 and in the section on metabolic pathways for GAOs (3.4.3.6).

- *Polyphosphate degradation and phosphate transport across the cell membrane*

Smolders *et al.* (1994a) accounted for a yield of 1 mol ATP and 1 mol phosphate for the hydrolysis of polyphosphate. It is assumed that no energy is produced during the efflux of phosphate. However, the same authors assume energy is necessary for the reverse reaction, i.e. aerobic uptake of phosphate.

- *Overall representation of anaerobic cell internal metabolism*

In Figure 24 the cell internal anaerobic mechanisms are presented. ATP values mentioned by Smolders *et al.* (1994a) have been omitted since controversy without conclusive answers prevents final acceptance of one or another statement. The overall representation as presented in the figure is now generally accepted.

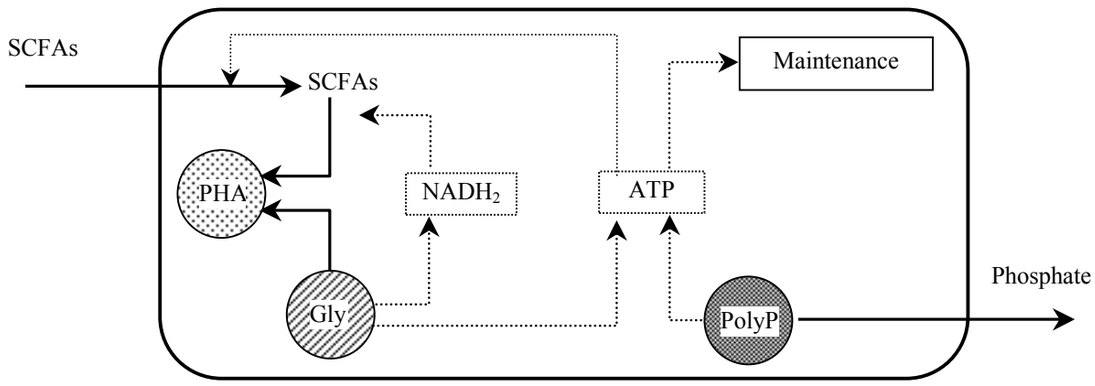

Figure 24 Anaerobic mechanism after Smolders et al. (1995a)

3.4.3.1.2. *Non-SCFAs as carbon source*

Going through specific EBPR related literature, no information was encountered regarding the transport of these substances across the cell membrane. Schlegel (1993) states that sugars, e.g. glucose, fructose, manose, are transported by means of group translocation.

Using *lactate* as carbon source, no carbohydrates have to be degraded since reducing power is not necessary. However, in their experiments, Satoh *et al.* (1992) did observe consumption of intracellular carbohydrates. To account for the observed phenomena, the authors proposed a model for the direct uptake of lactate and concomitant consumption of glycogen (Figure 25).

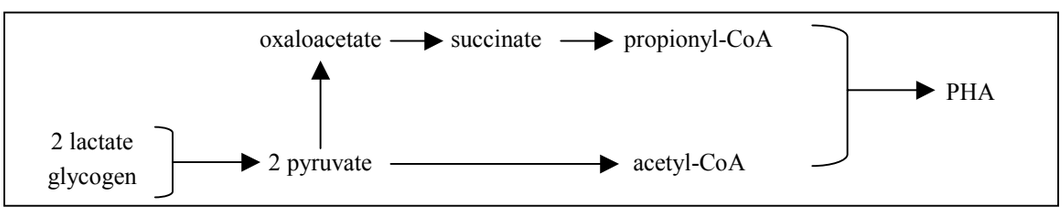

Figure 25 Model for anaerobic lactate uptake with glycogen consumption (after Satoh et al. 1992)

Tracy and Flammino (1987) postulated a biochemical pathway for EBPR that involves direct uptake of *glucose* by PAOs where poly-P provides energy for glucose uptake and its storage as glycogen. In this case the characteristics of PHA storage by PAOs were not observed.

According to Jeon and Park (2000), glucose is partially accumulated as glycogen and partially broken down to lactic acid by so-called Lactic acid Producing Organisms (LPOs). The lactic acid is then used by PAOs. The metabolic pathway suggested is presented in
Figure 26. At first, the LPO rapidly accumulate glucose as glycogen in the cell. The requirement of ATP for the glycogen storage is supplied by lactate formation through glycolysis of glucose rather than consumption of poly-P. Glycogen in the LPO is mainly converted to storage polymers in the anaerobic phase, but part of the glycogen is also converted to other storage polymers. The fate of glucose during the anaerobic/aerobic phases was investigated by supplying ^{13}C-labelled glucose at the beginning of the anaerobic phase which was traced by ^{13}C-NMR spectroscopy.

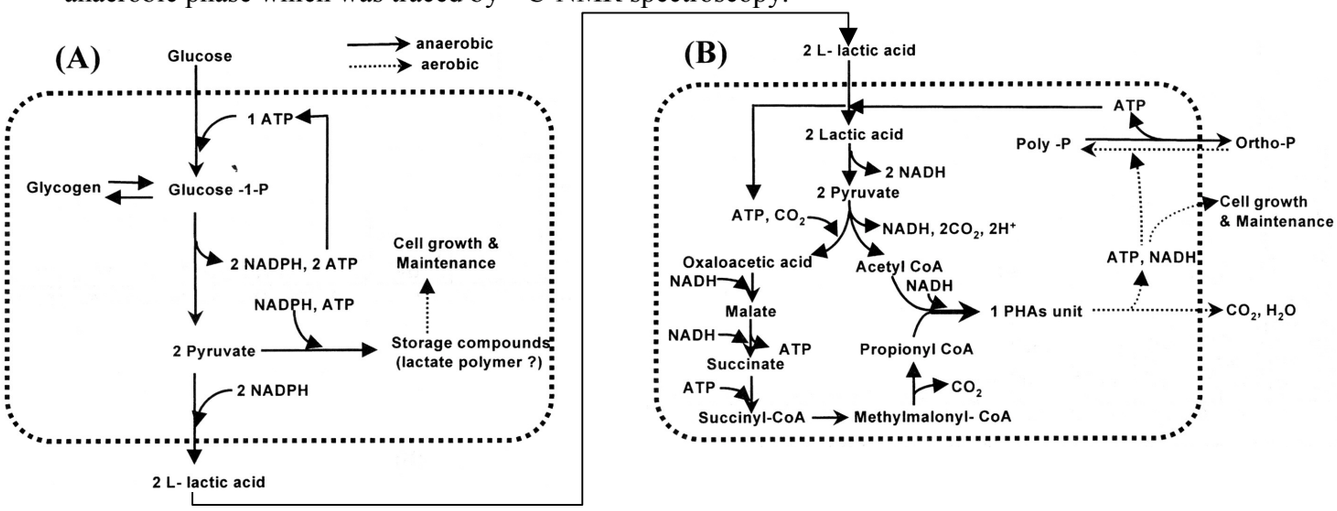

Figure 26 Proposed metabolic pathways for conversion of glucose to lactate polymer by LPO (part A) and for conversion of lactate to PHAs and phosphorus removal by PAO (part B) (Jeon and Park, 2000)

3.4.3.2. *Observed cell internal-external reactions for PAOs in the anaerobic phase*

For the development of their metabolic model, Smolders *et al.* (1995a) used metabolic reactions taking into account the metabolism occurring in the anaerobic and aerobic phases (Table 9). All relevant metabolic reactions underlying the metabolism, considering also components like ATP and $NADH_2$, are described based on the biochemical pathways.

In Table 8 the elemental compositions of the components as used by Smolders *et al.* (1995a) are presented. In their paper Filipe and Daigger (1998) remark that the elemental composition for PAOs used by Smolders *et al.* (1995) differs from the general formula for bacteria, i.e. $C_5H_7O_2NP_{0.09}$, often observed (Grady and Lim, 1980). Smolders *et al.* (1994a, 1995a) derived the formula from experimentally observed and predicted PHB consumption on a C-molar basis.

Table 8 Elemental formulas for the components considered by Smolders (1995)

Component		Elemental composition	Component		Elemental composition
Acetate	(S_A)	CH_2O	Glycogen	(X_{GL})	$CH_{1.67}O_{0.83}$
Phosphate	(S_{PO4})	H_3PO_4	Ammonia	(S_{NH4})	NH_3
PAOs	(X_{PAO})	$CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$	Oxygen	(S_{O2})	O_2
PHAs	(X_{PHA})	$CH_{1.5}O_{0.5}$	Carbon dioxide	(S_{CO2})	CO_2
Polyphosphate	(X_{PP})	HPO_3	Water	(S_{H2O})	H_2O

Table 9 Metabolic reactions for the anaerobic phase (Smolders et al., 1994a) (for α_{tot} see Figure 13)

(Uptake of S_A) Storage of X_{PHA}	$- CH_2O - 0.5\ CH_{1.67}O_{0.83} - \alpha_{tot}\ HPO_4 - (\alpha_{tot} - 5/12)\ H_2O + 1.33\ CH_{1.5}O_{0.15} + 0.17\ CO_2 + \alpha_{tot}\ H_3PO_4 = 0$
Anaerobic maintenance	$- HPO_3 - H_2O + H_3PO_4 = 0$

3.4.3.3. ***Cell internal metabolic pathways for PAOs in the aerobic phase***

Whereas the anaerobic metabolic reactions are extensively studied, the aerobic phase is getting much less attention. In what follows the ideas put forward by Smolders *et al.* (1995a) are presented (Figure 28). This model is based on acetate as the sole carbon source fed to the anaerobic phase. As such only PHB is regarded as PHA available aerobically.

- *PHB degradation for maintenance, biomass production and glycogen replenishment*

PHB is degraded to acetyl-CoA which is partially converted to CO_2 in the TCA cycle with concomitant production of $NADH_2$ and ATP. Partially the acetyl-CoA is used to produce new biomass. PHB is partially converted to oxaloacetate through the glyoxylate cycle. The produced oxaloacetate is then used for the replenishment of glycogen through gluconeogenesis (Figure 27).

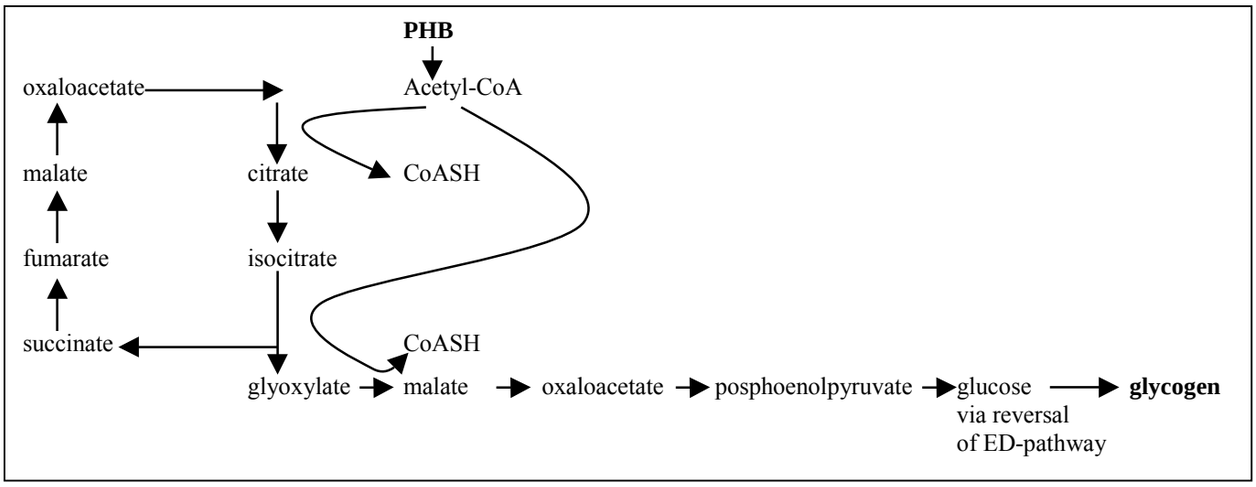

Figure 27 Schematic diagram showing a possible pathway for regeneration of glycogen from PHB (after Wentzel et al., 1991b)

- *Phosphate transport across the cell membrane and polyphosphate synthesis*

According to Smolders *et al.* (1994b), phosphate transport across the cell membrane is a process requiring energy. Phosphate is negatively charged and has to be taken up against an electrical potential difference. According to the same authors, the positive ions required for polyphosphate synthesis (Mg^{2+}, K^{2+}) are taken up without energy need. The energy required for phosphate transport is generated by the import of protons which are subsequently exported over the cell membrane linked to the oxidation of $NADH_2$.

For the synthesis of polyphosphate from phosphate 1 ATP is necessary (Smolders *et al.*, 1994b).

- *Overall representation of aerobic cell internal metabolisms*

In Figure 28 the cell internal aerobic mechanism is presented.

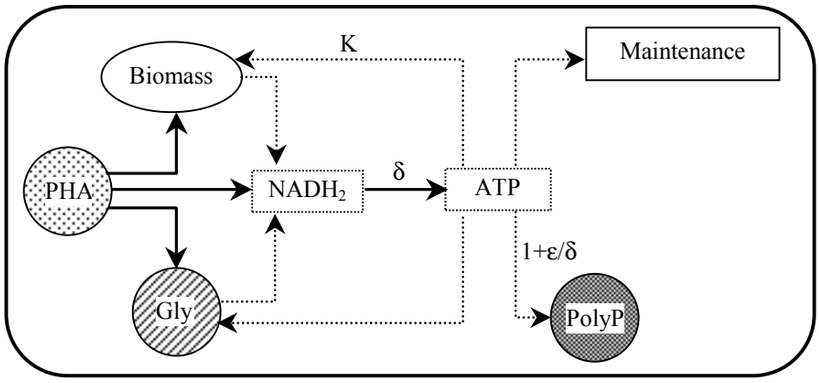

Figure 28 Aerobic mechanism according to Smolders et al. (1995a)

3.4.3.4. ***Observed cell internal-external reactions for PAOs in the aerobic phase***

In Table 10 the metabolic reactions for the aerobic phase are presented. With this representation, the kinetic equation chosen by Smolders *et al.* (1995a) are based on product formation (biomass, phosphate and glycogen), with PHB consumption the result (Figure 29A). Murnleitner *et al.* (1997) adapted this view point, taking biomass formation as a resultant, proposing kinetic equations for PHB consumption, phosphate uptake and glycogen formation (Figure 29B). In the original presentation by Murnleitner *et al.* (1997) maintenance is not presented, but in both models it is accounted for. In Figure 29 maintenance has been added.

Table 10 Metabolic reactions for the aerobic phase (Smolders, 1995)

Biomass synthesis	- 1.37 $CH_{1.5}O_{0.15}$ - 0.20 NH_3 - 0.015 H_3PO_4 - 0.42 O_2 + $CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$ + 0.37 CO_2 + 0.305 H_2O = 0
Phosphate uptake	- 0.27 $CH_{1.5}O_{0.15}$ - 0.306 O_2 - H_3PO_4 + HPO_3 + 0.27 CO_2 + 1.20 H_2O = 0
Glycogen formation	- 1.12 $CH_{1.5}O_{0.15}$ - 0.26 O_2 + $CH_{1.67}O_{0.83}$ + 0.12 CO_2 + 0.007 H_2O = 0
Maintenance	- $CH_{1.5}O_{0.15}$ - 1.125 O_2 + CO_2 + 0.75 H_2O = 0

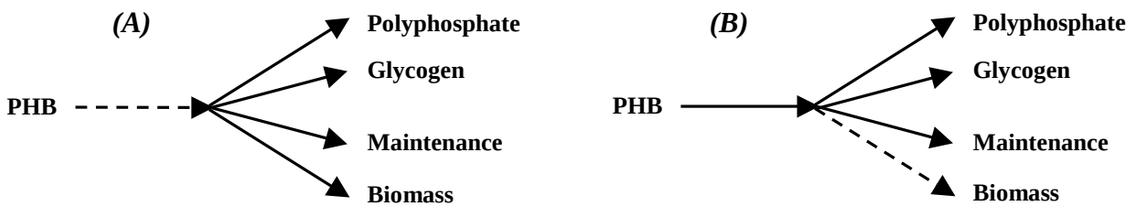

Figure 29 Kinetic structure of the models as proposed by Smolders et al. (1995a) (A) and by Murnleitner et al. (1997) (B) (after Murnleitner et al, 1997)

3.4.3.5. ***Metabolic pathways observed for DPAOs in the anoxic phase***

3.4.3.5.1. *True anoxic phase*

When DPAOs can proliferate in an anaerobic-anoxic or anaerobic-anoxic-aerobic system, it is observed denitrification and phosphorus uptake occur simultaneously (see 3.4.1.2), the final electron acceptor in the oxidative phosphorylation of ATP now being nitrate. The mechanism presented in Figure 28 can thus be used.

3.4.3.5.2. *Anoxic phase with acetate breakthrough or anaerobic phase with nitrate breakthrough*

Special attention was focussed by several authors regarding the influence of acetate breakthrough to the anoxic zone (Chuang *et al.*, 1996) or nitrate breakthrough to the anaerobic zone (Kuba *et al.*, 1994) in systems with DPAOs. For both systems, micro-organisms are faced with simultaneous availability of acetate and nitrate. Chuang *et al.* (1996) reported glycogen accumulation both in the absence and presence of acetate in the anoxic phase. The preliminary mechanism for DPAOs presented by the authors differs from anaerobic uptake of acetate by PAOs by the fact that glycogen is accumulated instead of broken down for production of reducing equivalents (Figure 29). It is doubted that both glycogen and PHA formation can occur simultaneously. Kuba *et al*. (1994) did not record glycogen profiles during their batch experiments. Chuang *et al.* (1996) assume denitrification occurred both with and without acetate uptake, the kinetic competition controlled by the internal poly-P content.

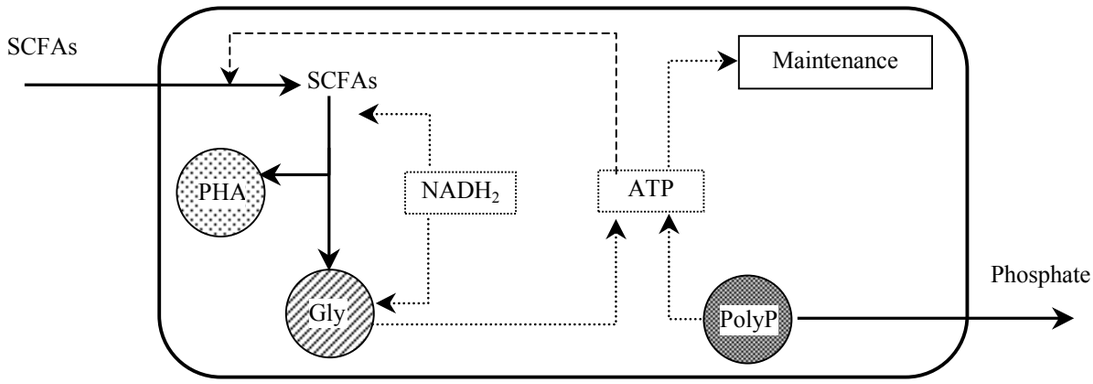

Figure 30 Preliminary metabolism for simultaneous phosphorus release and denitrification under anoxic conditions (after Chuang et al. 1996)

Kuba *et al.* (1994) reported the ratio of phosphorus released to acetate consumed to be smaller in the presence of nitrate than in the absence of nitrate. When both mechanisms proposed by Chuang *et al.* (1996) occur simultaneously as stated by the authors, the observation made by Kuba *et al*. (1994) is easy to explain. Kuba *et al.* (1994) also observed a difference in the ratio of PHB produced to acetate consumed, i.e. 0.8 and 1.33 mol C-X_{PHB}/mol C-X_{PAO} in the presence and absence of nitrate respectively. The authors remark that the value of 0.8 mol C-X_{PHB}/mol C-X_{PAO} corresponds to the value obtained assuming the Comeau-Wentzel model would be valid. However, assuming the competition proposed by Chuang *et al*. (1996) is active, PHB will be produced and accumulated concomitantly resulting in a lower observed ration of PHB produced to acetate consumed than expected when the Mino model is valid.

3.4.3.6. ***Metabolic pathways for GAOs in the anaerobic phase***

3.4.3.6.1. *SCFAs as carbon source*

Satoh *et al.* (1992) were the first to propose an anaerobic metabolism in which only energy is generated and no reducing equivalents. In their model conversion of glycogen to acetyl-CoA and CO_2 generates reducing power, whereas conversion to propionyl-CoA via the succinate-propionate pathway consumes reducing power (Figure 24 and Figure 32). Satoh *et al.* (1994) later took into account the overall production of 3 mol ATP in the glycolysis step. Acetate or propionate activation to acetyl-CoA and incorporation of the latter in the PHA co-polymer is thus possible without poly-P degradation for energy supply (Figure 31).

	12 acetate + 12 ATP	→	12 acetyl-CoA + 12 ATP
	2 glycosyl-units + 6 ADP	→	4 acetyl-CoA + 6 ATP + 16 [H]
	3 glycosyl-units + 6 ADP	→	3 acetyl-CoA + 3 propionyl-CoA+ 6 ATP + 6 [H]
	19 acetyl-CoA + 3 propionyl-CoA + 22 [H]	→	11 3-hydroxy-alkanoate units
•	Overall reaction for acetate: 12 acetate + 5 glycosyl units	→	11 3-hydroxy-alkanoate units
•	Overall reaction for propionate: 12 propionate + 5 glycosyl units	→	11 3-hydroxylalkanoate units

Figure 31 Anaerobic acetate uptake without poly-P utilisation (Satoh et al., 1994)

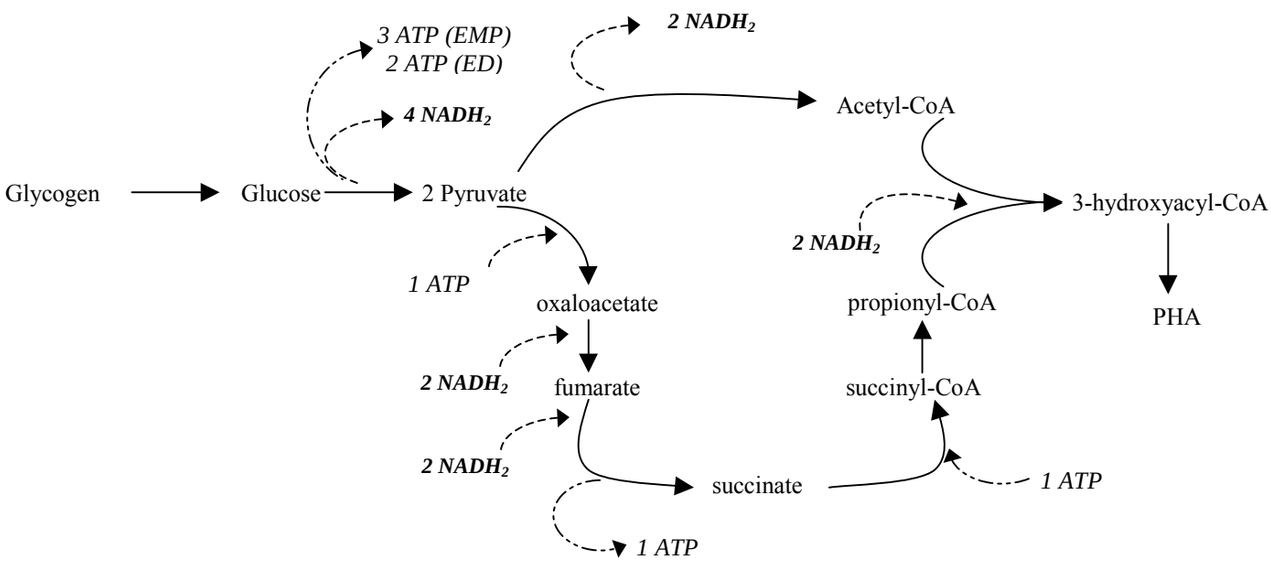

Figure 32 Metabolic pathway for glycogen degradation with energy supply, without production of reducing power (after Satoh et al., 1992)

Satoh *et al.* (1992) stated that their metabolic model might be an explanation for the phenomena observed by Fukase *et al.* (1985) and Cech and Hartman (1990) where micro-organisms dominated that get energy other than from polyphosphates hydrolysis. Their assumption was later supported by the study of Cech and Hartmann (1993). In 1994, Satoh *et al.* used their model to explain observed breakdown of EBPR activity. Other authors also reported on the anaerobic degradation of glycogen where anaerobic acetate or glucose uptake is observed without concurrent release of phosphate in systems where phosphorus feeding was limited to prevent poly-P accumulation (Liu *et al.*, 1994).

The hypothesis put forth by Satoh *et al.* (1992) was supported when Liu *et al.* (1994) performed experiments using N,N'-dicyclohexylcarbodiimide (DCCD) as an ATPase membrane inhibitor, preventing only proton-motive-force driven processes (Keevil *et al.*, 1986). This product has no influence on the hydrolysis of ATP for example produced through the glycolysis pathway (Strobel and Russel, 1989). Addition of DCCD had no influence on the anaerobic acetate uptake, glycogen breakdown and PHA synthesis. The model proposed by the authors accounts for acetate uptake (Figure 33).

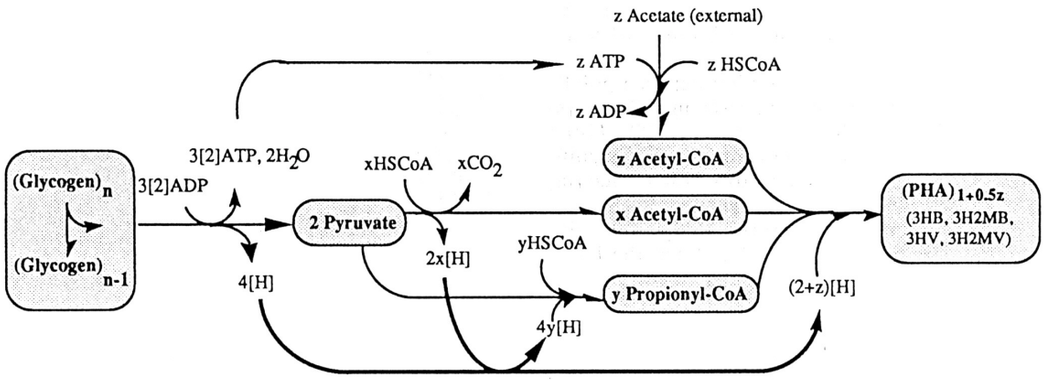

Figure 33 Proposed metabolic pathway for acetate uptake by GAOs (Liu et al., 1994)

The metabolism for GAOs is more complicated than the metabolism for PAOs (Satoh *et al.*, 1994), since less enzymes are needed in the latter. The regulation of the system is also more difficult because there is a competition for the same substrate between two enzymes.

If the mechanisms proposed by Satoh *et al.* (1992) and by Liu *et al.* (1994) are compared to the mechanism presented by Jeon and Park (2000), GAOs perform the whole pathway in one organism and no extracellular intermediate lactic acid is formed.

3.4.3.6.2. *Non-SCFAs as carbon sources*

According to Satoh *et al.* (1994) glucose can be taken up and can be accumulated as PHA just as glycogen is converted to PHA. According to the authors the GAOs metabolic pathway may be preferred.

When feeding sugars to a system not showing EBPR activity, Liu *et al.* (1996b) indicated uptake of sugar (e.g. glucose) causes glycogen and PHA accumulation. No metabolic model is presented, but the authors state that ATP and $NADH_2$ generated from glycolysis of glucose are used for synthesis of PHA from glycolysis products. In order to keep the energy balance within the system in order, excessive ATP produced is used for synthesis of glycogen from glucose. When feeding the system with glucose and acetate, PHA alone is synthesised and only very little concurrent consumption of internal glycogen is observed. The authors stated the micro-organisms prefer use of an external energy source (glucose) to an internal energy source (glycogen) for acetate uptake.

3.4.4. The microbiology of biological P-removal

From the beginning and actually still on many occasions, biological phosphorus removal is linked with *Acinetobacter* spp. activity. The first published research results (Fuhs and Chen, 1975) stated that a single micro-organism, *Acinetobacter* spp. was responsible for EBPR. Using culturing dependent identification methods such as the Analytical Profile Index (API), several authors subsequently reported predominance of *Acinetobacter* spp. in EBPR systems (Buchan, 1983; Lötter, 1985; Wentzel *et al.*, 1988).

In general, however, it has to be mentioned that most experimental data nowadays point out that phosphorus removing sludges are not dominated by a single bacteria but are composed of a few dominant bacterial taxa (Wagner *et al.*, 1994; Bond *et al.*, 1997; Mino *et al.*, 1998). Therefore, *Acinetobacter* spp. should no longer be considered as the dominant organism (Mino *et al.*, 1998). In addition, former experimental observations indicate that the EBPR community may not be stable but changes in time and in location. Moreover, diverse organisms performing functions other than EBPR affect the relative number of PAOs in EBPR processes (Mino *et al.*, 1998).

Many studies have been performed to determine the organisms involved in EBPR. Sludges were taken from full scale or pilot scale phosphorus removing plants. Alternatively, laboratory scale reactors were

operated in such conditions that they result in extremely high phosphorus removing performance. In the latter case phosphorus removing bacteria are strongly selected for and it is thus likely that a low bacterial diversity remains. These reactors, however, provide good research material to identify the phosphorus removing bacteria (Bond *et al.*, 1997).

In the literature the organisms responsible for enhanced biological phosphorus removal are mostly defined as Polyphosphate-Accumulating Organisms (PAOs) or PolyPhosphate-bacteria (PP) whereas in other articles they are referred as Biological Phosphorus removing organisms (Bio-P). Although these terminologies are insufficient to describe all characteristics the organisms should reveal, the first description can lead to some controversy. The key characteristics of the involved organisms under anaerobic conditions are acetate uptake and its conversion to PHA for storage coupled with hydrolysis of stored poly-P and consequent release of ortho-phosphate (Jenkins and Tandoi, 1991). In the subsequent aerobic phase, stored PHA is consumed and phosphate is taken up and converted to poly-P for storage. Organisms revealing poly-P accumulation under aerobic conditions without the anaerobic processes occurring, are not responsible for EBPR and therefore do not belong to PAOs. In the following sections the terminology PAOs, or better PAO sludges, is reserved to organisms not only accumulating large amounts of poly-P under aerobic conditions, but also revealing the key characteristics described above under anaerobic conditions.

In the following section the characteristics of PAO sludges are described. This section is followed by a similar one describing the characteristics of *Acinetobacter* spp. Much evidence is gathered that *Acinetobacter*, although capable of accumulating large amounts of polyphosphate, is not the only micro-organism strain responsible for EBPR. In many cases the key characteristics for the anaerobic acetate metabolism are lacking in the isolates (Jenkins and Tandoi, 1991, Tandoi *et al.*, 1998, van Loosdrecht *et al*., 1997a,d). Many researchers also indicated that *Acinetobacter* spp. encountered in wastewater treatment plants, did not show sufficient phosphorus uptake and release to account for the observed EBPR activity in the treatment plant (Deinema *et al.*, 1980; Deinema *et al.*, 1985; Meganck *et al.*, 1985; Murphy and Lötter, 1986 and Tandoi *et al.*, 1987; van Groenestijn *et al.*, 1988). The section on characteristics of PAO sludges is followed by information gained concerning possible other micro-organisms contributing to EBPR. Finally, the last section deals with possible micro-organisms, so called GAOs, causing unexpected deterioration of EBPR performance.

Morphological characteristics of PAO sludges were first described by Fuhs and Chen (1975) based on microscopic observations of a PAO enriched sludge and concerned: non-motile rods or cocci, usually forming clusters, staining positive for PHB and containing Neisser positive granules in the cell. Initially they were believed to be gram-negative bacteria, but later the possibility has been formulated that they are gram-positive (Wagner *et al.*, 1994; Liu, 1995; Kawaharasaki *et al.*, 1999). Wagner *et al.* (1994) also reported that micro-organisms belonging to a beta-subclass of *Proteobacteria* were present in high concentrations, whereas according to Kawaharasaki *et al.* (1999) the dominant organisms belonged to the alpha-subclass of *Proteobacteria.* According to Mudaly *et al.* (2000), using Fluorescence *In Situ* Hybridisation (FISH) technology, bacterial predominance in the PAO sludges appeared, in descending order, to be: beta-subclass of *Proteobacteria* (22%), alpha-subclass of *Proteobacteria* (19%), gamma-subclass of *Proteobacteria* (17%) and *Actinobacter* (11%). Mudaly *et al.* (2000), referring to Roller *et al.* (1994), indicated that *Actinobacter* was formerly referred to as gram-positive bacteria with high Guanine+Cytosine-(G+C)-content. To avoid confusion (*Actinobacter* versus *Acinetobacter*), this new name is not used in this review.

Chemotaxonomic characteristics of PAO sludges. The dominant respiratory quinones in PAO-enriched sludges are quinone-8 (Q-8) and menaquinone-8(H_4) (MK-8(H_4)) (Hiraishi *et al.*, 1989; Hiraishi and Morishita, 1990; I Made *et al.*, 1998). Wagner *et al.* (1994) showed that PAO sludges contain a very high G+C-content. Using rRNA-probe techniques, Wagner *et al.* (1994) stated that bacteria with a high G+C-DNA-content are important for the enhanced biological P-removal. Auling *et al.* (1991) using

diaminopropane (DAP) showed that EBPR sludges had nearly no DAP in the polyamine pattern. van Groenestijn (1987) correlated the concentration of the AMP:phosphotransferase enzyme to with the EBPR activity of the PAO sludge.

Phosphorus accumulating/releasing activity of PAO sludges. The phosphorus content of activated sludge with good phosphate removal capacities is around 4-10% (Comeau *et al.*, 1986; Deinema *et al.*, 1985; Lowson and Tonhazy, 1980; Ohtake *et al.*, 1985). Bond *et al.* (1999) observed values ranging between 8.5 and 12.5% P/MLSS. Wentzel *et al.* (1989b) reported the maximal phosphorus content of the cells in the range of 0.35 mg P/mg active VSS. Comeau *et al.* (1986) reported the maximum releasable concentration to be 33 mg P/g VSS which corresponded to 55% of the total phosphorus accumulated. The anaerobic release rate observed by Satoh *et al.* (1992) and Smolders *et al.* (1994a) varied between 27 and 76 mg P/g MLSS/h.

Since *Acinetobacter* spp. was first attributed EBPR capacities, the following section deals with the characteristics of these strains. Traditional research performed on *Acinetobacter* spp. has finally led to new developments for characterisation of activated sludge.

Morphological characteristics of Acinetobacter spp. They belong to the group of gram-negative bacteria. They belong to the gamma-subclass of *Proteobacteria* (Kawaharasaki *et al.*, 1999). The average length of the cells was estimated by Juni (1995) to be between 1.5 and 2.5 µm and by Bosch (1992) to be between 1.0 and 1.5 µm. According to Cloete and Steyn (1988) the volume of the polyphosphate containing cells is between 0.5 and 0.59 µm³. Bosch (1992) indicated that this volume was between 0.1 and 1.9 µm³. These results indicate that *Acinetobacters* are relatively small and can only remove approximately 10^{-10} mg phosphate/cell (Momba and Cloete, 1996).

Chemotaxonomic characteristics of Acinetobacter spp. The dominant respiratory quinone for *Acinetobacter* is ubiquinone 9 whereas for PAO sludges this was Q-8 and MK-8(H_4). The presence of polyamines with diaminopropane (DAP) as a monomer was indicated by Auling *et al.* (1991). Since DAP was not encountered in PAO sludges, this is one of the evidences that *Acinetobacter* can not be the dominant micro-organism. Van Groenestijn (1987) showed the presence of AMP:phosphotransferase, an enzyme necessary for the energy conservation during polyphosphate degradation.

Phosphorus accumulating/releasing activity of Acinetobacter spp. The phosphorus content of *Acinetobacter* spp. can increase from 4 to 10% of its dry weight (Bosch, 1992). Deinema *et al.* (1985) and Pauli (1994) mentioned too that *Acinetobacter* spp. are able to accumulate large amounts of phosphorus - 2 to 10w% - depending on temperature, pH, growth rate and substrate limitations. Tandoi *et al.* (1998) specified that values of 4 to 10% intracellular phosphorus were obtained subjecting *Acinetobacter* spp. to both batch and continuous aerobic conditions, i.e. not by subjecting them to alternating aerobic-anaerobic conditions. Extremely high concentrations of cellular phosphorus (18.8%) were obtained under "overplus" conditions, i.e. following addition of phosphate to phosphate-starved cells (Tandoi *et al.*, 1998).

Using pure cultures Rustrian *et al.* (1997) observed that the overall removal efficiency depends strongly on the kind of strain used, e.g. *Acinetobacter lwoffi* ATC 21130 and *Acinetobacter calcoaceticus* NRRL 8058 are able to remove large amounts of phosphorus with either acetic, propionic or butyric acid (6.5 - 9w% stored P/cell). However, *Acinetobacter* SUCT-5 could only reach high phosphorus removal efficiencies with acetic acid (9.5w% stored P/cell).

The anaerobic release rate of phosphate by *Acinetobacter* spp. varies considerably with the highest value amounting 5.5 mg P/g MLSS/h (Tandoi *et al.*, 1998) being much lower than the release rate observed in PAO sludges. This is another indication of the limited role of *Acinetobacter* spp. in EBPR processes.

In two strains of *Acinetobacter*, isolated from treatment plants exhibiting EBPR, Tandoi *et al.* (1998) were able to indicate aerobic accumulation of high levels of poly-P or a "lipid" reserve, in one strain identified as PHA. However, when subjected to cyclic anaerobic/aerobic conditions in a chemostat, neither strain showed release of phosphate or uptake of acetate under anaerobic conditions.

Going into more detail Rustrian *et al.* (1997) studied the effect of cell harvesting time on the poly-P release characteristics. It was shown for *Acinetobacter* that the releasable phosphorus was 5-38% of the phosphorus consumed by cells in the logarithmic phase and up to 17-58% of the phosphorus consumed by cells in the stationary phase. Making no distinction Kuba *et al.* (1993) found a ratio between 45 and 63%.
In general it is reported that phosphorus release by pure cultures of *Acinetobacter* strains occurs very slowly with respect to phosphate accumulation (Deinema *et al.*, 1985; Ohtake *et al.*, 1985; Nakamura *et al.*, 1989; Appeldoorn *et al.*, 1992; Rustrian *et al.*, 1997).
Research has been focussed on the *effect of the growth phase* on phosphate release and uptake. Cloete and Bosch (1994) observed that phosphorus is accumulated at the end of the log phase and during stationary phase, once active growth had ceased. They concluded that the number of cells and their growth phase are crucial factors governing biological phosphorus removal. Rustrian *et al.* (1997) indicated that in stationary growth, cells generally released larger quantities of phosphate than in logarithmic growth. Phosphate uptake amounts for log or stationary cells were very similar.

Presence of Acinetobacter* spp. *in PAO sludges. Several techniques have been developed and tested to evaluate quantitatively the fraction of *Acinetobacter* members in relation to the total microbial population in activated sludges showing EBPR. API, the first method used (Fuhs and Chen, 1975), is a culturing dependent identification method. In these methods, only those bacteria which are cultivable on the artificial media used and under the defined conditions will be isolated. Wagner *et al.* (1993) and Kampfer *et al.* (1996) stated that it is likely that only a minor portion of the bacteria in activated sludges can grow under such conditions. Studies using fluorescent antibodies (Cloete *et al.*, 1985; Cloete and Steyn, 1988) and API tests (Meganck *et al.*, 1985 and Lötter and Murphy, 1985) to count bacteria in activated sludge systems showed that there is no link between the number of *Acinetobacter* spp. present and the phosphorus removal capacity of the plant. According to Cloete and Steyn (1988), *Acinetobacter* could remove at most 34% of the phosphorus removed in the activated sludge systems. More recent research is focussed on the application of a dual staining technique. Whereas the culturing independent methods show the phylogenetic position of bacteria in the community, the physiological characteristics of those bacteria cannot be shown by these methods. Combining rRNA targeted oligonucleotide probes and 4'-6-diamidino-2-phenylindol (DAPI), this problem is overcome. DAPI is a fluorescent dye usually used for DNA staining. When DAPI is used at high concentrations, it also stains polyphosphate granules (volutin) and lipid inclusions (Tijssen *et al.*, 1982; Streichan *et al.*, 1990). Polyphosphate-DAPI fluorescence is bright yellow whereas lipid-DAPI fluorescence is weak yellow and fades in a few seconds. Using this dual staining technique Kawaharasaki *et al.* (1999) could not detect any presence of *Acinetobacter* in activated sludge samples from an anaerobic/aerobic batch type reactor for EBPR.
Adding more arguments to the controversy on the role of *Acinetobacter* spp. in EBPR processes, Converti *et al.* (1995) reported high numbers of *Acinetobacter* in sludges from a bench scale system exhibiting good EBPR removal and Knight *et al.* (1995b) reported high numbers as well in full-scale nutrient removal plants with high EBPR capacity. Using screening methods (Biolog GN system) to assess for ability of the selected cultures to utilise different carbon sources, Knight *et al.* (1995a) observed considerable variations even within individual genospecies of a large number of isolates of *Acinetobacter* obtained from the modified UCT plant. This result suggests that further strain characterisation is required to increase the reliability of these identification systems to assess the members of *Acinetobacter* genospecies.
As a further proof for the still existing controversy it has to be noted that an article by Zafiri *et al.* (1999) elaborates on the kinetic modelling of biological phosphorus removal using a pure culture of *Acinetobacter* sp. ATCC 11171. The authors stated that the bacterium exhibited the ability to accumulate intracellularly large amounts of organic carbon, especially when it was grown under polyphosphate limiting conditions. Under anaerobic conditions the bacterium was capable of taking up acetate with simultaneous phosphorus release. A decrease of acetate uptake and phosphorus release rates was observed as the intracellular carbon content of the cells was getting higher. However, no measurement of PHA was

performed. The acetate uptake and phosphorus release rates were limited by the amount of releasable phosphorus from the cells resulting from internally stored polyphosphate hydrolysis.

As a last remark it should be said that whatever the experimental controversy in research results, *Acinetobacter* is able to accumulate high levels of poly-P and thus might play a role in EBPR. However, its role is still to be defined and should probably not be referred to as PAO.

Presence of other candidate PAO micro-organisms in PAO sludges. Besides *Acinetobacter* several authors also mentioned *Arthrobacter* and *Pseudomonas* (Kavanaugh, 1991; Okada *et al.*, 1992) as possible responsible micro-organisms is PAO sludges. Shoda *et al.* (1980) indicated that *Arthrobacter globiformis*, having a phosphorus storage capacity of 7w%, does not store phosphorus as polyphosphate. Phosphorus is mainly stored in the RNA fraction. *Pseudomonas vesicularis* (now *Brevundi monas*) has a shown storage capacity of 31w%, but phosphorus uptake and release was not observed. Randall *et al.* (1994) observed significant numbers of *Pseudomonas* and *Bacillus* in SBRs fed with glucose and with at least some EBPR activity. However, phosphorus uptake and release profiles are not discussed explicitly in the paper. PHB and/or glycogen profiles were not recorded either. According to the polyamine profiles, species belonging to the pseudomonads, the *Proteobacteria* groups and the *Flavobacterium-Cytophaga* group can play an important role in biological phosphorus removal too (Kortstee *et al.*, 1994). According to the results obtained by Kawaharasaki *et al.* (1999) with a dual staining technique, staining with rRNA-targeted oligonucleotide probes showed that four major bacterial groups were present in EBPR sludge: the beta-subclass of *Proteobacteria*, gram-positive bacteria with a high G+C-content, the alpha-subclass of *Proteobacteria* and bacteria belonging to the *Cytophaga-Flavobacterium* cluster of the *Cytophaga-Flavobacterium-Bacteriodes* phylum. When staining with DAPI alone, many of the gram-positive bacteria with high G+C-content and the organisms belonging to the alpha-subclass of the *Proteobacteria* fluoresced brightly yellow, indicating accumulation of large amounts of polyphosphate. Stante *et al.* (1996, 1997) isolated a PHB storing strain from a SBR designed for EBPR and identified it as *Lampropedia* spp. This bacterium has the ability to take up acetate and store it as PHA under anaerobic conditions. Morphologically it has a very unique sheet-like cell arrangement which is not common in EBPR processes. The presence of this bacterium is thus doubted.

- *Microlunatus phosphovorus*

Nakamura *et al.* (1991, 1995a, 1995b) and Kawaharasaki and Nakamura (1995) isolated a micro-organism from a biological phosphorus removing activated sludge plant and named it *Microlunatus phosphovorus* strain NM-1^T (Nakamura *et al.*, 1995a). It concerns a gram-positive, coccus shaped, non-spore forming, non-motile micro-organism. The cells are 0.8 to 2.0 µm in diameter, with a G+C-DNA-content of 67.9 mol%. The physiological and biochemical characteristics of the strain are obligate aerobic and chemo-organotrophic. Chemotaxonomically strain NM-1^T has MK-9(H_4) as major quinone, different from the observations of the dominant respiratory quinones in PAO sludges (Hiraishi *et al.*, 1989; Hiraishi and Morishita, 1990; I Made *et al.*, 1998). As for the cultural characteristics, it was observed that the growth rate of the strain (16.5 d^{-1}) was 10 times slower than that of *Acinetobacter* 210A. The phosphorus accumulating activity observed depended on the moment of harvesting. Cells harvested during stationary phase had decreased phosphorus uptake activity, especially in the late stationary phase. By increasing the intracellular carbohydrate content beforehand, cells harvested in early stationary phase exhibited recovered phosphorus uptake activity, while cells harvested in the late stationary phase did not take up phosphorus although they did accumulate carbohydrate. Kawaharasaki and Nakamura (1995) revealed that the moment the bacteria are harvested from the activated sludge system is an important factor determining the phosphorus uptake capacity of the organism, cf. *Acinetobacter*. Analysis of hydrolysed cells (cooked for 10 min in a 1N HCl solution) showed that the internal phosphorus mainly consisted of polyphosphate. In the enriched culture the internal phosphorus content increased from an initial value of 2% to 6%. In pure culture, however, the organism seemed able to store 12.6 w% phosphorus under micro-aerophilic conditions. The intracellular phosphorus content then was 16.6%.

Changing to anaerobic conditions with glucose as carbon source, a clear phosphorus release and glucose uptake occurred. With acetate, glutamate and lactate, phosphorus release still occurred but no substrate uptake was observed. Mino *et al*. (1998) state in their review article that observation implies that *Microlunatus phosphovorus* is not responsible for EBPR. In the early logarithmic phase the organism contains 2.4% phosphorus, whereas in the stationary growth phase 6% phosphorus was found. In the logarithmic phase the cells contain 8% internally stored carbohydrates, whereas this amount decreased to 2.4% in the stationary phase. Cells harvested in the logarithmic phase showed remarkable phosphorus uptake activity, while cells harvested in the stationary growth phase were characterised by decreased phosphorus removal capacity under aerobic conditions. The results indicate that the growth phase and/or intracellular carbohydrates significantly affected the phosphorus uptake activity. Using a dual staining technique described above, Kawaharasaki *et al.* (1999) only detected a rather small amount of *Microlunatus phosphovorus* in EBPR sludge originating from a lab scale batch-type anaerobic/aerobic reactor.

- <u>*Nocardia*</u>

One of the common problems in activated sludge wastewater treatment plants is the incidence of foaming due to the excessive growth of filamentous actinomycetes, especially *Nocardia amarae*. Consequently, Kim and Pagilla (2000) studied the occurrence of *Nocardia* phosphorus removing sequencing batch reactors. It was shown that *Nocardia*, due to its lower maximum specific growth rate (μ_{max} = 2.3 d^{-1}) and observed yield (Y_{obs} = 0.23 g VSS produced/g COD removed) than those of *Acinetobacter* (μ_{max} = 6.2 d^{-1} and Y_{obs} = 0.35 g VSS produced/g COD removed) will not be able to compete in a dual culture fed with the same carbon source. Since only acetate was used as carbon source, extrapolation of these results to full scale SBR systems is not possible. Extrapolation of these results to continuous systems is even more questionable.

In a study by Chuang and Ouyang (2000) the biomass fractions of heterotrophs (X_H) and phosphate accumulating organisms (X_{PAO}) were estimated through experimental and theoretical calculations on the mass balance of organic matter, nitrogen and phosphorus in a pilot scale biological nutrient removal process. The yield fraction of X_H growth in anoxic and aerobic conditions were 48.4% and 39.4%, respectively. The yield fraction of X_{PAO} growth in aerobic conditions was 12.2%. X_H showed to be the major community, whereas X_{PAO} was only present in a minute amount. In the study the basic assumptions underlying ASM2 (Henze *et al.*, 1995) were used in the mass balance calculation. As such it is assumed that X_{PAO} only grows on stored PHA in the aerobic phase. Moreover, it is assumed that the phosphate accumulating organisms do not possess denitrifying capability as was later accepted in ASM2d (Henze *et al.*, 1999).

Presence and possible dominance of competing micro-organisms, GAOs, in PAO sludges

Cech and Hartman (1990, 1993) were the first to report on so-called G-bacteria causing deterioration of EBPR activity. Later, Matsuo (1994) and Satoh (1994) reported similar observations. The responsible group of bacteria were then called Glycogen Accumulating bacteria (GAOs) (Liu *et al.*, 1994). In 1988, Mino *et al.* added to this that these bacteria are different from PAOs by referring to them as Glycogen Accumulating non-poly-P bacteria, however still with the same abbreviation, i.e. GAOs. The latter definition clearly demonstrates the narrow difference between PAOs and GAOs. Indeed, both micro-organisms have the ability of aerobically storing glycogen and using its degradation under anaerobic conditions for uptake of carbon sources and storage as PHAs. However, PAOs have the possibility of also using poly-P hydrolysis as energy source. Since the discovery of GAOs is much more recent than that of PAOs, less information is yet available on these organisms. To allow better study of these organisms some authors have artificially grown them by limiting the phosphorus content in the feed (Liu *et al.*, 1994, 1996b; Sudiana *et al.*, 1997). Whether these organisms are the same organisms as encountered by Cech and Hartman (1990, 1993) still needs proper verification. In 1997, Carucci *et al.* stated it is not yet possible to say the G-bacteria described by Cech and Hartman (1993) behave as GAOs. Carucci *et al.*

(1997) observed micro-organisms morphologically resembling the G-bacteria, but anaerobic PHA accumulation was not observed.

Cech and Hartman (1993) described the G-bacteria encountered in their system as large coccoid cells packed in tetrads or aggregates like *Methanosarcina* spp., 2-3μm in size, non-motile, Neisser positive (whole cell blue). Matsuo (1994) observed two dominant G-bacteria: Neisser staining showed one was internally coloured purple and the other yellow with some tiny granules. Both occurred mostly in packets of tetrads, 6-8 μm in size and surrounded by thick and dense slime. Liu *et al.* (1996) observed at least three different morphological types of GAOs. The most abundant type was described as a coccus with 0.8 -1.2 μm diameter, mostly appearing in pair, tetrads or aggregates, non-motile, Neisser variable (whole blue cells, light blue or pink) and PHB staining positive (black granules, orange red cell). The next most abundant type was described as a large coccus (ϕ = 2 - 2.5 μm), occurring in packets of 8 cells or more having a thick cell, non-motile, several tiny granules within the cell, Neisser variable (whole blue cells, light blue or pink) and PHB staining positive (black granules, orange red cell). This type resembles the micro-organisms described by Cech and Hartman (1993) but were in contrast not the most abundant ones. The last category consisted of large oval rods, appearing as single cells with a diameter of 2 to 3 μm, usually non-motile and having several large PHA granules inside the cells. This last category was Neisser negative (light pink or colourless) and stained PHB positive (whole cell black).

From a sludge with poor phosphorus removing capabilities but that continued to utilise the carbon substrate, to accumulate PHA and to degrade carbohydrate in the anaerobic phase (Bond *et al.*, 1997) it was shown with FISH probing that bacteria resembling the G-bacteria according to the description of Cech and Hartman (1993) became abundant in the system. Since these cultures were obtained from a reactor fed with only small amounts of phosphorus, this result is not a proof that G-bacteria compete with the PAOs.

3.4.5. Process configurations for biological P-removal

Based on the characteristics of the EBPR process a lot of different process configurations have been developed. The processes can be divided in two main groups: mainstream processes and side-stream processes. Mainstream processes are characterised by the fact that the anaerobic phase is in the waterline of the process and the phosphate is removed while being inside the PAOs. In the side-stream process, the phosphate removal is performed in the sludge line of the process and the PAOs are only used to concentrate the phosphate which is finally removed through precipitation after release out of the PAOs. To this end, an anaerobic tank is placed in the sludge line to select for PAOs. In the latter case the biological process is only used to concentrate the phosphate in that section of the process where it is efficiently precipitated.

Whereas the division between mainstream and side-stream processes is traditionally used, processes can also be divided in two groups according to the operating mode, i.e. continuous processes or batch processes. For the latter group especially the so-called sequencing batch reactors (SBRs) are often used for wastewater treatment processes. Whereas these processes can generally be categorised as mainstream processes, i.e. the phosphate is removed while being inside the PAOs, they are very often separately mentioned in textbooks or listed under the cyclic process design systems. In this literature review the latter subdivision is adopted.

The combined biological/chemical process is especially advantageous for those wastewaters where the influent COD to phosphate ratio is low. An evaluation of biological and biological/chemical phosphate processes revealed that for the first case approximately 20 g COD/g P was necessary, whereas for the combined process the minimal COD requirement dropped to 2 g COD/g P (Smolders *et al.*, 1996).

In the following paragraphs those configurations most encountered in practise are discussed. This list is not meant to be an exhaustive one, but to provide the reader with sufficient information to understand easily other schemes not discussed.

3.4.5.1. ***Side-stream processes***

3.4.5.1.1. *Phostrip process*

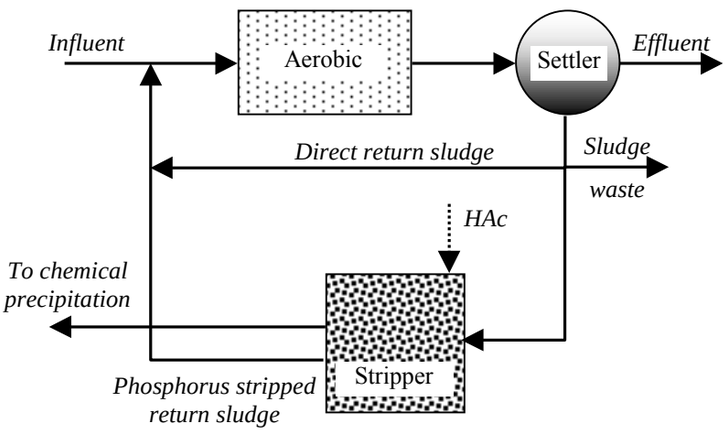

Figure 34 Schematic representation of the Phostrip process

Based on observations by Shapiro (1965) at the Baltimore treatment plant, where phosphorus uptake was followed by its release at the bottom of secondary clarifiers, Shapiro proposed to expose return sludge to such conditions prior to return to the aeration basin in order to strip out phosphorus. This was a predecessor to the Phostrip process, patented by Levin (1966) and applied in the 1970's in the United States (Figure 33). The proprietary Phostrip became the most popular biological phosphorus removal process at that time. The side-stream flow is diverted to the anaerobic phosphorus stripper at a ratio of approximately 5-30% of the influent flow (Metcalf and Eddy, 1991; Brett, 1997).

Besides phosphorus release the main purpose of the anaerobic stripper is sludge thickening. The retention time in this tank generally ranges from 8 to 12 hours (Metcalf and Eddy, 1991; Tetreault *et al.*, 1986). The release of phosphorus in the stripper tank occurs with the same mechanisms as for an anaerobic-aerobic sequence in the activated sludge process described earlier. Addition of acetic acid or influent stimulates the phosphate release. Soluble phosphorus is transferred to the supernatant either by recycling the stripper underflow or by passing an elutriation stream (primary effluent, secondary effluent or supernatant return) through the stripper. The stripper overflow is then fed to a chemical tank where phosphorus is precipitated by lime addition (Metcalf and Eddy, 1991; Johansson, 1994; Brett, 1997).

With the proprietary Phostrip design Levin (1965) did not yet understand the relation between anaerobic substrate storage and the concurrent phosphorus release, nor did it account for the detrimental effects of nitrate penetrating the anaerobic phase (Reddy, 1998). Initially these systems were operated at sludge ages so low that nitrification did not occur (Alexander *et al.*, 1994). A modification of the original Phostrip design incorporates an anoxic reactor ahead of the aerobic reactor, with an internal recirculation from the aerobic to the anoxic phase, to minimise nitrate in the effluent from the aerobic reactor (Brett, 1997).

The first full scale applications of biological phosphorus removal were based on the side-stream process (Phostrip). The most important advantages were the controllability, reliable effluent phosphate concentrations of less than 1.5 mg/ℓ (Metcalf and Eddy, 1991) and the production of a phosphate rich side product, eventually applicable for other purposes. However, this process is labour intensive and the use of chemicals is very high. According to De Jong, (1999) a market for the by-products has not really been found, although changes are expected. It is thought that the application of this process will remain limited because of its high cost and complexity. Moreover, the necessity to add chemicals makes the process non sustainable.

A variant to the traditional Phostrip process is the combination with the so-called Crystalactor® where the precipitation unit is replaced with the crystallisation of phosphorus compounds in a fluidised bed reactor (Giesen, 1998).

3.4.5.1.2. *The BCFS® process*

By the Dutch Waterboard "Groot Salland", the Netherlands, the BCFS® (translated from Dutch: *biological chemical phosphate nitrogen removal*) process has been developed. The process design is based on the ideas of the Phostrip process, but avoiding the large investments in extra infrastructure. The process is specially designed to optimise the activity of DPAOs (van Loosdrecht *et al.*, 1998).

Nitrification is optimised by operating the treatment plant at long SRT (45 days). The associated low sludge productions limits the capacity for intracellular polyphosphate accumulation in the sludge (van Loosdrecht, 1997c). Phosphorus that can not be accumulated in the sludge has is removed chemically. Therefore an in-line "stripper" is provided by placing baffles at the end of the anaerobic reactor compartment to induce a quiescent zone in which the sludge partly settles. The phosphate-rich supernatant can be pumped from the activated sludge tank and precipitated in the sludge thickener (van Loosdrecht *et al.*, 1997c, 1998). A practical application of this process configuration is implemented at the Holten WWTP in the Netherlands (Van Veldhuizen, 1999). A schematic representation of the process configuration is presented in Figure 35.

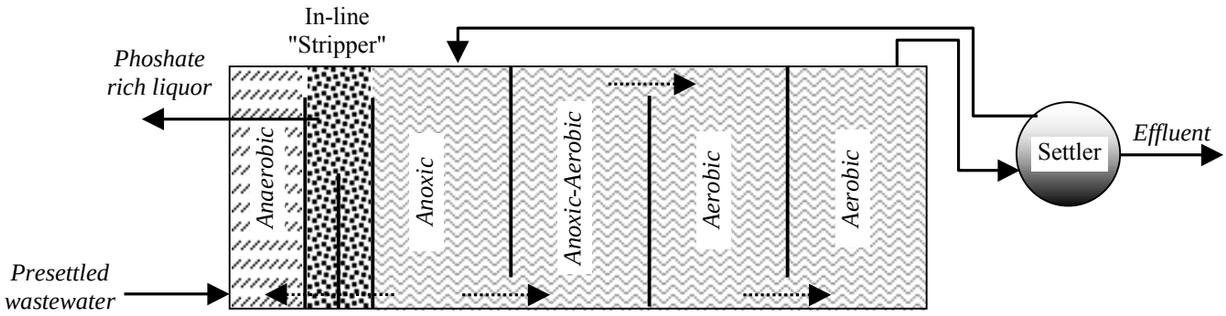

Figure 35 Schematic presentation of the aeration basin and secondary clarifier of the Holten WWTP implementing the BCFS® process (Van Veldhuizen et al., 1999)

3.4.5.1.3. *Renpho- and Modified Renphosystem*

In the Renphosystem (Rensink *et al.*, 1988), influent and direct return sludge come together in the anaerobic phase where phosphate is released. The mixed liquor then flows into an aerobic phase where phosphate is taken up and nitrification takes place. In the next, anoxic phase, the nitrate formed is reduced, electrons being provided by adding some fresh influent that contains readily biodegradable organic compounds. In a final additional aerobic phase the last traces of phosphate and organic matter are removed. After settling of the sludge, one part is returned to the front end of the plant and the second part is pumped into the anaerobic stripper where phosphate is released from the cells. The stripped cells are then pumped back to the first aerobic phase (Figure 36).

The supernatant of the stripper tank is generally sent through a Crystalactor® (see 3.6) (Rensink *et al.*, 1997). In the stripper tank a detention time of 4 hours was sufficient. Dosage of acetate (10-20 mg COD/g MLSS) stimulate phosphorus release and growth of PAOs (Rensink *et al.*, 1997). The efficiency of the P-removal on the basis of ortho-phosphate and total phosphate amounted 97 and 87% respectively (Rensink *et al.*, 1997).

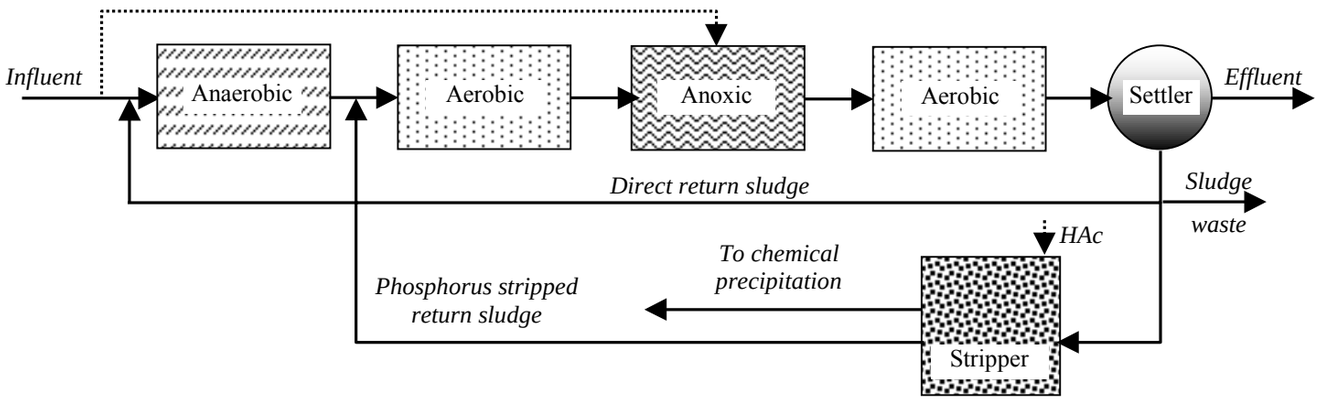

Figure 36 Schematic representation of the Renphosystem

Although the nitrogen removal in the Renphosystem achieved values of 70% at low temperatures and 80% at high temperatures, the process configuration was not capable of reaching very low nitrogen values in the effluent. To allow for improved nitrification-denitrification, the Modified Renphosystem was designed comprising an additional phosphorus stripped sludge return to the anoxic reactor. This modified process design allowed for growth of DPAOs resulting in saving of COD and energy (Rensink *et al.*, 1997).

Both the Renphosystem and the modified Renphosystem are combined sidestream and mainstream processes. Because of the stripper tank, generally they are listed along with the sidestream processes.

3.4.5.2. ***Mainstream processes***

3.4.5.2.1. *Bardenpho™ and Phoredox process*

Barnard (1975) first achieved phosphorus removal in a mainstream process later called the Bardenpho™ process. A four phase anoxic-aerobic-anoxic-aerobic configuration, originally designed for nitrogen removal, was used. Sludge from the secondary clarifier and the mixed liquor from the first aerobic basin are recirculated to the first anoxic reactor (Figure 37).

Although significant phosphorus removal was achieved, the effluent concentration was very sensitive to maintenance conditions. Looking back at the prerequisites for phosphorus removal, the Bardenpho™ process is violating the first rule, i.e. the feed stream has to come into contact with an oxygen and nitrate free stream. Since the first reactor in the Bardenpho™ configuration is an anoxic reactor, some nitrate will be present.

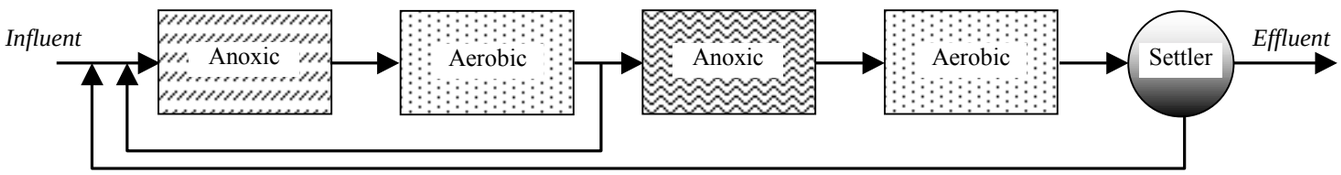

Figure 37 Original Bardenpho™ configuration

Including an anaerobic reactor ahead of the first anoxic reactor in the Bardenpho™ configuration, fed with sludge from this first anoxic reactor, created favourable conditions for phosphorus removal. This configuration is known as the five stage Bardenpho™ configuration, or the modified Bardenpho™ process. In South Africa and Europe this process is known as the Phoredox process.

Further developments in South Africa led to additional modifications, i.e. a Modified or 3-phase Phoredox system where the post-denitrification and re-aeration steps are omitted. A description of such a plant in the Johannesburg area was presented where an effluent concentration of 2.1 mg P/ℓ was achieved (Pitman, 1983) and in Olifantsfontein where a 1.0 mg P/ℓ level was reported by van Huyssteen *et al.* (1990).

3.4.5.2.2. *The A/O and A^2/O configuration*

The proprietary A/O and A^2/O processes, patented by Air Products, are very similar to the Phoredox concept, except that the anaerobic and aerobic stages are divided into a number of equally sized, completely mixed compartments.

3.4.5.2.3. *(Modified) UCT process*

Further modifications of the Phoredox plant were done by the research team of Prof. Marais at the University of Cape Town in South Africa. The main idea here was to return the activated sludge to the anoxic instead of the anaerobic phase to avoid any negative effects on the initial phosphorus removal efficiency by nitrate present in the return sludge. Here the nitrate-containing sludge is first introduced into a denitrification reactor, after which the nitrate-free sludge/water mixture is partly recycled to the anaerobic tank (Figure 38).

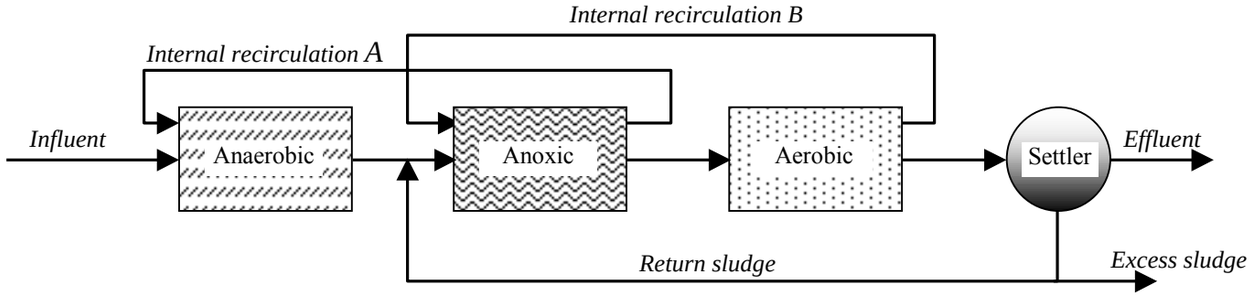

Figure 38 Schematic representation of the University of Cape Town (UCT)-type process

In the modified UCT configuration, the anoxic reactor from the UCT process is divided in two compartments. The anoxic reactor following the anaerobic phase receives the nitrates recycled with the return sludge. The mixed liquor is recycled to the anaerobic phase. The second anoxic reactor allows denitrification of the recycled mixed liquor from the aerobic phase.

It has been shown that denitrifying bacteria can contribute significantly in the UCT-type process (Kuba *et al.*, 1997a; van Loosdrecht *et al.*, 1997c). Indeed, in these processes, organisms are cycled between anaerobic and denitrifying conditions, which stimulate their growth.

3.4.5.2.4. *Virginia Initiative Plant (VIP™)*

Operating the UCT process as a high rate system (MCRT of 10 days or less) resulted in superior phosphorus removal capability compared to the low rate UCT process (Daigger *et al.*, 1987). Operation at a lower MCRT resulted in a greater net observed yield and thus increased sludge production. This concept was patented as a public domain patent by CH2M/HILL as the VIP™ process (Reddy, 1998). A staged reactor configuration is provided by using at least two complete mix cells in series for each zone of the biological reactor (Daigger *et al.*, 1987).

3.4.5.2.5. *Johannesburg process*

With the process configuration proper to the Johannesburg process, nitrates are prevented from entering the anaerobic phase. Therefore the sludge return line first passes through an anoxic phase before entering the anaerobic phase (Figure 39). Comparing this system with the UCT configuration reveals that the advantage mentioned by van Loosdrecht *et al.* (1997c) no longer exists because the organisms are not cycled anymore between anaerobic and denitrifying conditions.

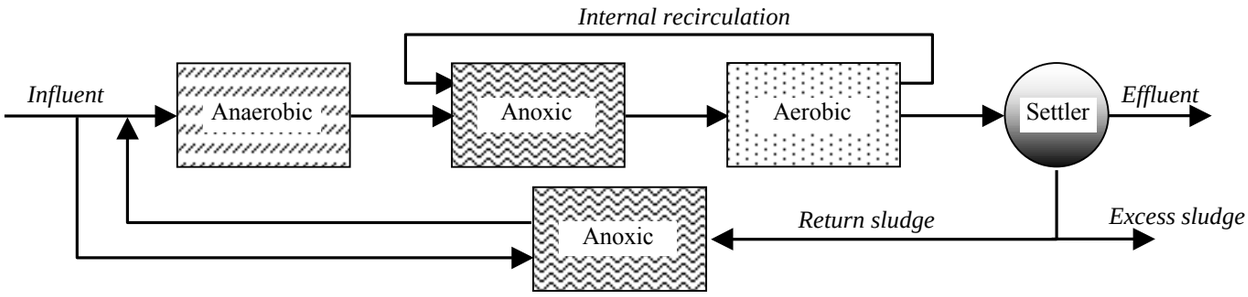

Figure 39 Schematic representation of the Johannesburg process

3.4.5.2.6. *Two sludge systems*

In order to overcome difficulties related to optimising combined nitrogen and phosphorus removal, novel process configurations have been worked out using two sludges instead of a single sludge system. With these process configuration, slow growing nitrifiers and fast growing heterotrophs are separated (Wanner *et al.*, 1992; Kuba *et al.*, 1996b; Sorm *et al.,* 1996). With the proposed process scheme (Figure 40) phosphorus release is performed under strict anaerobic conditions in a mixed tank. Most soluble organics are stored by PAOs and the fine and coarse particles are entrapped in the floc. A settler allows to separate a supernatant with high ammonia and phosphorus concentrations and a COD rich sludge. The supernatant is then nitrified in a fixed film reactor. This reactor type is preferred due to the very low yield of the nitrifiers. The COD rich sludge bypasses the nitrification reactor and is re-mixed with the nitrified effluent in an anoxic phase, where denitrification and biological phosphorus removal occur simulta neously. An aerobic phase is placed as last for safety reasons, in order to insure that the PAOs regenerate the poly-P and glycogen pool and to avoid rising problems in the final settler (Wanner *et al.*, 1992).

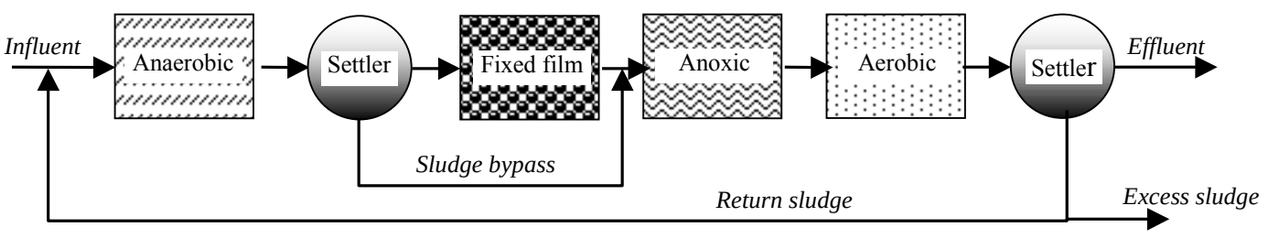

Figure 40 Schematic representation of a two sludge system
for combined nitrogen and phosphorus removal (Wanner et al. 1992)

3.4.5.3. ***Cyclic process design***

Whereas for an important period of time continuous flow configurations have received most attention, cyclic process designs are gaining importance again because of their operating flexibility. Actually, continuous systems are derived from the simple fill-and-draw operation developed by Ardern and Lockett as early as 1914. These researchers established the concept of operating a single reactor basin using

repetitive cycles of aeration, settling, effluent withdrawal and sludge recycle. To allow for continuous operating conditions, several reactors can be operated in parallel.

Actually, in Belgium, two different cyclic process designs are in operation, namely the Unitank® system (Gerards *et al.*, 1996) and the Biodenipho™ process (Bundgaard *et al.*, 1989). Because of the growing importance of these cyclic processes, the evolution of these cyclic designs is described in the following sections.

3.4.5.3.1. *Oxidation ditch design*

The oxidation ditch technology, or endless loop activated sludge reactor, played an important role in the development of the clarifier-less variable volume activated sludge systems. Within these systems, mixing and aeration is provided by means of horizontal axis paddles or rotating brush aerators. The concept can easily be compared with an induced river flow action. A clarifier is provided by means of a separate zone in the oxidation ditch. This zone is separated from the endless loop by means of baffles. Mixed liquor is allowed in this zone at regular time intervals. After sludge settling, the mixed liquor is withdrawn and new mixed liquor flows into this zone. Notable developments were reported by Pasveer and co-workers in the late 1950s and early 1960s (Irvine, 1996). To allow for higher flow rates, a separate settler was foreseen in later designs.

To allow for phosphorus removal in an oxidation ditch system, an anaerobic reactor is placed upstream of the oxidation ditch (Reddy, 1998). In Figure 41 is visualised, though the distinct zones do not exist because of the very high internal recirculation rate. Wastewater typically circulates at velocities varying between 0.25 and 0.35 m/s (Metcalf and Eddy, 1991).

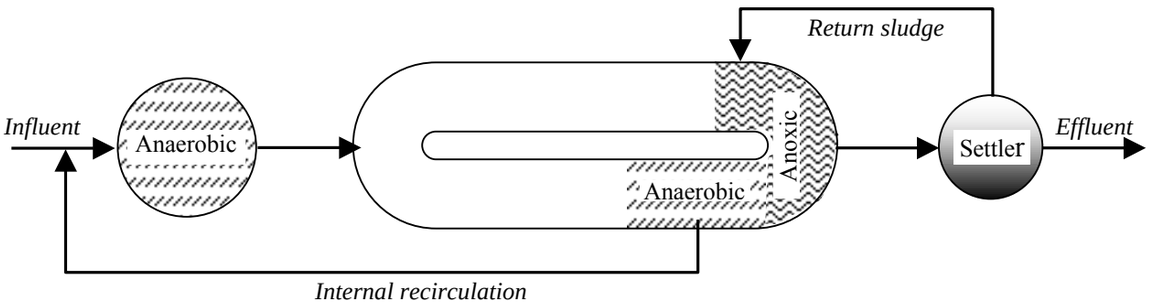

Figure 41 Oxidation ditch design for nitrogen and phosphorus removal (Reddy, 1998)

Well known oxidation ditch processes comprising EBPR activity are the Carrousel 2000 (Janssen *et al.* 1996) (commercialised in the US under the name deniTR). The process contains an additional pre-denitrification phase following the anaerobic phase. A modification of the latter processes incorporates a selector instead of an anaerobic reactor ahead of the oxidation ditch to enhance growth of denitrifying PAOs (Janssen and van der Roest, 1996).

3.4.5.3.2. *Biodenipho process™*

The Danish nutrient removal processes - Bio-Denitro™ and Bio-Denipho™ - are performed in phased isolation ditches which are continuous flow activated sludge systems with phased or intermittent operations (Bundgaard *et al.*. 1989). In Figure 42 the operating conditions of this process configuration are presented and explained below. Only the first half of the cyclic operation is presented, while the next half is performed with interchanged flow directions.

The influent enters the anaerobic phase from where it passes through an anoxic phase during the first phase. The aerobic phase at that moment is not in line. This phase takes half an hour or can be operated on the basis of effluent ammonia concentration. Indeed, during this phase the influent is never subjected

to aerobic conditions, nitrification does not occur. During the following phase, the mixed liquor from the anoxic phase flows to the aerobic phase allowing nitrification. This phase takes 1.5 hours or can be controlled now on the basis of effluent nitrate concentration. For the following phase the aeration in the anoxic phase is switched on to allow aerobic conditions. In the second half of the cyclic operation, what was the anoxic tank is now the aerobic tank and what was originally the aerobic tank is operated anoxically by switching off the aeration. This interchanging of flows and processes allows for uniform sludge concentration in both the aerobic and anoxic phases.

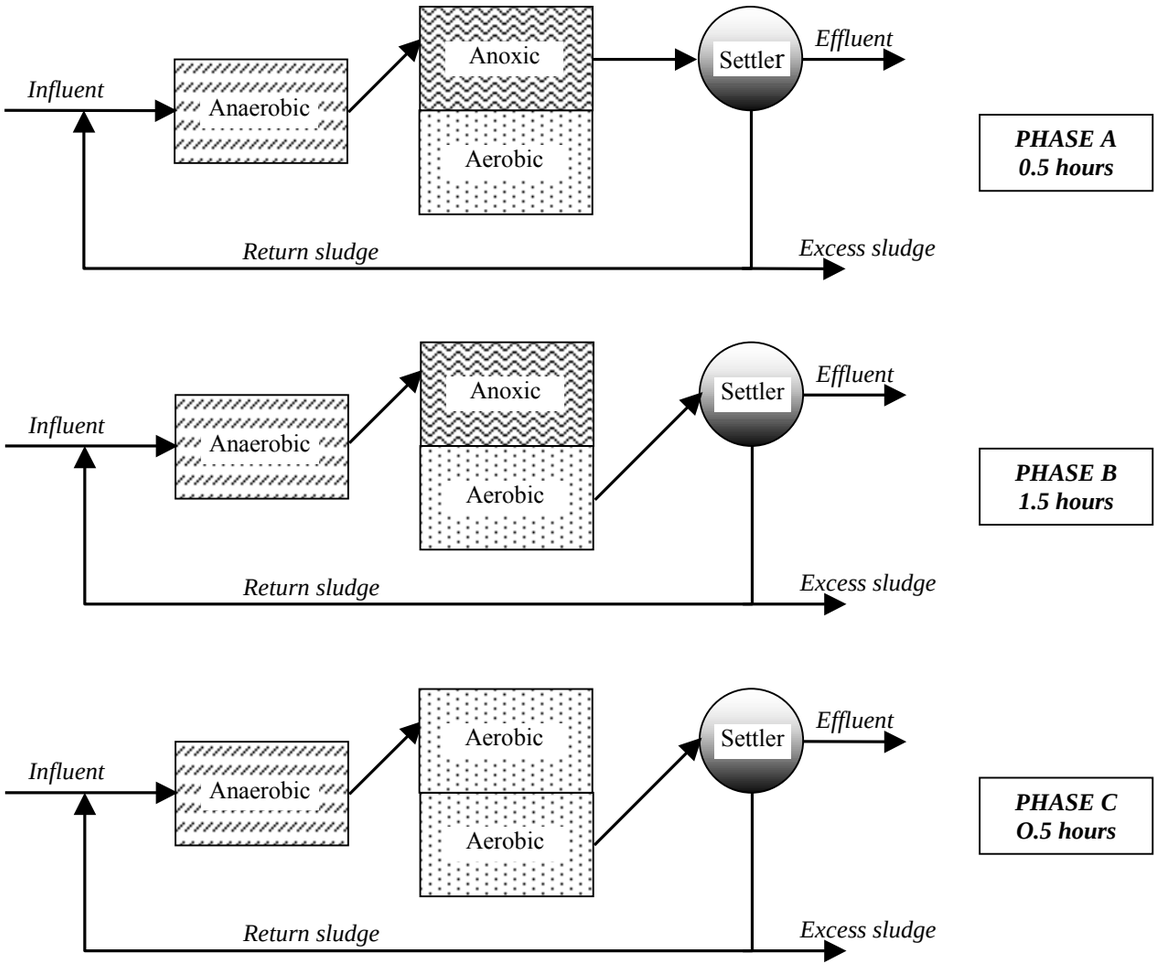

Figure 42 Schematic representation of the Bio-Denipho process (Bundgaard et al., 1989)

3.4.5.3.3. *Sequencing batch reactors (SBRs)*

With the SBR design, the developments of the shallow oxidation ditch were translated to a deep rectangular basin. Mixed liquor now remains in the reactor during all steps of the activated sludge process, thereby eliminating the need for separate secondary sedimentation tanks (Metcalf and Eddy, 1991).

For sequencing batch operating conditions different phases can be distinguished (Figure 43). The fill phase may be static, mixed or aerated, depending on treatment objectives. A static fill results in minimum energy input and high substrate concentration at the end of the fill phase. A mixed fill results in denitrification, if nitrates are present, and provides anaerobic conditions required for biological

phosphorus removal. Aerated fills result in the beginning of aerobic reactions, a reduction in the overall cycle time, and holds substrate concentrations low, which may be of importance if biodegradable constituents exist which are toxic at high concentrations (Ketchum, 1996).

Comparing the SBR operating conditions, it is noticed that Pasveer's configuration differed from the earlier fill-and-draw systems and from the SBR configuration, in that the influent was continuously supplied to the aeration tank while the treated effluent was periodically discharged.

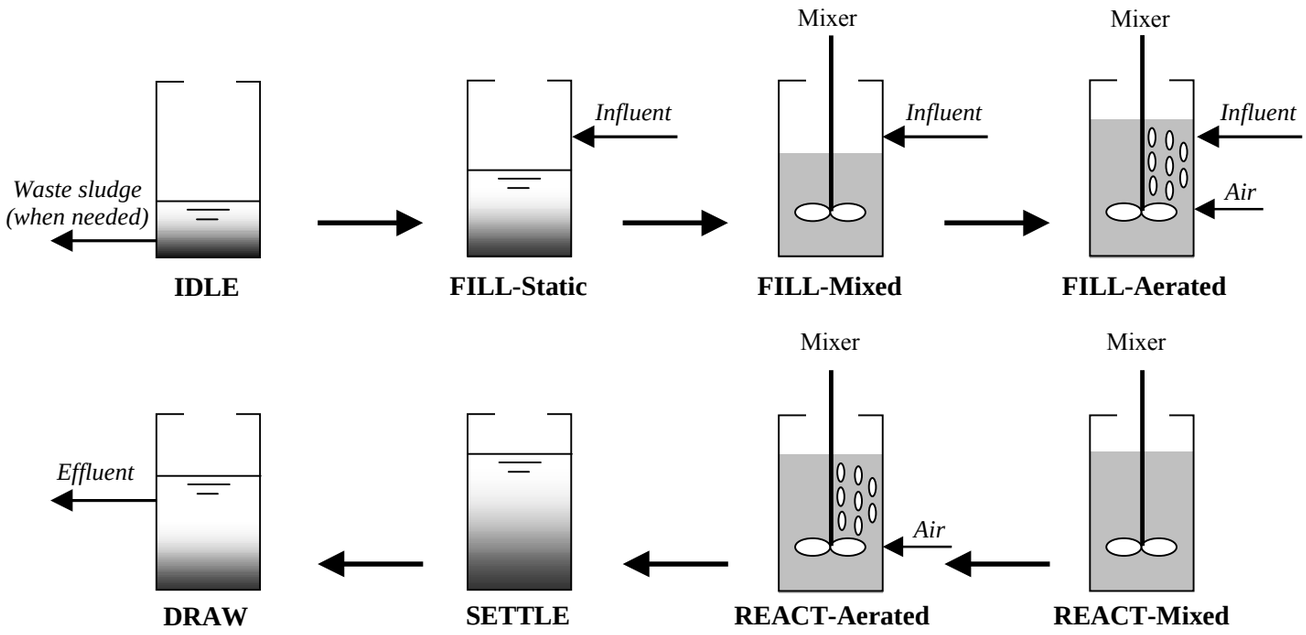

Figure 43 Illustration of an SBR reactor during one complete cycle and definition of phases (Ketchum, 1996)

3.4.5.3.4. *Cyclic Activated Sludge System (CASS^{TM})*

According to Goronszy (1994), this SBR combines self-regulating selector and variable volume reactor technology, providing an effective means for the control of filamentous sludge bulking often encountered in cyclic process designs. The system consists of a basin which comprises three reactor volumes, all in continuous fluid communication (Figure 44). Volume I refers to the captive selector, volume II to the secondary phase and volume III to the main aeration zone. Sludge is continuously returned from volume III to I in order to maintain the appropriate substrate to biomass (S_0/X_0) initial reaction conditions that favour growth of floc-forming micro-organisms (Goronszy, 1994). For typical domestic wastewater treatment applications, the sections are in the approximate proportions of 5%, 10% and 85% (www.sbrcass.com). During one cycle, the liquid level inside the reactor rises from a set bottom water level in response to a varying wastewater flow rate (www.sbrcass.com).

The CASS-configuration is a superposition of a complete-mix system (volume III) and a plug-flow-system (volume I). The general cyclic sequencing consists of fill-aeration, fill-settle, surface skim and fill-idle.

Cycles which drive EBPR include four and six hour periods with two and three hour sequenced air-on-off periods (Goronszy, 1994). There is little effect of residual nitrate on the EBPR performance due to the low nitrate concentration that remains in all volume sections of the basin through simultaneous nitrification-denitrification. Fermentation to produce VFAs, can be achieved by augmenting the air-off period (Goronszy, 1994).

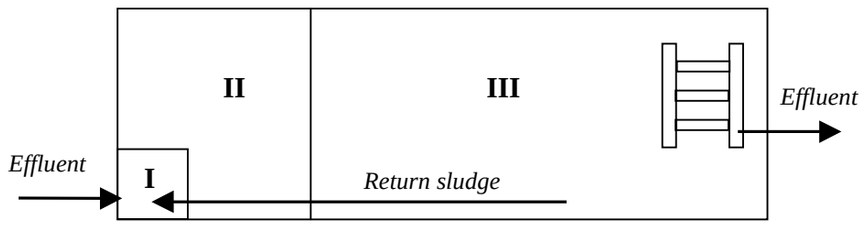

Figure 44 Schematic representation of the CASS^{TM} configuration (Goronszy, 1994) (Volume I: captive volume; volume II: secondary volume; volume III: oxidation zone)

3.4.5.3.5. *Submerged biofilm sequencing biofilm reactor (S-SBR)*

Whereas initially it was thought submerged biofilm reactors (SBR) were only efficient in organic and nitrogen removal, ammonia removal by nitrification in particular, Wang *et al.* (1998) were able to obtain good EBPR activity using an S-SBR.

3.4.5.4. ***Advantages and disadvantages of the different EBPR process configurations***

In the above paragraphs different process configurations have been elaborated on. Many different process configurations have been proposed by researchers and engineering companies. Although it is not easy to find true comparisons between these configuration in literature, based on pilot or full scale applications, some general advantages and disadvantages of the different configurations are listed below (Table 11).

Table 11 Advantages and disadvantages of the different EBPR process configurations (after Metcalf and Eddy, 1991)

Process	Advantages	Disadvantages
Phostrip	• Can be easily incorporated into existing systems • Process flexibility • Phosphorus removal does not depend on the BOD/P ratio	• Due to necessity of adding chemicals, the process is not sustainable • An additional reactor is needed for stripping • Lime scaling may be a maintenance problem
Bardenpho^{TM}	• Produces least sludge of all EBPR configurations • The wasted sludge has a high phosphorus content, thus has a fertiliser value	• Large internal cycle increases pumping energy and maintenance requirements • High BOD/P ratios are required
A^2/O	• The wasted sludge has a high phosphorus content, thus has a fertiliser value.	•
UCT	• Improved EBPR activity in combination with nitrification	• Large internal cycle increases pumping energy and maintenance requirements
VIP^{TM}	• Allows of high rate operation in comparison to UCT configuration	• Large internal cycle increases pumping energy and maintenance requirements
Cyclic process	• Processes are simple to operate • Process flexibility • No wash out of sludge by hydraulic surges	• To allow for continuous operation several parallel systems need to be operated.

It is stressed that many of the configurations are proprietary processes and each of the companies or research groups will mainly promote their own configuration. In general it can be said that, although cyclic process design necessitate more reactor volume to allow for continuous operation, these process configurations have a higher flexibility and controllability, allowing for more rapid adjustment to changing influent configurations.

3.4.6. Sludge handling and disposal

One of the drawbacks of biological phosphorus removal is that the phosphorus has accumulated into aerobic/anoxic sludge. This is susceptible to possible phosphorus release during anaerobic periods at any stage before effective disposal, e.g. in the thickener and the anaerobic sludge digester. Moreover, as digested sludge contains a high concentration of ammoniac nitrogen it is likely that struvite (magnesium ammonium phosphate) may be formed if the magnesium concentration is sufficiently high. Struvite formation can cause blockage of pumps and tubing. These problems can be overcome in the short term by dissolving the struvite in 10% sulphuric acid (Williams, 1998).

In Japan, limited landfill capacity and stringent regulations governing agricultural re-use of sewage waste have combined to make the incineration of sewage sludge the preferred method of disposal in many cases. Accordingly, this has presented the challenge of recovering phosphorus (3.6) vales from the incinerator ash, and laboratory scale work at Chuo University has shown this to be feasible (Morse *et al.*, 1998).

The phenomenon of phosphorus release could be used advantageously when organic waste sludge is spread on agricultural land. Indeed, phosphates present in the sewage sludge are in the organic polyphosphate form useful to agriculture as a slow release fertiliser (CEEP, 1998b). However, quantities of sewage sludge can considerably exceed the nutrient needs of local farm land. Moreover, farmers can refuse to use this sludge because of odour problems, or because the sludge contains too high concentration of pollutants, e.g. heavy metals, pathogens, …

3.5. *CHEMICAL OR BIOLOGICAL P-REMOVAL?*

Chemical removal systems have been in existence in Europe for many years longer than biological systems and this affects not only the current distribution of systems adopted in the EU, but also influences the further developments. Indeed, Member States tend to prefer systems with which they are already familiar (SCOPE, 1998).

In The Netherlands 80% of phosphorus removal was achieved in 1996 by chemical precipitation and 20% by biological nutrient removal (Bentvelsen, 1999). Germany has about 900 treatment plants with phosphorus removal, however, only 13% of these involve EBPR. In Sweden most phosphorus removal plants are either chemical treatment plants or combined systems. Denmark tends to have an increasing number of biological phosphorus removal systems, but here too usually combined with chemical precipitation. All new treatment plants in the United Kingdom are using chemical phosphorus removal (SCOPE, 1998).

Chemical processes produce around 5 to 7 kg of suspended solids per kilogram of precipitated phosphorus (SCOPE, 1998). The total sludge mass can increase by up to 50% and its volume by up to 150% depending on the chemicals used and the nature of the whole range of impurities present in the water-soluble phase (CEEP, 1998b). In pure EBPR processes only 3.4 kg of sludge is formed (SCOPE, 1998). Sludge treatment cost are thus higher for chemical processes. Moreover, despite the fact that costs of metal salts have fallen in recent years, chemical phosphorus removal costs are still at 6.1 €/kg P, compared to 2.4 €/kg P removed biologically and 3.5 €/kg P for combined biological + chemical removal. It is thus shown that the operating costs of biological removal are lower compared to chemical phosphorus removal (Bentvelsen, 1999) However, investment costs of chemical treatment plants are lower than biological treatment plants (SCOPE, 1998).

According to the Scientific Committee on Phosphates in Europe (SCOPE, 1998), it is unlikely that there will be any radical change in the types of systems preferred within the Member States, one of the reasons being the strict standards required by the urban wastewater treatment Directive. Opposite to this statement is the conclusion of an NVA conference (Bentvelsen, 1999), that because of sludge generation and salts discharges, chemical P-removal will be phased out over the coming 20-30 years and will be replaced with

biological systems. Now, already 40% of operators prefer biological systems, partly on grounds of sustainability (De Jong, 1999). Sustainability of biological phosphorus removal processes can further be improved by reductions in energy consumption (e.g. see 3.4.1.2), stricter discharge limits (effluent polishing) and the recovery of phosphates for recycling as calcium phosphates or struvite (Klapwijk, 1999). Since chemical phosphorus removal renders the phosphate impossible for re-use, this means an extra advantage for wider application of the biological phosphorus removal process.

3.6. *PHOSPHATE RECOVERY*

In the past a true phosphorus cycle existed, i.e. crops were consumed by animals and man close to their place of productions and the resulting animal and human manure, as well as crop wastes, were then applied to cultivated land, returning the nutrients to the soil. This traditional phosphorus cycle has been largely replaced by a linear throughput system, i.e. phosphates are extracted from a non-renewable resource (phosphate rock), pass through crops (via fertilisation of the soil), animals and man, and end up in landfill or in rivers and the see. As such only little is recycled to agricultural land (SCOPE, 1998).

Recovery of phosphate to a form applicable again in agriculture or in the non-fertiliser-phosphate industry could offer a possibility to obtain a sustainable use of phosphates again. Consequently, calcium phosphate, struvite (magnesium ammonium phosphate, $MgNH_4PO_4$ (MgAP)) and potassium struvite (potassium ammonium phosphate) recovery processes are already being operated on full scale (CEEP, 1998a). Although full scale application of calcium phosphate recovery, with well known applications as the DHV Crystalactor® (Giesen, 1998) and the Kurita process (Brett, 1997), the underlying processes are still not very well understood. Understanding of the dynamics could lead to improved ways of handling the reaction system (House, 1998). Necessary research for combined removal of heavy metals and phosphates from the sewage is pointed out.

The non-fertiliser-phosphate industry - metal and water products, flame retardants, corrosion inhibitors, paints, etc. - demand in general phosphate salts or phosphoric acid which has been substantially purified, but is equipped to handle heavy metal contaminants (CEEP, 1998a). However, currently industrial processes are incompatible to use struvite for the phosphate industry (CEEP, 1998a). Magnesium modifies the slag properties, thus furnace technology would have to be adapted for the thermal process route. For the wet acid process route magnesium would exclude the use of struvite as it does not crystallise out in the way calcium gypsum does (Brett, 1997). Moreover, ammonium would result in increased production of NO_X and N_2O. So, although struvite is an excellent material for removing phosphorus from waste water or sludge, its potential use in the non-fertiliser is not well known. Struvite is relatively insoluble in water, making it valuable as a slow release fertiliser with limited risks of leaching. Struvite is also citrate soluble rendering it a good nutrient source for plants (CEEP, 1998a). Gosh *et al.* (1996) indicated that struvite proved to be superior or equally effective as a source for phosphorus for gram plants compared to three commercial fertilisers tested.

Iron and aluminium, which are generally used for chemical precipitation of phosphates, are effective because they bind rapidly and tightly to the phosphates, rendering them insoluble. Unfortunately this "advantage" renders these phosphate precipitates poorly accessible for agricultural crops. Furthermore, application in industry is hampered too because iron and aluminium reactants prevent phosphorus recovery by established methods and interfere with industrial purification methods (CEEP, 1998b). According to Strickland (1998) phosphorus recovery is only possible if biological phosphorus removal is carried out. According to the author practical possibilities for phosphorus recovery exist, but will depend for implementation on the economic value of the recovered material, and the need to achieve other objectives, such as widespread implementation of BNR, and possibly low phosphate sludges. All options require further investigation, and may be site specific.

According to Woods *et al.* (1998) the economic incentives for implementing phosphorus recovery in the United States are minimal under current operating scenarios.

- The value of the recovered phosphorus product is insignificant relative to the cost of chemicals required for recovery and the capital cost of the facilities.
- Reductions in sludge handling costs do not provide for an attractive payback of the capital investment associated with implementing phosphorus recovery. Phosphorus recovery may be more cost-effective at locations where very high sludge handling costs are incurred.
- The economic boundary condition evaluation showed that phosphorus recovery becomes economically viable only at high influent concentrations and/or high sludge handling costs.

Sludge obtained from an EBPR system, containing on average 7-10% P on a dry weight basis, is typically dried and then incinerated at 670°C. It was found that the phosphorus contained in the incinerator ash could be leached with water and precipitated using ferric chloride to yield a mixture of ortho- and poly-phosphates, depending on the precise temperature (in the range 30-55°C) at which precipitation takes place. Although incineration is a potentially high-cost disposal method for sewage sludge, such a phosphate recovery method may prove to be of interest where no alternative to sludge incineration can be considered (Morse *et al.*, 1998).

In the year 2000, phosphate recovery is an emerging technology and still at an experimental stage. It is, however, noticed that the phosphate industry is actively supporting recovery for re-use (SCOPE, 1999). It will be important to closely follow up these trends to tune future wastewater treatment applications.

4. MATHEMATICAL MODELLING OF THE EBPR PROCESS

4.1. *MODELLING AS AN INHERENT PART OF THE DESIGN OF A WWTP*

Whenever researchers or engineers are confronted with the challenge of designing new systems, different reactor designs can be invented, based on experimental observation and, if existing, based on prior, partial, theoretical knowledge of the process. Looking back at the development of EBPR processes, it is noted that the original observation by Shapiro (1965) led to the Phostrip-configuration, a concept based on insufficient information, but EBPR activity was achieved. As such the conceptual model, derived from experimental observations at that time gave rise to a physical model, determined by the image gained from the conceptual model. Gaining more experimental and theoretical knowledge, researchers expanded their knowledge and thus the conceptual model. Several EBPR configurations were presented (3.4.5), tested on lab-scale or pilot plants. Meanwhile, researchers were confronted with time and money shortages, limiting the number of physical models that could be tested. As such, information gained form the conceptual and physical models was gathered in mechanistic and empirical mathematical models to facilitate the evaluation of possible EBPR configurations.

In Figure 45 the different model concepts possible for designing a reactor configuration are presented. When presented in this form, it becomes obvious that modelling in general is an inherent part also of the design of a WWTP, regardless the approach used. Both conceptual and physical models, although having their limitations, prove to be important for the development of a mathematical model to define the feasible design space. A mathematical model can be defined as a model where equations of various types are defined to relate inputs, outputs and characteristics of a system (Jeppsson, 1996). Mathematical models can be derived from physical models, leading to empirical models (i.e. models based on experimental experience) that incorporate a statistical approach to mimic the end results obtained by studies on the physical model. If the conceptual knowledge expands sufficiently, mechanistical models can be formulated that are more powerful because they allow for extrapolation of the design space.

The mathematical models obtained can be considered as heuristic models, i.e. models serving to find out or discover new information, thus continuously improving the models. The mathematical models thus are not static but dynamic and evolve in time with knowledge gained.

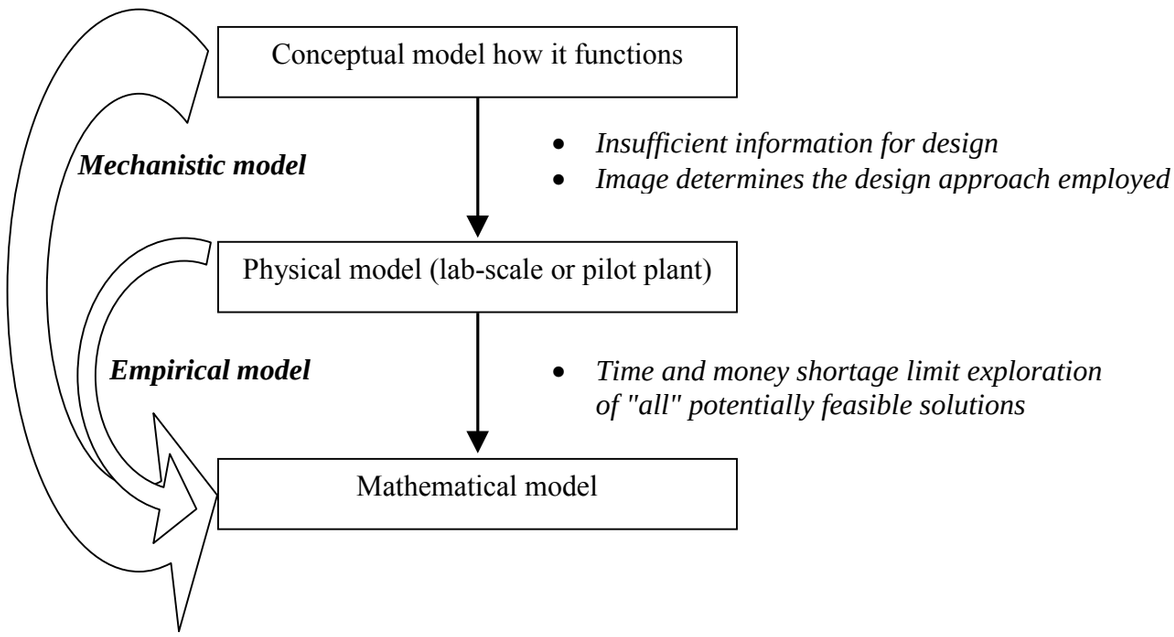

Figure 45 Different model concepts possible for the design of a reactor configuration

Mathematical modelling serves both the scientist and the engineer, gaining knowledge on the basic processes involved in EBPR and improving the EBPR activity in full scale plants. However, scientists and engineers might use different mathematical models, i.e. models with a different level of complexity. Whereas for scientists the model should comprise enough complexity to describe the processes according to the state-of-the-art knowledge, engineers need a mathematical model simple enough, but having the capability of realistic predictions. Indeed, mathematical models often comprise so many reactions that their complexity reaches far beyond the understanding of the practical users, although on the other hand the engineer using these models to design treatment plants has the most to gain from the mathematical models. Mathematical models should thus be subdivided in models applicable for a broad range of operating conditions but not too complex and expert models for the experienced scientist or engineer.

In general mathematical modelling of WWT processes is necessary:

- *to facilitate the design and operation of WWTP, i.e. to explore, through simulation, a very broad range of system configurations, inputs and operating conditions*
- *to predict the impact of changing wastewater characteristics (flow rate, loads, concentrations, …) and to propose new operating conditions*
- *for the development of control strategies by investigating the system response to a wide range of inputs without endangering the actual plant*
- *as a means of performance analysis, e.g. to evaluate the impact of new effluent requirements on plant design and operating cost*
- *to sensitise operators by indicating to them the influence of changed operating conditions*
- *as an essential part of research to expand the knowledge base and to pinpoint the need for more research, which in turn will lead to a new model based on the experience gained*

Initially, modelling efforts focussed on carbon removal and nitrifying-denitrifying systems. With the study of EBPR since 1976 by Barnard in South Africa, this process needed to be modelled too. However, initial conceptualisation extended little beyond the recognition of the necessity of an anaerobic/aerobic sequence and the adverse effects of nitrate entering the anaerobic phase. As long as the mechanism underlying the biological phosphorus removal remained unclear, only dynamic models for the EBPR process were developed that could predict the rates of phosphorus uptake and acetate uptake without a proper validation of the underlying biochemical reactions. It would take until 1986 for the first biochemistry based mathematical model of EBPR to be developed (Wentzel *et al.*, 1986, 1989a and 1989b). The various models becoming available at that time, however, were little used, partly due to a lack of trust in their predictions, partly due to limitations in computer power and partly due to the complicated way in which these models had to be presented in written form (Henze *et al.*, 2000).

4.2. *STANDARD MODEL FOR ACTIVATED SLUDGE PROCESSES*

In 1982 the International Association on Water Pollution Research and Control (IAWPRC) (later called International Association on Water Quality, IAWQ, and since 2000 called International Water Association, IWA) established a Task group on Mathematical Modelling for Design and Operation of Activated Sludge Processes. A matrix notation, based on the work of Petersen (1965) for chemical reactions, was introduced for activated sludge models together with the first Activated Sludge Model (ASM1) and became a common way of presenting models since then. This representation and the common nomenclature used, facilitated the communication of complex models and allowed the focusing of discussions on essential aspects of biokinetic modelling (Henze *et al.*, 2000). However, most unfortunate, researchers still kept using their own nomenclature and other units (molar units), rendering comparison difficult again.

The Activated Sludge Model No 1 (ASM1) was developed for biological carbon and nitrogen removal by activated sludge systems. Although at the time of its development and final release in 1987, biological phosphorus removal had already been studied extensively (Ekama *et al.*, 1984b; Wentzel *et al.*, 1985), the theoretical status was such that it was not considered ready for inclusion in a standard model. Because of the increasing popularity of and the insights in the EBPR process since the mid-1980s, the next version of the activated sludge model published in 1994, i.e. ASM2 (Henze *et al.*, 1995), did take into account the biological phosphorus removal as well. At the same time a simple model for chemical phosphorus removal was included too. It took again till 1999 before the status of understanding was such that it was warranted to extend this model further with denitrifying phosphorus removal. This model is referred to as ASM2d (Henze *et al.*, 1999). The development of these models didn't cease since then and in 1999 the ASM3 (Gujer *et al.*, 1999, Henze *et al.,* 2000) was released. This model takes into account further developed knowledge on internal storage compounds, which play an important role in the metabolism of the organisms. However, this model doesn't comprise biological phosphorus removal. New developments are soon expected to be published. Therefore, in the following section most attention is focussed on the ASM2 and ASM2d. Because of the importance of ASM2d for future reference this model is completely presented in Table 14 through Table 18.

4.2.1. Matrix structure

To improve transparency and easy comparison of different models, the task group suggested the matrix structure introduced by Petersen (1965) for model representation. This section is initiated with a general explanation, immediately referring to the tables used for ASM2d (Table 14 through Table 18). Due to this splitting in different tables, the initial "one matrix" notation is somewhat lost. However, by means of an example (Table 12) a simplified reaction set will be used in which the initial matrix structure is presented.

In the AS Models, all processes occurring in wastewater treatment plants are essentially brought back to N (transformation) processes, mainly subdivided according to the responsible micro-organisms (apart

from the hydrolysis reactions), and *M* components (sometimes called compounds in literature), the latter again subdivided in soluble and particulate components. The models quantify both the kinetics, i.e. the dependency of reaction rates on the concentrations of the components in the system (i.e. the state variables or the dependent variables), and the stoichiometry, i.e. the relation one component has to another in a reaction.

If the processes are characterised with index *j* and the components with the index *i*, the stoichiometric coefficients in the stoichiometric matrix (Table 12, Table 17) are presented as $\nu_{j,i}$. The process rates presented are characterised as ρ_j (Table 12, Table 14). As such, the net rate of conversion of the component *i*, r_i, in all parallel processes can be computed from the sum:

$$r_i = \sum \upsilon_{j,i} . \rho_j \quad \text{over all processes j} \qquad \textit{Equation 1}$$

Within the stoichiometric matrix one stoichiometric coefficient, $\nu_{j,k}$ of each process *j* may be chosen as dimensionless with the value +1 or -1. For all other stoichiometric coefficients algebraic equations may be given. Alternatively $\nu_{j,i}$ may be given in the form of absolute values with the dimension $M_i\ M_k^{-1}$ where M_k is the unit mass of the component *k* upon which stoichiometry is based (component which has $\nu_{j,k}$ = +1 or -1) (Henze *et al.*, 1999).

4.2.2. Conservation equations

The conservation equations (Henze *et al.*, 1987) are the mathematical equivalent of the principle that in chemical reactions, elements, electrons (or COD) and net charges may neither be formed nor destroyed. ASM2d is implicitly based on four conservation equations considering COD, electrical charges, nitrogen and phosphorus. Further, an equation is introduced which converts the different solid components $X_?$ from their unit of measurement to total suspended solids, X_{TSS}. The conversion factor to convert the units of component *i* to the units of the material *c*, to which conservation is to be applied, is characterised as $i_{c,i}$. A conservation reaction, valid for all processes *j* and all materials *c* subject to conservation, is written as:

$$\sum \upsilon_{j,i} . i_{c,i} = 0 \quad \text{over all components i} \qquad \textit{Equation 2}$$

4.2.3. Example for the use of the matrix structure and the conservation equations

The matrix structure and the conservation equations explained above are clarified by means of a simple example. Therefore a Monod-Herbert model is considered, quantifying the growth of the biomass component X_B at the expense of soluble substrate, S_S, accompanied by organism death (see 4.3.1.3 for more clarification on "the death" of organisms considered in the activated sludge models).

Continuity

mass balance

Table 12 Example of matrix structure (after Billing and Dold, 1988)

	Component *i* →	1	2	3	Rate expressions (ρ_j)
j	Process	X_B	S_S	S_O	
1	Growth	1 ($\nu_{1,1}$)	- 1/Y ($\nu_{1,2}$)	- (1-Y)/Y ($\nu_{1,3}$)	$\mu . X_B . \frac{S_S}{K_S + S_S}$ ρ_1
2	Decay	-1 ($\nu_{2,1}$)	($\nu_{2,2}$)	-1 ($\nu_{2,3}$)	$b\ X_B$ ρ_2
Observed conversion rates ($ML^{-3}T^{-1}$)		$r_i = \sum \upsilon_{j,i} . \rho_j$			Kinetic parameters: • Max. specific growth rate, μ • Half saturation constant, K_S • Specific decay rate, b
Stoichiometric parameters: • True growth yield, Y		Biomass $M(COD)L^{-3}$	Substrate $M(COD)L^{-3}$	Oxygen (negative COD) $M(COD)L^{-3}$	

The sign convention within the table is negative for consumption and positive for production.

In Table 12 the matrix structure is presented. Rate equations for both processes are presented on the right hand side, along with the kinetic parameters on the bottom right hand. For each reaction the stoichiometric parameters for each component involved in the reaction are given in the core of the table. The values for the kinetic and stoichiometric parameters are not mentioned within the table. For more complex models (see below) the stoichiometric matrix and the process rate equations are written down in separate tables.

The Monod equation ρ_1 expresses that growth of biomass is proportional to the biomass in a first order manner and to the substrate concentration in a mixed order manner. The rate of production of biomass X_B is obtained by summing the products of the stoichiometric coefficients $\nu_{j,i}$, times the process rate expressions ρ_j for the component *i* being considered, i.e. moving down along the matrix for the specific component X_B (Equation 1, Table 12):

$$r_{X_B} = \mu . \frac{S_S}{K_S + S_S} . X_B - b . X_B$$

The conservation principle can be demonstrated by considering the decay process. Noteworthy is the fact that oxygen is negative COD, so that its coefficient needs to be multiplied by -1. All three components are expressed in the same unit, so in this example the conversion factors $i_{c,i}$ all equal one. All COD lost from the biomass because of decay is balanced by oxygen utilisation (Equation 2).

4.3. *MODEL DEVELOPMENTS FOR EBPR PROCESSES*

In the following paragraphs activated sludge models are worked out in more detail, with special emphasis on those models comprising biological phosphorus removal. The ASM2d is used as a reference basis in the discussion. Units and nomenclature used by the different authors have been altered to a general basis to allow easy comparison.

Several compute packages have been developed that include the ASMs. Examples of such packages are GPS-X, Aquasim, Asim, Simba, Efor, West and Biowin. It is beyond the scope of this study to present an evaluation of these computer programmes. Despite all modelling efforts and available software, published results of applications of these models on full scale plants with EBPR are still scarce.

4.3.1. ASM2d

4.3.1.1. ***Model components***

For the development of ASM1, the task group based its notation on recommendations put forward by a previous task group (Grau *et al.*, 1982). Thus, since the beginning, the recommended symbol notation of the mathematical models has been X for particulate matter and S for soluble material. In general particulate components are assumed to be associated with the activated sludge (Henze *et al.*, 1995), i.e. particulate material by nature or components flocculated or adsorbed onto the floc. Despite the convention on symbol notation, the kinetic model for PAOs developed by Wentzel *et al.* (1989b) and used as a basis for ASM2d, was presented using different notations. These have been altered for introduction in ASM2d.

In Table 13 the model components used in ASM2d, are presented. Since ASM1 some changes and introduction of new components have occurred. For the development of ASM3 (see 4.3.2) again additional components were defined. Where possible reference is made to these changes to allow easy comparison.

In the models biodegradable organic matter is divided in two fractions (Billing and Dold, 1988)

- Readily biodegradable material (RBCOD), modelled as soluble material (S_S in ASM1, S_F since ASM2d)
- Slowly biodegradable material (SBCOD), modelled as particulate material (X_S). It is known that a fraction of the slowly biodegradable material is present as soluble material.

Table 13 Definition of model components in ASM2d (Henze et al., 1999)

Soluble components			***Particulate components***		
S_{O2}	Dissolved oxygen	g O_2/m^3	X_I	Inert, non-biodegradable organics	g COD/m^3
S_F	Readily biodegradable substrate (RBCOD)	g COD/m^3	X_S	Slowly biodegradable substrate (SBCOD)	g COD/m^3
S_A	Fermentation products (acetate)	g COD/m^3	X_H	Heterotrophic biomass	g COD/m^3
S_I	Inert, non-biodegradable organics	g COD/m^3	X_{PAO}	Phosphorus accumulating organisms	g COD/m^3
S_{NH4}	Ammonium	g N/m^3	X_{PP}	Stored poly-phosphate of PAO	g P/m^3
S_{N2}	Di-nitrogen (N_2)	g N/m^3	X_{PHA}	Organic storage products of PAO	g COD/m^3
S_{NO3}	Nitrate (plus nitrite)	g N/m^3	X_A	Autotrophic, nitrifying biomass	g COD/m^3
S_{PO4}	Phosphate	g P/m^3	X_{TSS}	Particulate material	g TSS/m^3
S_{ALK}	Bicarbonate alkalinity	mol HCO_3^-/m^3	X_{MeOH}	Metal-hydroxides	g TSS/m^3
			X_{MeP}	Metal-phosphate	g TSS/m^3

For ASM1 only carbon and nitrogen removal were considered, therefore only heterotrophic (formerly $X_{B,H}$, now X_H) and autotrophic bacteria (formerly $X_{B,A}$, now X_A, also often X_{AUT}) were considered. With the introduction of biological phosphorus removal, not only a separate biomass was introduced, X_{PAO}, but also internal storage components had to be introduced, i.e. poly-phosphate (X_{PP}) and the lump sum of organic storage components as PHAs, glycogen a.o. (X_{PHA}). With the introduction of EBPR also a separate carbon source was defined, i.e. fermentation products considered to be acetate (S_A). This component was introduced based on the accepted knowledge of SCFAs as major carbon source for PAOs.

4.3.1.2. ***Matrix notation for ASM2d***

In the following tables 14 to 18, the process rate equations, the stoichiometric matrix and typical values for the kinetic and stoichiometric parameters are presented. After the tables the reactions considered are elaborated on.

In this review the stoichiometric matrices (Table 17 and Table 18) are only provided for ASM2d. Because of the length of these tables, for the other models mentioned the reader has to consult the original papers. However, using the tables provided in this section, the stoichiometric matrices can easily be drawn based on the available information.

Table 14 Process rate equations for ASM2d (Henze et al., 1999)

Hydrolysis processes		
1	Aerobic hydrolysis	$K_h \cdot \frac{S_{O2}}{K_{LO2}+S_{O2}} \cdot \frac{X_S/X_H}{K_X+X_S/X_H} \cdot X_H$
2	Anoxic hydrolysis	$K_h \cdot \eta_{LNO3} \cdot \frac{K_{LO2}}{K_{LO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{LNO3}+S_{NO3}} \cdot \frac{X_S/X_H}{K_X+X_S/X_H} \cdot X_H$
3	Anaerobic hydrolysis	$K_h \cdot \eta_{fe} \cdot \frac{K_{LO2}}{K_{LO2}+S_{O2}} \cdot \frac{K_{LNO3}}{K_{LNO3}+S_{NO3}} \cdot \frac{X_S/X_H}{K_X+X_S/X_H} \cdot X_H$
Heterotrophic organisms: X_H		
4	Aerobic growth on ferm. substr, S_F	$\mu_H \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_F}{S_F+S_A} \cdot \frac{S_{NH4}}{K_{HNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{HP}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot X_H$
5	Aerobic growth on ferm. prod., S_A	$\mu_H \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_A}{K_{HA}+S_A} \cdot \frac{S_A}{S_F+S_A} \cdot \frac{S_{NH4}}{K_{HNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{HP}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot X_H$
6	Denitrif. with ferm. substr., S_F	$\rho_6 = \rho_4 \cdot \eta_{HNO3} \cdot \frac{K_{HO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
7	Denitrif. with ferm. prod., S_A	$\rho_7 = \rho_5 \cdot \eta_{HNO3} \cdot \frac{K_{HO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
8	Fermentation	$q_{fe} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \cdot \frac{K_{HNO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{S_F}{K_{fe}+S_F} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot X_H$
9	Lysis	$b_H \cdot X_H$
Phosphorus accumulating organisms (PAO): X_{PAO}		
10	Storage of X_{PHA}	$q_{PHA} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot X_{PAO}$
11	Aerobic storage of X_{PP}	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$
12	Anoxic storage of X_{PP}	$\rho_{12} = \rho_{11} \cdot \eta_{PNO3} \cdot \frac{K_{PO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
13	Aerobic growth on X_{PHA}	$\mu_{PAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
14	Anoxic growth on X_{PHA}	$\rho_{14} = \rho_{13} \cdot \eta_{PNO3} \cdot \frac{K_{PO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
15	Lysis of X_{PAO}	$b_{PAO} \cdot X_{PAO} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}}$
16	Lysis of X_{PP}	$b_{PP} \cdot X_{PP} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}}$
17	Lysis of X_{PHA}	$b_{PHA} \cdot X_{PHA} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}}$
Nitrifying organisms (autotrophic organisms): X_A		
18	Aerobic growth of X_A	$\mu_A \cdot \frac{S_{O2}}{K_{NO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{NNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{NP}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{NALK}+S_{ALK}} \cdot X_A$
19	Lysis	$b_A \cdot X_A$
Simultaneous precipitation of phosphorus with ferric hydroxide $Fe(OH)_3$		
20	Precipitation	$k_{PRE} \cdot S_{PO_4} \cdot X_{MeOH}$
21	Redissolution	$k_{RED} \cdot X_{MeP} \cdot S_{ALK} / (K_{ALK}+S_{ALK})$

Table 15 Definition and typical values for the kinetic parameters of ASM2d (10°C ≤ T ≤ 20°C) (Henze et al., 1999)

Hydrolysis of particulate substrate: X_S				
K_h	p(24)	hydrolysis rate constant	3.00*exp(-0.04*(20-T))	d^{-1}
η_{LNO3}	p(25)	anoxic hydrolysis reduction factor	0.60	-
η_{fe}	p(26)	anaerobic hydrolysis reduction factor	0.40	-
K_{LO2}	p(27)	saturation/inhibition coefficient for oxygen	0.20	g O_2 m^{-3}
K_{LNO3}	p(28)	saturation/inhibition coefficient for nitrate	0.50	g N-NO_3 m^{-3}
K_X	p(29)	saturation coefficient for particulate COD	0.10	g COD-X_S g^{-1} COD-X_H
Heterotrophic organisms: X_H				
μ_H	p(30)	maximum growth rate on substrate	6.00*exp(-0.07*(20-T))	d^{-1}
q_{fe}	p(31)	maximum rate for fermentation	3.00*exp(-0.07*(20-T))	g COD-S_F g^{-1} COD-X_H d^{-1}
η_{HNO3}	p(32)	reduction factor for denitrification	0.80	-
b_H	p(33)	rate constant for lysis and decay	0.40*exp(-0.07*(20-T))	d^{-1}
K_{HO2}	p(34)	saturation/inhibition coefficient for oxygen	0.20	g O_2 m^{-3}
K_F	p(35)	saturation coefficient for growth on S_F	4.00	g COD m^{-3}
K_{fe}	p(36)	saturation coefficient for fermentation of S_F	4.00	g COD m^{-3}
K_{HA}	p(37)	saturation coefficient for growth on acetate S_A	4.00	g COD m^{-3}
K_{HNO3}	p(38)	saturation/inhibition coefficient for nitrate	0.50	g N-NO_3 m^{-3}
K_{HNH4}	p(39)	saturation coefficient for ammonium (nutrient)	0.05	g N-NH_4 m^{-3}
K_{HP}	p(40)	saturation coefficient for phosphate (nutrient)	0.01	g P m^{-3}
K_{HAL}	p(41)	saturation coefficient for alkalinity (HCO_3^-)	0.10	mol HCO_3^- m^{-3}
Phosphorus accumulating organisms: X_{PAO}				
q_{PHA}	p(42)	rate constant for storage of X_{PHA} (base X_{PP})	3.00*exp(-0.04*(20-T))	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for storage of X_{PP}	1.50*exp(-0.04*(20-T))	g X_{PP} g^{-1} X_{PAO} d^{-1}
μ_{PAO}	p(44)	maximum growth rate of PAO	1.00*exp(-0.04*(20-T))	d^{-1}
η_{PNO3}	p(45)	reduction factor for anoxic activity	0.60	-
b_{PAO}	p(46)	rate for lysis of X_{PAO}	0.2*exp(-0.07*(20-T))	d^{-1}
b_{PP}	p(47)	rate for lysis of X_{PP}	0.2*exp(-0.07*(20-T))	d^{-1}
b_{PHA}	p(48)	rate for lysis of X_{PHA}	0.2*exp(-0.07*(20-T))	d^{-1}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.20	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	4.00	g COD m^{-3}
K_{PNH4}	p(51)	saturation coefficient for ammonium (nutrient)	0.05	g N m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage	0.20	g P m^{-3}
K_{PN}	p(53)	saturation coefficient for phosphate (nutrient)	0.01	g P m^{-3}
K_{PALK}	p(54)	saturation coefficient for alkalinity (HCO_3^-)	0.10	mol HCO_3^- m^{-3}
K_{PP}	p(55)	saturation coefficient for poly-phosphate	0.01	g P-X_{PP} g^{-1} COD-X_{PAO}
K_{MAX}	p(56)	maximum ratio of X_{PP}/X_{PAO}	0.34	g P-X_{PP} g^{-1} COD-X_{PAO}
K_{IPP}	p(57)	inhibition coefficient for PP storage	0.02	g P-X_{PP} g^{-1} COD-X_{PAO}
K_{PHA}	p(58)	saturation coefficient for PHA	0.01	g COD-X_{PHA} g^{-1} COD-X_{PAO}
Nitrifying organisms (autotrophic organisms): X_A				
μ_A	p(59)	maximum growth rate of X_A	1.00*exp(-0.098*(20-T))	d^{-1}
b_A	p(60)	decay rate of X_A	0.15*exp(-0.098*(20-T))	d^{-1}
K_{NO2}	p(61)	saturation coefficient for oxygen	0.50	g O_2 m^{-3}
K_{NNH4}	p(62)	saturation coefficient for ammonium (substrate)	1.00	g N m^{-3}
K_{NAL}	p(63)	saturation coefficient for alkalinity (HCO_3^-)	0.50	mol HCO_3^- m^{-3}
K_{NP}	p(64)	saturation coefficient for phosphate (nutrient)	0.01	g P m^{-3}
Simultaneous precipitation of phosphorus with ferric hydroxide $Fe(OH)_3$				
k_{PRE}	p(65)	rate constant for P precipitation	1.00	m^3 g^{-1} $Fe(OH)_3$ d^{-1}
k_{RED}	p(66)	rate constant for redissolution	0.60	d^{-1}
K_{ALK}	p(67)	saturation coefficient for alkalinity	0.50	mol HCO_3^- m^{-3}

Table 16 Definition and typical values for the stoichiometric coefficients of ASM2d (Henze et al., 1999)

Typical conversion factors for conservation equation				
Nitrogen				
i_{NSI}	p(2)	N content of inert soluble COD S_I	0.01	g N g^{-1} COD
i_{NSF}	p(3)	N content of fermentable substrates S_F	0.03	g N g^{-1} COD
i_{NXI}	p(4)	N content of inert particulate COD X_I	0.02	g N g^{-1} COD
i_{NXS}	p(5)	N content of slowly biodegradable substrate X_S	0.04	g N g^{-1} COD
i_{NBM}	p(6)	N content of biomass, X_H, X_{PAO}, X_A	0.07	g N g^{-1} COD
Phosphorus				
i_{PSI}	p(7)	P content of inert soluble COD S_I	0.00	g P g^{-1} COD
i_{PSF}	p(8)	P content of fermentable substrates S_F	0.01	g P g^{-1} COD
i_{PXI}	p(9)	P content of inert particulate COD X_I	0.01	g P g^{-1} COD
i_{PXS}	p(10)	P content of slowly biodegradable substrate X_S	0.01	g P g^{-1} COD
i_{PBM}	p(11)	P content of biomass, X_H, X_{PAO}, X_A	0.02	g P g^{-1} COD
Total suspended solids				
i_{TSSXI}	p(12)	TSS to COD ratio for X_I	0.75	g TSS g^{-1} COD
i_{TSSXS}	p(13)	TSS to COD ratio for X_S	0.75	g TSS g^{-1} COD
i_{TSSBM}	p(14)	TSS to COD ratio for X_H , X_{PAO} , X_A	0.90	g TSS g^{-1} COD
Typical stoichiometric parameters				
Hydrolysis				
f_{SI}	p(15)	production of S_I in hydrolysis	0.00	g COD g^{-1} COD
Heterotrophic biomass: X_H				
Y_H	p(16)	yield coefficient	0.625	g COD g^{-1} COD
f_{XIH}	p(17)	fraction of inert COD generated in lysis	0.10	g COD g^{-1} COD
Phosphorus-accumulating organisms: X_{PAO}				
Y_{PAO}	p(18)	yield coefficient (biomass/PHA)	0.625	g COD g^{-1} COD
Y_{PO4}	p(19)	PP requirement (PO_4 release) per PHA stored	0.40	g P g^{-1} COD
Y_{PHA}	p(20)	PHA requirement for PP storage	0.20	g COD g^{-1} P
f_{XIP}	p(21)	fraction of inert COD generated in lysis	0.10	g COD g^{-1} COD
Nitrifying organisms: X_A				
Y_A	p(22)	yield of autotrophic biomass per NO_3-N	0.24	g COD g^{-1} N
f_{XIA}	p(23)	fraction of inert COD generated in lysis	0.10	g COD g^{-1} COD

Table 17 Stoichiometric matrix for ASM2d for soluble and particulate components (Henze et al., 1999)

Stoichiometric matrix for soluble components											
	Process component	S_{O2}	S_F	S_A	S_I	S_{NH4}	S_{N2}	S_{NO3}	S_{PO4}	S_{ALK}	
	Expressed as →	O_2	COD	COD	COD	N	N	N	P	mol	
Hydrolysis process											
1	Aerobic hydrolysis		1-f_{SI}		f_{SI}	$\nu_{1,NH4}$			$\nu_{1,PO4}$	$\nu_{1,ALK}$	
2	Anoxic hydrolysis		1-f_{SI}		f_{SI}	$\nu_{2,NH4}$			$\nu_{2,PO4}$	$\nu_{2,ALK}$	
3	Anaerobic hydrolysis		1-f_{SI}		f_{SI}	$\nu_{3,NH4}$			$\nu_{3,PO4}$	$\nu_{3,ALK}$	
Heterotrophic organisms: X_H											
4	Aerobic growth on ferm. substr, S_F	1-1/Y_H	-1/Y_H			$\nu_{4,NH4}$			$\nu_{4,PO4}$	$\nu_{4,ALK}$	
5	Aerobic growth on ferm. prod., S_A	1-1/Y_H		-1/Y_H		$\nu_{5,NH4}$			$\nu_{5,PO4}$	$\nu_{5,ALK}$	
6	Denitrif. with ferm. substr., S_F		-1/Y_H			$\nu_{6,NH4}$	$\frac{1-Y_H}{2.86.Y_H}$	$-\frac{1-Y_H}{2.86.Y_H}$	$\nu_{6,PO4}$	$\nu_{6,ALK}$	
7	Denitrif. with ferm. prod., S_A			-1/Y_H		$\nu_{7,NH4}$	$-\frac{1-Y_H}{2.86.Y_H}$	$-\frac{1-Y_H}{2.86.Y_H}$	$\nu_{7,PO4}$	$\nu_{7,ALK}$	
8	Fermentation		-1	1		$\nu_{8,NH4}$			$\nu_{8,PO4}$	$\nu_{8,ALK}$	
9	Lysis					$\nu_{9,NH4}$			$\nu_{9,PO4}$	$\nu_{9,ALK}$	

Stoichiometric matrix for soluble components (continued)											
	Process component	S_{O2}	S_F	S_A	S_I	S_{NH4}	S_{N2}	S_{NO3}	S_{PO4}	S_{ALK}	
	Expressed as →	O_2	COD	COD	COD	N	N	N	P	mol	
Phosphorus accumulating organisms: X_{PAO}											
10	Storage of X_{PHA}			-1					Y_{PO4}	$\nu_{10,ALK}$	
11	Aerobic storage of X_{PP}	$-Y_{PHA}$							-1	$\nu_{11,ALK}$	
12	Anoxic storage of X_{PP}						$-\nu_{12,NO3}$	$\nu_{12,NO3}$	-1	$\nu_{12,ALK}$	
13	Aerobic growth on X_{PHA}	$\nu_{13,O2}$				$\nu_{13,NH4}$			$-i_{PBM}$	$\nu_{13,ALK}$	
14	Anoxic growth on X_{PHA}					$\nu_{14,NH4}$	$-\nu_{14,NO3}$	$\nu_{14,NO3}$	$-i_{PBM}$	$\nu_{14,ALK}$	
15	Lysis of X_{PAO}					$\nu_{15,NH4}$			$\nu_{15,PO4}$	$\nu_{15,ALK}$	
16	Lysis of X_{PP}								1	$\nu_{16,ALK}$	
17	Lysis of X_{PHA}			1						$\nu_{17,ALK}$	
Nitrifying organisms (autotrophic organisms): X_A											
18	Aerobic growth of X_A	$-\frac{4.57-Y_A}{Y_A}$				$\nu_{18,NH4}$		$1/Y_a$	$-i_{PBM}$	$\nu_{18,ALK}$	
19	Lysis					$\nu_{19,NH4}$			$\nu_{19,PO4}$	$\nu_{19,ALK}$	
Simultaneous precipitation of phosphorus with ferric hydroxide $Fe(OH)_3$											
20	Precipitation								-1	$\nu_{20,ALK}$	
21	Redissolution								1	$\nu_{21,ALK}$	

Stoichiometric matrix for particulate components											
	Process component	X_I	X_S	X_H	X_{PAO}	X_{PP}	X_{PHA}	X_A	X_{TSS}	X_{MeOH}	X_{MeP}
	Expressed as →	COD	COD	COD	COD	P	COD	COD	TSS	TSS	TSS
Hydrolysis process											
1	Aerobic hydrolysis		-1						$\nu_{1,TSS}$		
2	Anoxic hydrolysis		-1						$\nu_{2,TSS}$		
3	Anaerobic hydrolysis		-1						$\nu_{3,TSS}$		
Heterotrophic organisms: X_H											
4	Aerobic growth on ferm. substr, S_F			1					$\nu_{4,TSS}$		
5	Aerobic growth on ferm. prod, S_A			1					$\nu_{5,TSS}$		
6	Denitrif. with ferm. substr., S_F			1					$\nu_{6,TSS}$		
7	Denitrif. with ferm. prod., S_A			1					$\nu_{7,TSS}$		
8	Fermentation								$\nu_{8,TSS}$		
9	Lysis	f_{XI}	1- f_{XI}	-1					$\nu_{9,TSS}$		
Phosphorus accumulating organisms: X_{PAO}											
10	Storage of X_{PHA}					$-Y_{PO4}$	1		$\nu_{10,TSS}$		
11	Aerobic storage of X_{PP}					1	$-Y_{PHA}$		$\nu_{11,TSS}$		
12	Anoxic storage of X_{PP}					1	$-Y_{PHA}$		$\nu_{12,TSS}$		
13	Aerobic growth on X_{PHA}				1		$-1/Y_{PAO}$		$\nu_{13,TSS}$		
14	Anoxic growth on X_{PHA}				1		$-1/Y_{PAO}$		$\nu_{14,TSS}$		
15	Lysis of X_{PAO}	f_{XI}	1- f_{XI}		-1				$\nu_{15,TSS}$		
16	Lysis of X_{PP}					-1			$\nu_{16,TSS}$		
17	Lysis of X_{PHA}						-1		$\nu_{17,TSS}$		
Nitrifying organisms (autotrophic organisms): X_A											
18	Aerobic growth of X_A							1	$\nu_{18,TSS}$		
19	Lysis	f_{XI}	1- f_{XI}					-1	$\nu_{19,TSS}$		
Simultaneous precipitation of phosphorus with ferric hydroxide $Fe(OH)_3$											
20	Precipitation								1.42	-3.45	4.87
21	Redissolution								-1.42	3.45	-4.87

Table 18 Example of a stoichiometric matrix for ASM2d for soluble and particulate components and for the precipitation process (Henze et al., 1999)

Stoichiometric matrix for soluble components											
	Process component	S_{O2}	S_F	S_A	S_I	S_{NH4}	S_{N2}	S_{NO3}	S_{PO4}	S_{ALK}	
	Expressed as →	O_2	COD	COD	COD	N	N	N	P	mol	
Hydrolysis process											
1	Aerobic hydrolysis		1.00			0.010				0.001	
2	Anoxic Hydrolysis		1.00			0.010				0.001	
3	Anaerobic Hydrolysis		1.00			0.010				0.001	
Heterotrophic organisms: X_H											
4	Aerob. growth on ferm. substr, S_F	-0.60	-1.60			-0.022			-0.004	-0.001	
5	Aerobic growth on ferm. prod, S_A	-0.60		-1.60		-0.070			-0.020	0.021	
6	Denitrif. with ferm. substr., S_F		-1.60			-0.022	0.21	-0.21	-0.004	0.014	
7	Denitrif. with ferm. prod., S_A			-1.60		-0.070	0.21	-0.21	-0.020	0.036	
8	Fermentation		-1.00	1.00		0.030			0.010	-0.014	
9	Lysis					0.031			0.010	0.002	
Phosphorus accumulating organisms: X_{PAO}											
10	Storage of X_{PHA}			-1.00					0.400	0.009	
11	Aerobic storage of X_{PP}	-0.20							-1.000	0.016	
12	Anoxic storage of X_{PP}						0.07	-0.07	-1.000	0.021	
13	Aerobic growth on X_{PHA}	-0.60				-0.070			-0.020	-0.004	
14	Anoxic growth on X_{PHA}					-0.070	0.21	-0.21	-0.020	0.011	
15	Lysis of X_{PAO}					0.031			0.010	0.002	
16	Lysis of X_{PP}								1.000	-0.016	
17	Lysis of X_{PHA}			1.00						-0.016	
Nitrifying organisms (autotrophic organisms): X_A											
18	Aerobic growth of X_A	-18.0				-4.240		4.17	-0.020	-0.600	
19	Lysis					0.031			0.010	0.002	
Simultaneous precipitation of phosphorus with ferric hydroxide $Fe(OH)_3$											
20	Precipitation								-1.000	0.048	
21	Redissolution								1.000	0.048	

Stoichiometric matrix for particulate components											
	Process component	X_I	X_S	X_H	X_{PAO}	X_{PP}	X_{PHA}	X_A	X_{TSS}	X_{MeOH}	X_{MeP}
	Expressed as →	COD	COD	COD	COD	P	COD	COD	TSS	TSS	TSS
Hydrolysis process											
1	Aerobic hydrolysis		-1.00						-0.75		
2	Anoxic hydrolysis		-1.00						-0.75		
3	Anaerobic hydrolysis		-1.00						-0.75		
Heterotrophic organisms: X_H											
4	Aerob. growth on ferm. substr, S_F			1.00					0.90		
5	Aerobic growth on ferm. prod, S_A			1.00					0.90		
6	Denitrif. with ferm. substr., S_F			1.00					0.90		
7	Denitrif. with ferm. prod., S_A			1.00					0.90		
8	Fermentation										
9	Lysis	0.10	0.90	-1.00					-0.15		
Phosphorus accumulating organisms: X_{PAO}											
10	Storage of X_{PHA}					-0.40	1.00		-0.69		
11	Aerobic storage of X_{PP}					1.00	-0.20		3.11		
12	Anoxic storage of X_{PP}					1.00	-0.20		3.11		
13	Aerobic growth on X_{PHA}				1.00		-1.60		-0.06		
14	Anoxic growth on X_{PHA}				1.00		-1.60		-0.06		
15	Lysis of X_{PAO}	0.10	0.90		-1.00				-0.15		
16	Lysis of X_{PP}					-1.00			-3.23		
17	Lysis of X_{PHA}						-1.00		-0.60		
Nitrifying organisms (autotrophic organisms): X_A											
18	Aerobic growth of X_A							1.00	0.90		
19	Lysis	0.10	0.90					-1.00	-0.15		
Simultaneous precipitation of phosphorus with ferric hydroxide $Fe(OH)_3$											
20	Precipitation								1.42	-3.45	4.87
21	Redissolution								-1.42	3.45	-4.87

4.3.1.3. ***Practical meaning of reactions defined in ASM2d***

- *Hydrolysis reactions*

The first set of reactions appearing in Table 14 for ASM2d are hydrolysis reactions (solubilisation reaction after Billing and Dold, 1988) comprising extracellular conversion of X_S to S_S with simultaneous production of a small fraction of soluble inert material. In ASM1 only aerobic and anoxic hydrolysis were considered, whereas since ASM2 anaerobic hydrolysis is also taken into account. The reactions are supposed to be surface reactions and are thus modelled on the basis of Levenspiel's surface reactions kinetics (Levenspiel, 1972). In the reaction rate equations the concentration of SBCOD is written as the ratio of two bulk concentrations, i.e. X_S/X_H.

$$K_h \cdot \eta_{LNO3} \left(\frac{K_{LO2}}{K_{LO2} + S_{O2}} \cdot \frac{S_{NO3}}{K_{LNO3} + S_{NO3}} \right) \frac{X_S / X_H}{K_X + X_S / X_H} \cdot X_H$$

Considering the rate equations for hydrolysis it is observed that all rates are dependent on oxygen and eventually on nitrate concentrations. These Monod-like terms are introduced to eliminate problems of numerical instability in simulating the system behaviour, and were referred to in initial works as switching functions (Billing and Dold, 1988). The Monod-like structure was chosen more for its mathematical convenience than for conformity to any fundamental rate laws.

Observed lower anoxic than aerobic and again lower anaerobic than anoxic hydrolysis rates are modelled by incorporating the reduction factors η_{LNO3} and η_{fe}.

- *Reactions related to heterotrophic organisms*

The second block within the matrix structure in Table 14 comprises the heterotrophic organisms. They are assumed to be "all rounder" heterotrophic organisms. They are supposed to grow aerobically and anoxically and to be active under anaerobic condition for fermentation of RBCOD to acetate. Endogenous mass loss of heterotrophs is modelled according the death-regeneration concept introduced by Dold *et al.* (1980) (see 4.3.2) and is denoted as lysis of biomass. The hydrolysis reactions mentioned above are considered to be performed by heterotrophic organisms as well (Henze *et al.*, 1995). Growth of heterotrophic organisms under anaerobic conditions is not considered.

- *Reactions related to PAOs*

The following block accounts for the processes occurring in PAOs. The kinetic model developed by Wentzel *et al.* (1989b), formed the basis of this incorporation of EBPR in the former ASM1 model. The organisms are supposed to store fermentation products as cell internal storage products, including PHA, glycogen, etc. This storage capacity is only present for PAOs and can proceed under all conditions, i.e. anaerobically, anoxically and aerobically (Henze *et al.*, 1995). The storage process is accounted for as a surface reaction. Possible direct storage of SBCOD is not considered. Under practical conditions it is supposed that no fermentation products are available under aerobic conditions, and when they should become available it is assumed that the maximum growth rate on fermentation products by heterotrophic organisms is higher. Moreover, the maximum growth rate on fermentable products by heterotrophic organisms is supposed to be higher than the fermentation rate. So, to conclude, although the aerobic storage of fermentation products as PHA by PAOs is included, it is not likely to occur. Wentzel *et al.* (1989b) used a switching function, only to account for anaerobic storage of PHA. Where ASM2 only comprised aerobic growth of PAOs (denitrifying capacity of PAOs was not observed by Wentzel *et al.* (1989b), denitrifying capacity of PAOs (3.4.1.2) is taken into account for ASM2d. In the model it is assumed that only a fraction of the PAOs, i.e. DPAOs, can grow under anoxic conditions. Therefore, a reduction factor, η_{LNO3}, is introduced. Aerobic/anoxic poly-P accumulation is also accounted for as a surface reaction. Endogenous mass loss of PAOs is modelled according to the death-regeneration concept introduced by Dold *et al.* (1980) (see 4.3.2) and denoted as lysis of biomass.

- *Reactions related to nitrifiers*

In ASM2d the block of reactions following the PAOs related reactions is the nitrifier block where aerobic growth of nitrifying organisms is considered. Endogenous mass loss of nitrifiers is modelled according the death-regeneration concept introduced by Dold *et al.* (1980, see 4.3.2) and denoted as lysis of biomass.

- *Chemical precipitation*

ASM2d also comprises precipitation and redissolution. The processes are introduced based on the assumption that X_{MeOH} and X_{MeP} are composed of ferric hydroxide and ferric phosphate respectively.

- *General remark concerning death/decay principles*

For all organisms endogenous mass loss of active biomass is accounted for in ASM2d. In ASM1 this process was called decay whereas with the introduction of PAOs and the simultaneous introduction of cell internal components, the process was called lysis rather than decay. The process is considered to follow the principle of death-regeneration, i.e. biomass is transformed to slowly biodegradable material (X_S) and inert material (X_I), the former used by active micro-organisms for growth of new biomass (after hydrolysis). In ASM1 a differentiation was even made between inert particulate material originating from the influent or inert particulate products arising from biomass decay (X_P), the latter inert to further attack by micro-organisms as modelled by Dold *et al.* (1980). With this decay no direct utilisation of oxygen is involved. However, when, after hydrolysis of SBCOD, the generated RBCOD is used by heterotrophic organisms for growth, oxygen will be used. In paragraph 4.3.2 more attention is focussed to the phenomenon of "decay/lysis".

4.3.1.4. *Relationship between PAO-reactions in ASM2d and the model proposed by Wentzel et al. (1989b)*

The kinetics and stoichiometry for PAOs in ASM2d are largely based on the kinetic model developed by Wentzel *et al.* (1989b). Since this initial work is often referred to, the essential differences not yet mentioned in the previous paragraph, are elaborated on below. To allow easy comparison the notation of ASM2d is used. Reactions similar in both models are given the same number. The model developed by Wentzel *et al.* (1989b) comprised 12 processes and 13 components, whereas ASM2d, not taking into account the anoxic reactions only comprises and 6 processes and 16 components (not taking into account precipitation and redissolution reactions and their components and not taking into account S_I since not produced or generated for PAO linked processes). Components not considered by Wentzel *et al.* (1989b) are alkalinity, di-nitrogen and total suspended material. Upon decay of PAOs endogenous mass is created according to Wentzel *et al.* (1989b), which can best be compared with particulate inert material in ASM2d. Upon decay of PAOs, so-called non-biodegradable soluble substrate, i.e. not biodegradable by PAOs but possibly biodegradable by other organisms, is formed too. This component can best be regarded as slowly biodegradable particulate substrate (X_S) used in ASM2d. In the Wentzel model, decay of PAOs is linked with production of degradable soluble organic nitrogen. This component is indirectly accounted for in ASM2d. Indeed, in the latter model, upon decay of organisms, X_S is produced containing nitrogen fraction. This fraction is liberated during hydrolysis.

The number of reactions taken into account by Wentzel *et al.* (1989b) is double the number implemented in ASM2d. Additional reactions taken into account by Wentzel *et al.* (1989b) are:

- Differentiation between *aerobic growth on X_{PHA} using ammonia or using nitrate as nitrogen source.*
- In the model, *aerobic growth* is directly linked with polyphosphate production, whereas in ASM2d, storage of polyphosphate is considered as a separate reaction.
- To account for possible external ortho-phosphate depletion, Wentzel *et al.* (1989b) introduced aerobic *growth using the internal polyphosphate pool as phosphorus source* for aerobic growth (with nitrate or ammonia as nitrogen source).

- Differentiation is made between ***aerobic and anaerobic decay***, both for PAOs and the internal storage components X_{PP} and X_{PHA}. This is different from ASM2d, as in the latter lysis is independent of the oxygen and/or nitrate concentration.
- As an additional reaction ***anaerobic cleavage of polyphosphate for anaerobic maintenance*** purposes is foreseen. It was later indicated by the authors (Wentzel *et al.*, 1991a) that the phosphorus release for anaerobic maintenance requirements is always small compared to phosphorus release for the anaerobic the PHA storage energy requirements.

As can be seen when comparing the reaction rates mentioned in Table 14 and Table 19, equations are very similar. One remark is to be made:

- For the polyphosphate limitation function in the ***storage of X_{PHA}*** Wentzel *et al.* (1989b) did not use a surface reaction as suggested in ASM2d, but a Monod-Herbert equation in polyphosphate (X_{PP}) is used.

For the stoichiometric and kinetic coefficients (Table 20 and Table 21), one remark is to be made:

- For the kinetic coefficients in the ***storage of X_{PHA}*** two phases are proposed with different uptake rates, according to their experimental observation. Practically they suggested phase I will be mostly encountered. The switching concentration between both phases is determined at 0.32 g P/g COD active biomass.

Table 19 Process rate equations for PAOs in the kinetic model of Wentzel et al. (1989b)

10	Storage of X_{PHA} (sequestration of S_A)	$q_{PHA} \cdot \frac{K_{PO2}}{K_{PO2}+S_{02}} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{X_{PP}}{K'_{PP}+X_{PP}} \cdot X_{PAO}$
13	Aerobic growth on X_{PHA} with S_{NH4}	$\mu_{PAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
13 bis	Aerobic growth on X_{PHA} with S_{NO3}	$\mu_{PAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{K_{PNH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{NO3}}{K_{PNO3}+S_{NO3}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
	Aerobic growth on X_{PHA} with S_{NH4} under S_{PO4} limitations	$\mu_{PAO}^{PO4\,lim} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{K_{PN}}{K_{PN}+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
	Aerobic growth on X_{PHA} with S_{NO3} under S_{PO4} limitations	$\mu_{PAO}^{PO4\,lim} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{K_{PNH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{NO3}}{K_{PNO3}+S_{NO3}} \cdot \frac{K_{PN}}{K_{PN}+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot X_{PAO}$
	Anaerobic cleavage of PP for maintenance	$b_{PP,cleav}^{Anaer} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} X_{PP}$
15	Aerobic decay of X_{PAO}	$b_{PAO}^{Aer} \cdot X_{PAO}$
16	Aerobic lysis of X_{PP}	$b_{PAO}^{Aer} \cdot X_{PP}$
17	Aerobic lysis of X_{PHA}	$b_{PAO}^{Aer} \cdot X_{PHA}$
	Anaerobic decay of X_{PAO}	$b_{PAO}^{Anaer} \cdot X_{PAO}$
	Anaerobic lysis of X_{PP}	$b_{PAO}^{Anaer} \cdot X_{PP}$
	Anaerobic lysis of X_{PHA}	$b_{PAO}^{Anaer} \cdot X_{PHA}$

Table 20 Typical values for the conversion factors and the stoichiometric coefficients defined by Wentzel et al. (1989b)

Typical conversion factors for conservation equations				
Nitrogen				
i_{NXI}	p(4)	N content of inert particulate COD X_I (endogenous)	0.07	g N g^{-1} COD-X_I
i_{NXS}	p(5)	N content of slowly biodegradable substrate X_S	0.07	g N g^{-1} COD-X_S
i_{NBM}	p(6)	N content of biomass X_{PAO}	0.07	g N g^{-1} COD-X_{PAO}
Phosphorus				
i_{PXI}	p(9)	P content of inert particulate COD X_I (endogenous)	0.02	g P g^{-1} COD-X_I
i_{PBM}	p(11)	P content of biomass X_{PAO}	0.02	g P g^{-1} COD-X_{PAO}
Typical stoichiometric parameters				
Phosphorus-accumulating organisms : X_{PAO}				
Y_{PAO}	p(18)	yield coefficient (biomass/PHA)	0.639	g COD g^{-1} COD
$Y_{PO4,1}$	p(19)	PP requirement (PO_4 release) per PHA stored for phase 1	0.48-0.55	g P g^{-1} COD
$Y_{PO4,2}$		PP requirement (PO_4 release) per PHA stored for phase 2	0.8 - 1.0	g P g^{-1} COD
Y_{PHA}	p(20)	PHA requirement for PP storage [1]	0.9 - 1.1	g COD g^{-1} P
f_{XIP}	p(21)	fraction of inert COD generated in decay (lysis)	0.25	g COD-X_I g^{-1} COD-X_{PAO}
f_{XSP}		fraction of X_S generated in decay (lysis)	0.20	g COD-X_S g^{-1} COD-X_{PAO}
f_{cv}		Ratio COD/VSS	1.42	g COD g^{-1} VSS

[1] Defined by Wentzel *et al.* (1989b) as the ratio of P uptake to COD stored.

Table 21 Typical values for the kinetic parameters defined by Wentzel et al. (1989b)

Phosphorus accumulating organisms: X_{PAO}				
$q_{PHA,1}$	p(42)	rate constant for storage of X_{PHA} for phase 1	6	g COD g^{-1} COD-X_{PAO} d^{-1}
$q_{PHA,2}$		rate constant for storage of X_{PHA} for phase 2	2.6	g COD g^{-1} COD-X_{PAO} d^{-1}
μ_{PAO}	p(44)	maximum growth rate of PAO	0.9 - 1.1	d^{-1}
$\mu_{PAO}^{PO4-lim}$		maximum growth rate of PAO under PO_4 limitation	0.42	d^{-1}
b_{PAO}^{Aer}	p(46)	aerobic rate for decay of X_{PAO}	0.03 - 0.04	d^{-1}
b_{PAO}^{Anaer}		anaerobic rate for decay of X_{PAO}	0.03	d^{-1}
$b_{PP,cleav}^{Anaer}$		anaerobic rate for cleavage of X_{PP} for maintenance	0.03	d^{-1}
K_{HNO3}	p(38)	saturation coefficient for nitrate	1.0	g N-NO_3 m^{-3}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.002	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate	1.0	g COD m^{-3}
K_{PNH4}	p(51)	saturation coefficient for ammonium (nutrient)	0.05	g N-NH_4 m^{-3}
K_{PN}	p(53)	saturation coefficient for phosphate (nutrient)	0.1	g P m^{-3}
K_{PHA}	p(58)	saturation coefficient for PHA	0.18	g COD-X_{PHA}^{-1} m^{-3} (*)
K'_{PP}		saturation coefficient for poly-phosphate	1.0	g P m^{-3}

(*) Unit mentioned by Wentzel *et al*. (1989b). However, taking into account the form of the equations, it is more likely the unit needs to be g COD-X_{PHA}^{-1} g^{-1} COD-X_{PAO}

4.3.1.5. *"First" general model for biological nutrient removal (Barker and Dold, 1997)*

After the publication of ASM1 (Henze *et al.*, 1997) and with the development of the kinetic model for biological phosphorus removal proposed by Wentzel *et al.* (1989b) and prior to the publication of ASM2 (Henze *et al.*, 1995) and ASM2d (Henze *et al.*, 1999), researchers developed own models (see 4.3.3) or combined the ideas formulated in ASM1 and by Wentzel *et al.* (1989b) in one general model. Additionally, Barker and Dold (1997) extended the Wentzel-model by incorporating denitrifying phosphorus removal. Since the publication of the Wentzel-model experimental evidence provided sufficient proof to allow incorporation of this reaction into a general model. As such, in 1997 Barker and Dold proposed a unified model for carbon, nitrogen and phosphorus removal. Differences between their model and ASM2d are listed below. The discussion is mainly focussed on the phosphorus related reactions.

Whereas in ASM1 and later ASM2d components are divided into particulate and soluble components, Barker and Dold (1997), following Wentzel *et al.* (1989b), defined biomass components and substrate concentrations. In Table 22 the model components taken into account by Barker and Dold (1997) are presented using the notation of ASM2d. The number of components (19) is higher than the number of model components considered in ASM2d (15, not considering precipitation, total particulate material and di-nitrogen gas). The observed differences are:

- Separate introduction of the *endogenous mass* of the micro-organisms. When the model is compared to ASM2d, this component corresponds to the inert, non-biodegradable component used in ASM2d. However, the authors state endogenous mass is not accounted for in ASM2d. On the other hand the authors state that the particulate non-biodegradable substrate is equivalent to the inert, non-biodegradable component used in ASM2d. This statement should be given some doubt. Moreover, particulate non-biodegradable substrate is not consumed nor produced in the Barker and Dold's model.
- The stored poly-phosphate content is divided in a fraction which can be released and a fixed fraction.
- Soluble and particulate organic nitrogen and non-biodegradable soluble nitrogen is accounted for.

Table 22 Model components defined by Barker and Dold (1997)

Soluble components			***Particulate components***		
S_{O2}	Dissolved oxygen	g O_2/m^3	$X_{I,1}$	Endogenous mass	g COD/m^3
S_F	Readily biodegradable substrate (RBCOD)	g COD/m^3	$X_{I,2}$	Particulate non-biodegradable matter	g COD/m^3
S_A	Fermentation products (acetate)	g COD/m^3	X_S	Slowly biodegradable substrate (SBCOD)[1]	g COD/m^3
S_I	Inert, non-biodegradable organics	g COD/m^3	X_H	Heterotrophic biomass	g COD/m^3
S_N	Soluble biodegradable organic nitrogen	g N/m^3	X_{PAO}	Phosphorus-accumulating organisms	g COD/m^3
S_{NH4}	Ammonium	g N/m^3	$X_{PP,1}$	Releasable stored poly-phosphate of PAO	g P/m^3
S_{NO3}	Nitrate (plus nitrite)	g N/m^3	$X_{PP,2}$	Fixed stored poly-phosphate of PAO	g P/m^3
S_{PO4}	Phosphate	g P/m^3	X_N	Particulate biodegrad. organic nitrogen	g N/m^3
S_{ALK}	Bicarbonate alkalinity	mol HCO_3^- /m^3	X_{PHA}	Organic storage products of PAO	g COD/m^3
			X_A	Autotrophic, nitrifying biomass	g COD/m^3

[1] The authors use the terminology *enmeshed slowly biodegradable substrate*.

Barker and Dold (1997) take into account 19 reactions related for PAOs, whereas Wentzel *et al.* (1989b) only considered 12 reactions. All reactions accounted for by Wentzel *et al.* (1997) are considered as defined by these authors. The 7 new reactions deal with the anoxic reactions not accounted for in the Wentzel-model and the introduction of the fixed part of the stored poly-phosphate which is released as soluble phosphate upon decay of the organisms. For modelling denitrification by PAOs, Barker and Dold (1997) assumed, as in ASM2d, that a fraction of the PAOs can use nitrate as an electron acceptor. In the

Wentzel-model, 4 aerobic growth processes are considered. However, not all reactions are duplicated for the anoxic stage as it is presumed growth is likely to occur in the presence of sufficient ammonia and sufficient soluble phosphorus. So, only that aerobic growth process was duplicated for the anoxic phase related reactions. In ASM2d lysis is considered in all phases, i.e. anaerobically, anoxically and aerobically lysis of all cell related components are considered. These reactions are not mentioned below as new reactions. The newly introduced reactions and additional typical values for the stoichiometric coefficients are listed in Table 23 and Table 24 respectively. It is observed that taking into account the anoxic reactions by Barker and Dold (1997) in the Wentzel-model, boils down to introduction of the anoxic growth on X_{PHA}, using the same kinetic expression as later used in ASM2d.

Table 23 Additional process rate equations for PAOs in the kinetic model of Barker and Dold (1997)

14	Anoxic growth on X_{PHA} with S_{NH4}	$\mu_{PAO} \cdot \eta_{PNO3} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{PNO3}+S_{NO3}} \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PAO}/X_{PAO}} \cdot X_{PAO}$
	Decay of $X_{PP,2}$	$b_{PP,2} \cdot X_{PP,2}$

Table 24 Additional typical values for the stoichiometric coefficients defined by Barker and Dold (1997)

Phosphorus-accumulating organisms : X_{PAO}				
Y_{PO4}	p(19)	PP requirement (PO_4 release) per PHA stored for phase 1	0.52	g P g^{-1} COD
f_{PP}		Fraction of P taken up which can be released	0.94	g Pg^{-1} P

4.3.1.6. ***Limitations and proposed modification for ASM2d***

According to the authors (Henze *et al.*, 2000), the more important limitations for ASM2d are:

- The model is only valid for municipal wastewater. Extending the model beyond these limits asks for at least the incorporation of specific rate equations to account for the additional reactions occurring (e.g. Ky (1999) and Ky *et al.* (2000), see 4.3.6).
- Processes with overflow of acetate to the aeration phase can not be modelled. Suggestion are proposed by Johansson (1994) and Brdjanovic (1998) (see 4.3.3 and 4.3.5.6 respectively).
- The wastewater must contain sufficient Mg^{2+} and K^{+}. Whereas under normal circumstances this condition is fulfilled, Ky (1999) and Ky *et al.* (2000) accounted for possible Mg-limitations (see 4.3.6).
- pH should be near neutral
- The temperature is expected to be in the range of 10-25°C.

Not mentioned by the authors, the possible competition between PAOs and GAOs should be accounted for. Manga *et al.* (2000) proposed a model to account for this competition (4.3.4).

More general, storage by non-PAOs is not accounted for in ASM2d. Carucci *et al.* (1998) proposed a lumped anaerobic yield factor Y'_H including both anaerobic growth and storage by non-PAO. The resulting mathematical simulation indicated that competition under anaerobic conditions of non-PAO heterotrophs against PAOs significantly affects the effluent phosphate concentration and can be a decisive factor for explaining EBPR. With the publication of ASM3 (Henze *et al.*, 2000) the storage concept has been introduced, however this model does not yet account for EBPR (see 4.3.2). Ky *et al.* (2000) proposed a combined model accounting for the storage phenomena considered in ASM3 and accounting for EBPR using a metabolic model (see 4.3.6)

4.3.2. ASM3: new insight on storage components involved in non-PAOs

Since the publication of ASM2d a revised version of ASM1 has been developed and published, as ASM3 (Gujer *et al.*, 1999 and Henze *et al.*, 2000). Although this model does not comprise biological phosphorus removal, important new features have been introduced which will influence later inclusion of EBPR activity in this model. Therefore, ASM3 is presented below. In Figure 46, a schematic presentation of the different reactions accounted for in ASM3 is provided. The model components considered and the rate equations are presented in Table 25 and Table 26 respectively.

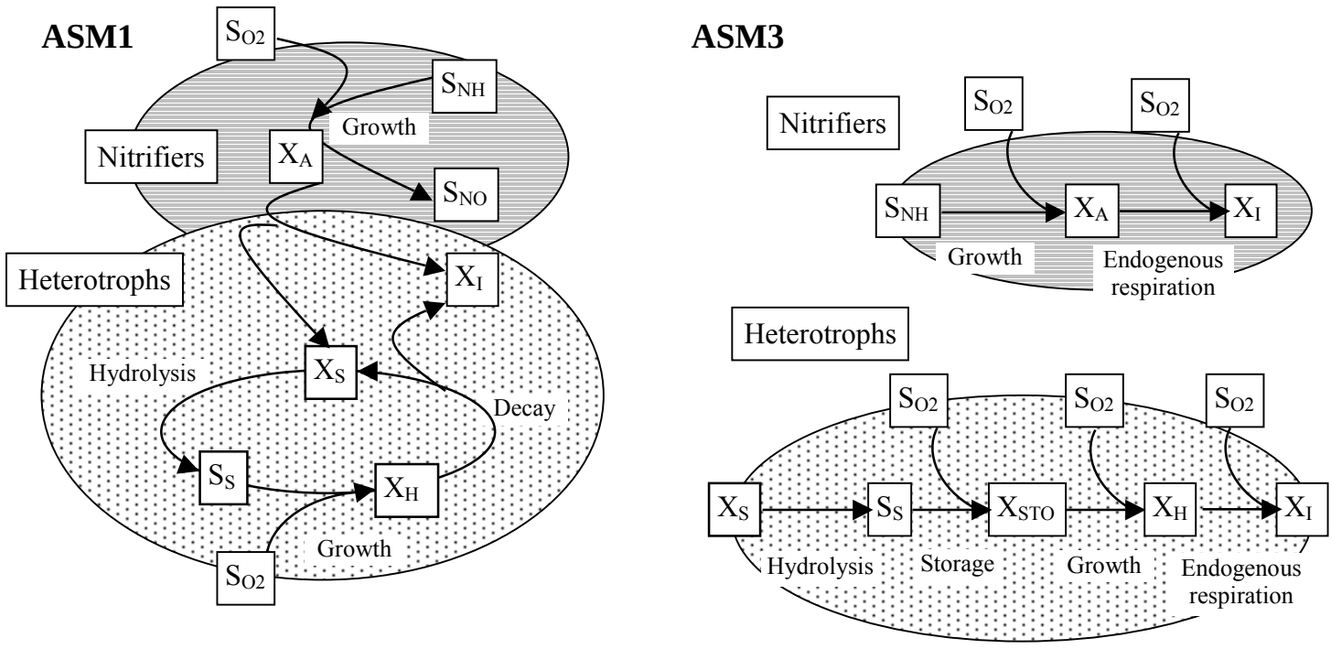

Figure 46 Flows of COD in ASM1 and ASM3 (Henze et al, 2000)

Shortcomings in ASM1 have been taken into account in ASM3 but are not discussed hereafter. However, the most important change is the introduction of storage components for all organisms, similar to internal storage components for PAOs in ASM2. With ASM3 a shift was made from hydrolysis to storage of organic substrates (Henze *et al*., 2000). This shift was caused by essentially two reasons. First, with the research on EBPR processes, much emphasis has been put on understanding the fate of internal storage components (van Loosdrecht and Henze, 1999).Also, new analysis methods for internal storage components allowed better insight in the "true" biochemical processes occurring. It soon appeared that not only PAOs are able to store carbon sources internally. Indeed, whereas this idea was already proposed much earlier (Dawes and Ribbons, 1964), only more recent analysis techniques allowed experimental verifications of the different hypothesis. Secondly, with the development of respirometric analysis techniques to determine the wastewater characteristics often high yield coefficients are obtained. Even if only soluble, readily biodegradable substrates such as acetate are added, it appeared from the respiration tests that this substrate would include a slowly biodegradable substrate. This observation, however, can easily be explained accounting for internal storage of carbon sources.

Experimentally it is observed that aerobic biomass also consumes oxygen in the absence of external substrate. Porges *et al.* (1953) concluded that cells oxidise their own cell material, denoted as ***endogenous respiration***, whereas Dawes and Ribbons (1964) proposed that micro-organisms contain intracellular reserve material which is used for ***maintenance*** purposes resulting in oxygen consumption in the absence of external substrate. Later, it was suggested that the observed endogenous respiration is due to ***decay of***

cells and subsequent consumption of these decayed cells to form new biomass, a concept referred to as cryptic growth. This assumption was mainly based on the observation of inert material accumulating in the sludge. It was thought this material originated from decay of biomass (Grady and Roper, 1974). Exactly this concept was introduced by Dold *et al.* (1980) as the death-regeneration concept and was incorporated in all prior ASMs. To make the concept even more complicated to non-microbiologists, Grady and Roper (1974) conceptualised the decay process to consist of decay and *lysis*, where decay is the process in which cell functions are halted and lysis is the process in which cell material is broken down to smaller pieces.

Table 25 Model components in ASM3 (Henze et al. 2000)

Soluble components			***Particulate components***		
S_{O2}	Dissolved oxygen	g O_2/m^3	X_I	Inert, non-biodegradable organics	g COD/m^3
S_F (*)	Readily biodegradable substrate (RBCOD)	g COD/m^3	X_S	Slowly biodegradable substrate (SBCOD)	g COD/m^3
S_I(**)	Inert, non-biodegradable organics	g COD/m^3	X_H	Heterotrophic biomass	g COD/m^3
S_{NH4}	Ammonium	g N/m^3	X_{STO}	Organics stored by heterotrophs	g COD/m^3
S_{N2}	Di-nitrogen (N_2)	g N/m^3	X_A	Autotrophic, nitrifying biomass	g COD/m^3
S_{NO3}	Nitrate (plus nitrite)	g N/m^3	X_{TSS}	Particulate material	g TSS/m^3
S_{ALK}	Bicarbonate alkalinity	mol HCO_3^-/m^3			

(*) Denoted by Henze *et al.* (2000) as S_S, for comparison the same notation as for ASM2d has been used here.

(**) Inert soluble material is still considered in the model. The stoichiometric parameter for the production of S_I, however, is considered to be zero (Table 27) but any value can be given. Especially in South Africa, production of S_I during hydrolysis is considered to be important (personal communication van Loosdrecht)

Table 26 Process rate equations for ASM3 (Henze et al., 2000)

Hydrolysis processes		
1-3	Hydrolysis	$K_h \cdot \frac{X_S/X_H}{K_X + X_S/X_H} \cdot X_H$
Heterotrophic organisms: X_H		
	Aerobic storage of S_F	$k_{STO} \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_F}{K_F+S_F} \cdot X_H$
	Anoxic storage of S_F	$k_{STO} \cdot \eta_{HNO3} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{S_F}{K_F+S_F} \cdot X_H$
	Aerobic growth on X_{STO}	$\mu_H \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{HNH4}+S_{NH4}} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot \frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H} \cdot X_H$
	Anoxic growth on X_{STO}	$\mu_H \cdot \eta_{HNO3} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{S_{NH4}}{K_{HNH4}+S_{NH4}} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot \frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H} \cdot X_H$
	Aerobic endogenous respiration	$b_{H,O2} \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot X_H$
	Anoxic endogenous respiration	$b_{H,NO3} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_H$
	Aerobic respiration of X_{STO}	$b_{STO,O2} \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot X_{STO}$
	Anoxic respiration of X_{STO}	$b_{STO,NO3} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{STO}$

Table 27 Typical values for the stoichiometric coefficients of ASM3 (Henze et al., 2000)

Typical stoichiometric parameters				
Hydrolysis				
f_{SI}	p(15)	production of S_I in hydrolysis	0.00	g COD-S_I g^{-1} COD-X_I
Heterotrophic biomass: X_H				
$Y_{STO,O2}$		aerobic yield of stored product per S_F	0.85	g COD-X_{STO} g^{-1} COD-S_F
$Y_{STO,O2}$		anoxic yield of stored product per S_F	0.80	g COD-X_{STO} g^{-1} COD-S_F
$Y_{H,O2}$	p(16)	aerobic yield coefficient of heterotrophic biomass	0.63	g COD-X_H g^{-1} COD-X_{STO}
$Y_{H,NO3}$		anoxic yield coefficient of heterotrophic biomass	0.54	g COD-X_H g^{-1} COD-X_{STO}
f_{XIH}	p(17)	fraction of inert COD generated in endogenous respiration	0.20	g COD-X_I g^{-1} COD-X_H

Table 28 Typical values for the kinetic parameters at 20°C of ASM3 (Henze et al., 2000)

Hydrolysis of particulate substrate: X_S				
K_h	p(24)	hydrolysis rate constant	3.00	g COD-X_S g^{-1} COD-X_H d^{-1}
K_X	p(29)	saturation coefficient for particulate COD	1.00	g COD-X_S g^{-1} COD-X_H
Heterotrophic organisms: X_H				
μ_H	p(30)	maximum growth rate on substrate	2.00	d^{-1}
η_{HNO3}	p(32)	reduction factor for denitrification	0.60	-
$b_{H,O2}$	p(33)	rate constant for aerobic endogenous respiration	0.20	d^{-1}
$b_{H,NO3}$		rate constant for anoxic endogenous respiration	0.10	d^{-1}
$b_{STO,O2}$		rate constant for aerobic respiration of X_{STO}	0.20	d^{-1}
$b_{STO,NO3}$		rate constant for anoxic respiration of X_{STO}	0.10	d^{-1}
K_{HO2}	p(34)	saturation/inhibition coefficient for oxygen	0.20	g O_2 m^{-3}
K_F	p(35)	saturation coefficient for growth on S_F	2.00	g COD-S_F m^{-3}
K_{HNO3}	p(38)	saturation/inhibition coefficient for nitrate	0.50	g N-NO_3 m^{-3}
K_{HNH4}	p(39)	saturation coefficient for ammonium (nutrient)	0.01	g N m^{-3}
K_{HALK}	p(41)	saturation coefficient for alkalinity (HCO_3^-)	0.10	mol HCO_3^- m^{-3}
k_{STO}		rate constant for storage of S_S	5.00	g COD-S_S g^{-1} COD-X_H d^{-1}
K_{STO}		saturation constant for X_{STO}	1	g COD-X_{STO} g^{-1} COD-X_H

Evaluating the process rate equation proposed in ASM3 (Table 26) the following observations can be made:

- Whereas the death-regeneration concept was taken into account for ASM1 through ASM2d, **ASM3** takes into account the *endogenous respiration* (decreasing biomass and production of inert material, X_I) and (endogenous) *respiration of internal storage components* (decreasing internal storage components but no decrease of biomass or production of inert material). However, this endogenous respiration concept only comprises anoxic and aerobic degradation of internal storage components. Anaerobic decrease of biomass or internal storage components is not accounted for. The endogenous respiration is supposed to account for all forms of biomass loss and energy requirements not associated with growth: decay, endogenous respiration, lysis, predation, motility, death and so on. Anoxic endogenous respiration is supposed to be slower, especially since protozoa are considered to be less active under denitrifying conditions.
- *Heterotrophic growth* is using internal storage components, i.e. directly using readily biodegradable material is not considered in ASM3 (Figure 46).
- *Anaerobically only hydrolysis* is considered. Compared to ASM2d, hydrolysis is supposed to occur at the same rate in all phases, i.e. anaerobic, anoxic or aerobic, in accordance with observations by Mino *et al.* (1994). Production of soluble inert material during hydrolysis is omitted.

- Whereas in ASM3 storage compounds are considered, *anaerobic storage of the carbon source* is not modelled. As such, although it is explicitly mentioned (Henze *et al.*, 2000) that PAOs are not yet considered in the model, also GAOs or other micro-organisms with the ability to anaerobically store carbon sources are not accounted for.

For typical values of the stoichiometric coefficients, the reader can refer to Table 16. In Table 27 only newly introduced coefficients are presented along with f_{XI}. The latter value changed because of the concept change from lysis to endogenous respiration.

Comparing Table 15 and Table 28 it can be seen that the maximum growth rate of heterotrophic biomass on storage components is modelled having a much lower maximum growth rate, i.e. 2 d^{-1} instead of 6 d^{-1} when considering growth on fermentable substrate. Comparing Table 16 and Table 27, it is noticed that production of inert material during endogenous respiration is supposed to be higher compared to production of inert material during lysis of heterotrophic biomass. Since biomass now partly consists of storage material and partly of "true" biomass, and since the internal storage component is supposed to create no inert material during (endogenous) respiration, this difference is easily understood.

For PAOs, the problem of decay was already addressed by Wentzel *et al.* (1985) who stated with the publication of their model that there is substantial evidence that PAOs are not, or only insignificantly, predated in mixed culture, and thus suffer endogenous mass loss only through maintenance. Wentzel *et al.* (1985) also stated that the endogenous mass loss rate for PAOs is far lower than for non-PAOs. This statement was put forward to explain observed ortho-phosphate uptake and release at different sludge ages. A specific endogenous mass loss rate of 0.03 - 0.04 d^{-1} is used in their model (Wentzel *et al.*, 1989; Table 21). The rate for lysis of X_{PAO} in ASM2d was taken to be 0.2 d^{-1} (20°C) whereas the rate constant for lysis of non-PAO heterotrophic biomass was taken to be 0.4 d^{-1} (20°C) (Table 15). In ASM3 the rate constant for aerobic endogenous respiration for heterotrophic biomass is taken to be 0.2 d^{-1} (20°C) (Table 28)

Again after Wentzel *et al.* (1989b), (for PAOs)-non-biodegradable soluble COD is generated by PAOs during endogenous mass loss. It is stated that it is very likely that this component will be used by other organisms in mixed cultures. If this statement is correct, the introduction of EBPR in ASM3 asks for careful application. Endogenous mass loss under anaerobic conditions then possibly generates a carbon source that can be hydrolysed and/or fermented by heterotrophic bacteria with subsequent uptake by PAOs as PHA. Endogenous mass loss under aerobic conditions, however, possibly generates a carbon source that can be assimilated by heterotrophic bacteria with growth of these organisms at the expense of PAOs.

Whereas ASM3 accounts for internal storage of carbon sources, only a lump sum of all possible internal storage components is considered. For EBPR is has been shown that both PHA and glycogen play an important role. Also for non-PAOs different internal storage components have been observed. Simultaneous storage and degradation of PHB and glycogen has been observed in activated sludge cultures (Carta *et al.*, submitted). These results indicate the necessity, at least for fundamental research, to include more sources of substrate and different storage components. This fundamental research should elucidate the necessity to include this subdivision in a standard model.

4.3.3. Upgrading ASM2d to account for ammonification and for hydrolysis of organic N and P

A general, standard ASM model accounting for ammonification and hydrolysis of soluble organic nitrogen and soluble and particulate organic phosphorus has not been developed yet. Average municipal wastewaters can be modelled, taking into account only the phosphorus and nitrogen content of the S_F, X_I, X_S and the biomass as fixed fractions. For certain wastewaters (industrial and mixed wastewaters) it can

be of importance to include specific organic nitrogen and phosphorus fractions. For his model development, Johansson (1994) included these fractions. Whereas his model is not an extension of ASM2d, ideas are formulated that can be translated to ASM2d.

4.3.3.1. ***Model representation***

The SIPHOR Model (Johansson, 1994), which stands for SImulation of biological PHOsphate Removal, was, as indicated by its name, especially developed for phosphorus removal. Besides EBPR, carbon oxidation, nitrification and denitrification are accounted for. The model was presented as a doctoral thesis the year ASM2 (Henze *et al.*, 1995) was getting finalised and many years before denitrifying capacities of PAOs were ever included in the AS Models (ASM2d, Henze *et al.*, 1999). Any remarks made below concerning comparison between the SIPHOR model and the ASM2d should therefore consider this chronological sequence. In what follows the differences between ASM2d and SIPHOR are elaborated on.

For the development of his model, Johansson (1994) only took a total number of 17 components into account. Some confusion arises concerning the number of soluble and the number of particulate components, i.e. on some occasions the sum of particulate and soluble organic phosphorus is written as a soluble term, on other occasions it is written as a particulate one. In Table 29 the soluble and particulate components are presented along with their eventual equivalent in ASM2d. When comparing the 17 model components included in SIPHOR with the 19 components accounted for in ASM2d (Table 13), the following differences can be listed (the notation of ASM2d has been used instead of the notation used by Johansson):

- *di-nitrogen gas* considered in ASM2d is not considered in SIPHOR
- *total suspended solids* is not considered as a separate component in SIPHOR
- *alkalinity* (bicarbonate) is not accounted for in SIPHOR. This is mainly because no practical bicarbonate limitations were encountered. In his conclusions Johansson (1994) remarks it is advised to include alkalinity in a standard model.
- *metal-hydroxides and metal-phosphates* are not considered
- additionally *soluble and particulate organic phosphorus* are considered as separate components in SIPHOR.
- *particulate and soluble organic phosphorus* is accounted for as a lump sum in SIPHOR

Table 29 Definition of model components in SIPHOR (Johansson, 1994)

Soluble components			***Particulate components***		
S_{O2}	Dissolved oxygen	g O_2/m^3	X_I	Inert, non-biodegradable organics	g COD/m^3
S_F	Readily biodegradable substrate	g COD/m^3	X_S	Slowly biodegradable substrate	g COD/m^3
S_A	Volatile Fatty Acids*	g COD/m^3	X_H	Heterotrophic biomass	g COD/m^3
S_I	Inert organics	g COD/m^3	X_{PAO}	Phosphorus accumulating organisms	g COD/m^3
S_{NH4}	Ammonium	g N/m^3	X_{PP}	Stored poly-phosphate of PAO	g P/m^3
S_{NO3}	Nitrate (plus nitrite)	g N/m^3	X_{PHA}	Organic storage products of PAO	g COD/m^3
S_{ND}	Organic nitrogen	g N/m^3	X_A	Autotrophic, nitrifying biomass	g COD/m^3
S_{PO4}	Phosphate	g P/m^3	X_{ND}	Organic nitrogen	g N/m^3
S_{PD}	Organic phosphate (part. + solub.)	g P/m^3			

* whereas in ASM2d S_A is written as acetate, in fact volatile fatty acids are considered

In the SIPHOR model 36 processes are considered, 9 active under anaerobic conditions, 13 under anoxic conditions and 14 under aerobic conditions. This high number of reactions compared to the 19 basic processes accounted for in ASM2d is partly to be attributed to the fact that processes are written for the different phases (anaerobic, anoxic and aerobic), whereas in ASM2d processes are mainly subdivided

according to the responsible micro-organisms (apart from the hydrolysis reactions) and only when different process rates are expected (e.g. reduced anoxic storage rate for X_{PP} compared to aerobic storage rate for X_{PP}) different reactions are considered. Comparing the process rate equations proposed by Johansson (1994) with the ones considered in ASM2d reveals the following differences:

- *lysis of the internal storage components X_{PP} and X_{PHA}* is not accounted for in SIPHOR.
- *aerobic and anoxic growth of PAOs directly on S_F as well as on S_A* is explicitly accounted for in SIPHOR. However, the maximum growth rate of PAOs to is significantly smaller than the one for non-PAOs (1.2 d^{-1} and 4.0 d^{-1} respectively). Moreover, an additional aerobic/anoxic reduction factor (α) is introduced on top if this. From experimental evidence (see 4.3.3.2) this parameter was set at 0.5, implying that growth on external substrate is lower than on internal substrate (PHA). A possible explanation for the introduction of these growth reactions can be the fact that the influents considered in the study by Johansson (1994) contain very large amounts of VFA. Residual amounts of this substrate can thus be present under anoxic and aerobic conditions, and as such the author wanted to account for any reaction possibly occurring. Possible phosphorus limitations are not accounted for in the rate equations.
- *aerobic and anoxic storage of S_A as PHA* is explicitly accounted for. The rate equation is the same as the one for the anaerobic phase and no reduction factors are included. Moreover, the rate coefficient for storage considered by Johansson (1994) (5 d^{-1}; Table 31) is higher than the one proposed in ASM2d (3 d^{-1}) and is higher than the maximum growth rate of heterotrophic organisms on S_A (4 d^{-1}). In accordance with these consideration, Johansson (1994) stated that, if any volatile fatty acids are present, they are taken up and stored as PHA by the PAOs. The necessity to account for aerobic and anoxic growth of PAOs directly on S_F as well as on S_A is thus rather limited, unless the poly-P content would be limited which is rather unlikely in the aerobic phase.
- *aerobic and anoxic growth* are directly linked in the model with polyphosphate production, whereas in ASM2d storage of polyphosphate is considered as a separate reaction. The approach followed by Johansson (1994) is similar to the approach of Wentzel (1989b) for aerobic growth. Since no growth limitation is accounted for in SIPHOR (see below), the difference between both expressions (ASM2d and SIPHOR) boils down to including an inverse Monod term to avoid more accumulation of poly-P than observed under experimental conditions. However, when growth limitations by nutrients would be included in the SIPHOR model, an ASM2d like approach for poly-P storage should be considered. Indeed, even though growth can cease due to nutrient limitation, poly-P can further be stored in already existing cells. This phenomenon asks for a decoupling of growth and storage, i.e. introduction of separate reactions, cf. Wentzel *et al.* (1989b).
- An important difference between ASM2d and SIPHOR is the addition of *ammonification reaction of soluble organic nitrogen components*. This reaction can be performed by heterotrophic as well as PAOs. Only one rate equation is considered in each phase. In the rate equation the biomass is written as the sum of X_H and X_{PAO}.
- The addition of *hydrolysis processes for particulate nitrogen and phosphorus components* in SIPHOR is another difference compared to ASM2d. This reaction can be performed by heterotrophic as well as PAOs. Only one rate equation is considered in each phase. In the rate equation the biomass is written as the sum of X_H and X_{PAO}.
- A final difference between the SIPHOR and ASM2d is the fact that *hydrolysis of SBCOD and fermentation* can not only be performed by heterotrophic organisms but by PAOs as well. Only one rate equation is considered in each phase. In the rate equation the biomass is written as the sum of X_H and X_{PAO}.

When using the subdivision presented in ASM2d, i.e. considering hydrolysis reactions, heterotrophic and phosphorus accumulating organisms, the number of additional reactions considered in SIPHOR and not accounted for in ASM2d, is 15. The process rate equations of these reactions are presented in Table 30, using the subdivision according to ASM2d.

Table 30 Process rate equations for reactions taken into account in SIPHOR and not in ASM2d (after Johansson, 1994)

Hydrolysis processes		
	Aerobic hydrolysis of X_{ND} to S_{ND}	$K_h \cdot \frac{X_S/(X_H+X_{PAO})}{K_X+X_S/(X_H+X_{PAO})} \cdot \frac{X_{ND}}{X_S} \cdot (X_H+X_{PAO})$
	Anoxic hydrolysis of X_{ND} to S_{ND}	$K_h \cdot \eta_{LNO3} \cdot \frac{X_S/(X_H+X_{PAO})}{K_X+X_S/(X_H+X_{PAO})} \cdot \frac{X_{ND}}{X_S} \cdot (X_H+X_{PAO})$
	Anaerobic hydrolysis of X_{ND} to S_{ND}	$K_h \cdot \eta_{fe} \cdot \frac{X_S/(X_H+X_{PAO})}{K_X+X_S/(X_H+X_{PAO})} \cdot \frac{X_{ND}}{X_S} \cdot (X_H+X_{PAO})$
	Aerobic hydrolysis of S_{PD} to S_{OP}	$K_h \cdot \frac{X_S/(X_H+X_{PAO})}{K_X+X_S/(X_H+X_{PAO})} \cdot \frac{S_{PD}}{X_S} \cdot (X_H+X_{PAO})$
	Anoxic hydrolysis of S_{PD} to S_{OP}	$K_h \cdot \eta_{LNO3} \cdot \frac{X_S/(X_H+X_{PAO})}{K_X+X_S/(X_H+X_{PAO})} \cdot \frac{S_{ND}}{X_S} \cdot (X_H+X_{PAO})$
	Anaerobic hydrolysis of S_{PD} to S_{OP}	$K_h \cdot \eta_{fe} \cdot \frac{X_S/(X_H+X_{PAO})}{K_X+X_S/(X_H+X_{PAO})} \cdot \frac{S_{ND}}{X_S} \cdot (X_H+X_{PAO})$
	Aerobic ammonification	$K_a \cdot S_{ND} \cdot (X_H+X_{PAO})$
	Anoxic ammonification	$K_a \cdot \eta_{LNO3} \cdot S_{ND} \cdot (X_H+X_{PAO})$
	Anaerobic ammonification	$K_a \cdot \eta_{fe} \cdot S_{ND} \cdot (X_H+X_{PAO})$
Phosphorus accumulating organisms: X_{PAO}		
	Aerobic storage of S_A	$q_{PHA} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot X_{PAO}$
	Anoxic storage of S_A	$q_{PHA} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot X_{PAO}$
	Aerobic growth on ferm. substrate, S_F	$\mu_{PAO} \cdot \alpha \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot X_{PAO}$
	Anoxic growth on ferm. substrate, S_F	$\mu_{PAO} \cdot \alpha \cdot \eta_{HNO3} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot X_{PAO}$
	Aerobic growth on ferm. products, S_A	$\mu_{PAO} \cdot \alpha \cdot \frac{S_A}{K_{HA}+S_A} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot X_{PAO}$
	Anoxic growth on ferm. products, S_A	$\mu_{PAO} \cdot \alpha \cdot \eta_{HNO3} \cdot \frac{S_A}{K_{HA}+S_A} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot X_{PAO}$

When comparing ASM2d and SIPHOR some general remarks finally need to be formulated:

- In ASM2d release of soluble nitrogen and phosphorus to the environment is considered, taking into account N and P content (i_{NXS} and i_{PXS}) of SBCOD which is released during hydrolysis. However, whereas in SIPHOR it is assumed that the internal nitrogen content is released as soluble nitrogen, in ASM2d it is assumed nitrogen is immediately released as ammonium. Both models assume internal phosphorus is released as soluble phosphate.

- In ASM2d, it is assumed fermentable substrate can contain a fraction of nitrogen and phosphorus. During fermentation nitrogen is released as ammonium and phosphorus as phosphate. This is not taken into account in the SIPHOR model.
- In the SIPHOR model growth limitation by the nutrients phosphorus and nitrogen has not been taken into account. Johansson (1994) remarked that for future developments these terms have to be taken into account. Monod type equations, as in ASM2d, are suggested for future applications.
- Alkalinity is not considered as a limiting factor in the SIPHOR model.

4.3.3.2. ***Model calibration and validation on pilot and full scale plants***

The SIPHOR model was run against experimental results mainly from pilot plants, i.e. the Sjölunda wastewater treatment plant in Malmö (Sweden) and the full scale plant in Helsingborg (Sweden). The treatment plant in Helsingborg was rebuilt in 1991 with the aim to remove BOD (to 10 mg/ℓ), nitrogen (to 12 mg N_{tot}/ℓ) and phosphorus (to 0.3 mg P_{tot}/ℓ) to a high degree. It has a design capacity of 3250 m³/h. Different operating modes were used during the investigation period which are fully documented in Johansson (1994). At Malmö two pilot plants (~2 m³/h) were operated, the oldest one as an A/O process and the new pilot plant as an UCT process.

Batch- and steady state experiments were run to obtain a set of kinetic constants which needed the minimum amount of alteration for successful simulation of the pilot plant under dynamic conditions. Anaerobic batch simulations were run to identify the constants q_{PHA}, K_{PA}, q_{fe}, $K_{PP,}$ $K_{HA,}$ Y_{PO4}, K_F and η_{fe}. Aerobic phosphorus uptake batch tests were performed to determine K_{PO2}, K_P and K_{PHA}. Simulation of the pilot plant under steady state condition was then used to get a complete set of kinetic constants.

In Table 31 the kinetic and stoichiometric constants used in the dynamic simulations are shown along with the values suggested in ASM2d. Both the notation used by Johansson (1994) and the ASM2d notation are used. This double notation allows to the reader upon consultation of Johansson's work to find the corresponding values more easily. Indeed, the notation used by Johansson differs so much from the ASM2d that it is sometimes difficult to compare both models.

In general it was observed that many variations exist, especially for the reduction factor, between the batch, steady state and dynamic profiles used for calibration and verification of the model.

As can be seen, almost the same parameter values can be used for the two plants studied. Those characteristics that needed alteration mainly depend on the characteristics of the influent wastewater.

- Since toxic agents in the influent wastewater inhibited nitrification processes in Helsingborg, i.e. the autotrophic growth rate was found to be approximately half the one found in Malmö.
- Influent wastewater in Helsingborg is hydrolysed, which, according to the author may make that S_s is more easily degradable. The fermentation rate observed in Helsingborg was twice as high as in Malmö.
- Due to the experimental circumstances, the author stated that the constants connected to phosphorus uptake are more accurate in Malmö.

Comparing the kinetic and stoichiometric parameters in the SIPHOR model and ASM2d the following observation are made:

- The experimental rate for the *anaerobic hydrolysis* was found to be nearly zero. Wentzel *et al*. (1994) and Van Haandel (1981) reported as well that the anaerobic release was very low. In contradiction to this Mino *et al.* (1994b) reported no significant difference between hydrolysis in the different phases.
- The *saturation coefficient for growth on acetate* is higher.
- The rate constants for lysis of X_H and X_A are lowered, whereas the one for X_{PAO} remained unchanged.

Table 31 Comparison of SIPHOR values used in dynamic simulations and ASM2d (at T=20°C) (after Johansson, 1994)

				Malmö	Helsing borg	ASM2d	
Conversion factors for conservation equation							
Nitrogen, Phosphorus							
ASM	SIPHOR						
i_{NBM}	i_{XB}	p(6)	N content of biomass, X_H, X_{PAO}, X_A	0.05	0.05	0.07	g N g^{-1} COD
i_{PBM}	i_{XP}	p(11)	P content of biomass, X_H, X_{PAO}, X_A	0.017	0.021	0.02	g P g^{-1} COD
Stoichiometric parameters							
Heterotrophic biomass: X_H							
Y_H	Y_h	p(16)	yield coefficient	0.67	0.67	0.625	g COD g^{-1} COD
f_{XIH}	f_p	p(17)	fraction of inert COD in lysis	0.06	0.06	0.1	g COD g^{-1} COD
Phosphorus-accumulating organisms : X_{PAO}							
Y_{PAO}	Y_P	p(18)	yield coefficient (biomass/PHA)	0.67	0.67	0.625	g COD g^{-1} COD
Y_{PO4}	f_{pp}	p(19)	PP requirement (PO_4 rel.) / PHA stored	0.35	0.30	0.40	g P g^{-1} COD
f_{XIP}	f_p	p(21)	fraction of inert COD generated in lysis	0.06	0.06	0.10	g COD g^{-1} COD
Nitrifying organisms: X_A							
Y_A	Y_a	p(22)	yield of autotrophic biomass per NO_3-N	0.24	0.24	0.24	g COD g^{-1} N
f_{XIA}	f_p	p(23)	fraction of inert COD generated in lysis	0.06	0.06	0.10	g COD g^{-1} COD
Kinetic parameters							
Hydrolysis of particulate and soluble components: X_S, X_{ND}, S_{PD}, S_{ND} and fermentation of S_S to S_A							
K_h	k_{hx}	p(24)	hydrolysis rate constant	3	3	3	d^{-1}
η_{LNO3}	δ	p(25)	anoxic hydrolysis reduction factor	0.6	0.6	0.6	-
η_{fe}	ε	p(26)	anaerobic hydrolysis reduction factor	0.008	0.008	0.4	-
K_X	K_X	p(29)	saturation coef. for particulate COD	0.027	0.027	0.1	g COD-X_S g^{-1} COD X_H
	k_a		ammonification rate	0.016	0.016		d^{-1}
q_{fe}	k_{ferm}	p(31)	maximum rate for fermentation	1.5	3.0	3.0	g S_F g^{-1} X_H d^{-1}
K_{fe}	K_s	p(36)	sat. coef. for fermentation on S_F	0.03	0.03	4.0	g COD g^{-1} COD g COD m^{-3}
Heterotrophic organisms: X_H							
μ_H	μ_h	p(30)	maximum growth rate on substrate	4.0	4.0	6.0	d^{-1}
η_{HNO3}	χ	p(32)	reduction factor for denitrification	0.5	0.9	0.8	-
b_H	d_h	p(33)	rate constant for lysis and decay	0.7	0.7	0.4	d^{-1}
K_{HO2}	K_{oh}	p(34)	saturation/inhibition coef. for oxygen	0.5	0.5	0.2	g O_2 m^{-3}
K_F	K_{hs}	p(35)	saturation coef. for growth on S_F	4.0	4.0	4.0	g COD m^{-3}
K_{HA}	K_{sva}	p(37)	sat. coefficient for growth on acetate S_A	20.0	20.0	4.0	g COD m^{-3}
K_{HNO3}	K_{no}	p(38)	sat./inhibition coefficient for nitrate	0.5	0.5	0.5	g N m^{-3}
Phosphorus accumulating organisms: X_{PAO}							
q_{PHA}	k_p	p(42)	rate const. storage of X_{PHA} (base X_{PP})	5.0	5.0	3.0	g X_{PHA} g^{-1} X_{PAO} d^{-1}
μ_{PAO}	μ_p	p(44)	maximum growth rate of PAO	1.2	1.2	1.0	d^{-1}
η_{PNO3}	β	p(45)	reduction factor for anoxic activity	0.9	0.8	0.6	-
b_{PAO}	d_p	p(46)	rate for lysis of X_{PAO}	0.2	0.2	0.2	d^{-1}
K_{PO2}	K_{op}	p(49)	sat./inhibition coefficient for oxygen	0.3	0.1	0.2	g O_2 m^{-3}
K_{PA}	K_{va}	p(50)	saturation coefficient for acetate, S_A	3.0	3.0	4.0	g COD m^{-3}
K_P	K_{po}	p(52)	sat. coef. for phosphorus in PP storage	0.5	0.1	0.2	g P m^{-3}
K_{PP}	K_{pop}	p(55)	sat. coefficient for poly-phosphate	0.01	0.01	0.01	g P m^{-3} g X_{PP} g^{-1} X_{PAO} d^{-1}
K_{PHA}	K_{xpa}	p(58)	saturation coefficient for PHA	0.06	0.05	0.01	g COD g^{-1} X_P g X_{PHA} g^{-1} X_{PAO} d^{-1}
	α		reduction factor for growth of PAOs on S_F and S_A	0.5	0.5	-	
Nitrifying organisms (autotrophic organisms): X_A							
μ_A	μ_a	p(59)	maximum growth rate of X_A	0.8	0.42	1.0	d^{-1}
b_A	d_a	p(60)	decay rate of X_A	0.1	0.1	0.15	d^{-1}
K_{NO2}	K_{oa}	p(61)	sat. coef. for oxygen	0.2	0.5	0.5	g O_2 m^{-3}
K_{NNH4}	K_{nh}	p(62)	sat. coef. for ammonium (substrate)	0.3	1.0	1.0	g N m^{-3}

4.3.4. Upgrading ASM2d to account for competition between PAOs and GAOs

With their research, Manga *et al.* (2000) aimed at the upgrading of ASM2 by including denitrifying PAOs and by accounting for possible competition between PAOs and GAOs. Since denitrifying PAOs have already been included in ASM2d, only the competition between PAOs and GAOs is addressed in this section.

The model proposed by Manga *et al.* (2000) and the model parameters deduced are based on observations, made on a pilot plant. The competition between PAOs and GAOs is attributed to the excess of acetate present in the influent, which causes a low phosphorus to carbon ratio (see also 3.4.2.2).

To account for the competition between PAOs and GAOs, glycogen is introduced as additional particulate component, present in PAOs and GAOs. With the inclusion of GAOs and the PHA content of GAOs, in total 4 new particulate components were introduced (Table 32).

Table 32 Definition of additional model components to account for competition between PAOs and GAOs in ASM2d (Manga et al., 2000)

Particulate components		
X_{GAO}	Glycogen accumulating organisms	g COD-X_{GAO}/m^3
X_{PHA}^{GAO}	PHA*, organic storage product of GAOs	g COD-X_{PHA}/m^3
X_{GLY}^{GAO}	Glycogen, organic storage product of GAOs	g COD-X_{GLY}/m^3
X_{GLY}^{PAO}	Glycogen, organic storage product of PAOs	g COD-X_{GLY}/m^3

* For GAOs, PHA is supposed to have the chemical formula of PHV

To account for the GAO related processes, Manga *et al.* (2000) included 8 additional processes to the already existing ASM2d. Additionally, 3 processes were included to model the glycogen related processes occurring in the PAOs. The process rate equations for the additional processes are presented in Table 33. The notations have been slightly altered to allow consistent notations with the other models and thus enhance easy comparison between the different models discussed.

- For the *anaerobic storage of PHA*, both for GAOs and PAOs, a yield coefficient expressing the acetate requirement for PHA production is introduced (Table 34). PHA is supposed to be produced directly from acetate (with yield Y_{SA}) and from glycogen breakdown (with yield 1-Y_{SA}). The same yield coefficient is presumed for both organisms.
- For the *aerobic formation of glycogen* on PHA and the *aerobic growth on PHA*, different saturation coefficients for X_{PHA} are introduced (both for PAOs and GAOs). In ASM2d this distinction is not made.
- In their model, Manga *et al.* (2000) introduced *yield coefficients* which are different for the *aerobic and anoxic reactions* (Table 34). In ASM2d yield coefficient are equal for aerobic or anoxic reactions.
- The *kinetic parameters* for PAOs proposed by Manga *et al.* (2000) differ from the ones proposed by Henze *et al.* (1999). The parameter set proposed by Manga *et al.* (2000) was deduced from pilot plant experiments. With the parameter set the authors were able to demonstrate an excellent concordance between model predictions and operating results.

Table 33 Additional process rate equations to account for competition between PAOs and GAOs in ASM2d (Manga et al., 2000)

Glycogen accumulating organisms (GAO): X_{GAO}		
	Storage of X_{PHA} (anaerobically)	$q_{PHA}^{GAO} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{GLY}^{GAO}/X_{GAO}}{K_{GLY}+X_{GLY}^{GAO}/X_{GAO}} \cdot X_{GAO}$
X1	Aerobic storage of X_{GLY} *	$q_{GLY}^{GAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PHA}^{GAO}/X_{GAO}}{K_{PHA}^{GAO,GLY}+X_{PHA}^{GAO}/X_{GAO}} \cdot \frac{K_{MAX,GLY}^{GAO} - X_{GLY}^{GAO}/X_{GAO}}{K_{IGLY}+K_{MAX,GLY}^{GAO}-X_{GLY}^{GAO}/X_{GAO}} \cdot X_{GAO}$
X2	Anoxic storage of X_{GLY}	$\rho_{X2} = \rho_{X1} \cdot \eta_{GNO3} \cdot \frac{K_{HO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
Y1	Aerobic growth on X_{PHA}	$\mu_{GAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PHA}^{GAO}/X_{GAO}}{K_{PHA}^{GAO,growth}+X_{PHA}^{GAO}/X_{GAO}} \cdot X_{GAO}$
Y2	Anoxic growth on X_{PHA}	$\rho_{Y2} = \rho_{Y1} \cdot \eta_{GNO3} \cdot \frac{K_{HO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
	Lysis of X_{GAO}	$b_{GAO} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot X_{GAO}$
	Lysis of X_{GLY}	$b_{GLY} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot X_{GLY}^{GAO}$
	Lysis of X_{PHA}	$b_{PHA} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot X_{PHA}^{GAO}$
Phosphorus accumulating organisms (PAO): X_{PAO}		
**	Storage of X_{PHA} (anaerobically)	$q_{PHA}^{PAO} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PP}}{K_{PP}+X_{PP}} \cdot \frac{X_{GLY}^{PAO}/X_{PAO}}{K_{GLY}+X_{GLY}^{PAO}/X_{PAO}} \cdot X_{PAO}$
Z1	Aerobic formation of X_{GLY} *	$q_{GLY}^{PAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot \frac{X_{PHA}^{PAO}/X_{PAO}}{K_{PHA}^{PAO,GLY}+X_{PHA}^{PAO}/X_{PAO}} \cdot \frac{K_{MAX,GLY}^{PAO} - X_{GLY}^{PAO}/X_{PAO}}{K_{IGLY}+K_{MAX,GLY}^{PAO}-X_{GLY}^{PAO}/X_{PAO}} \cdot X_{PAO}$
Z2	Anoxic formation of X_{GLY}	$\rho_{ZX2} = \rho_{Z1} \cdot \eta_{PNO3} \cdot \frac{K_{HO2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}}$
	Lysis of X_{GLY}	$b_{GLY} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot X_{GLY}^{PAO}$

*Remark: for the aerobic storage of glycogen, both for PAOs and GAOs, the dependency on the alkalinity is not accounted for in the paper presented by Manga et al. (2000). Presumably this is a typing mistake.

** This is not an additional reaction. Since the rate equation is different from ASM2d, i.e. a dependency on glycogen is supposed, the rate equation is mentioned in the table.

Table 34 Typical values used for the stoichiometric coefficients defined by Manga et al. (2000)

Glycogen accumulating organisms: X_{GAO}				
Y_{SA}		S_A requirement for PHA storage	0.75	g COD-S_A g^{-1} COD-X_{PHA}
$Y_{PO4,SA}$		PP requirement (PO_4 release) per S_A stored	0.40	g P-X_{PP} g^{-1} COD-S_A
Y_{GAO}		aerobic yield coefficient (biomass/PHA)	0.58	g COD-X_{PAO} g^{-1} COD-X_{PHA}
$Y_{GAO, NO}$		anoxic yield coefficient (biomass/PHA)	0.47	g COD-X_{PAO} g^{-1} COD-X_{PHA}
Y_{GLY}		PHA requirement for aerobic glycogen replenishment	1	g COD g^{-1} COD
$Y_{GLY, NO}$		PHA requirement for aerobic glycogen replenishment	1	g COD g^{-1} COD
Phosphorus accumulating organisms: X_{PAO}				
Y_{SA}		S_A requirement for PHA storage	0.75	g COD-S_A g^{-1} COD-X_{PHA}
Y_{PHA}	p(20)	PHA requirement for aerobic PP storage	0.32	g COD-X_{PHA} g^{-1} P-X_{PP}
$Y_{PHA, NO}$		PHA requirement for anoxic PP storage	0.57	g COD-X_{PHA} g^{-1} P-X_{PP}
Y_{PAO}	p(18)	aerobic yield coefficient (biomass/PHA)	0.58	g COD-X_{PAO} g^{-1} COD-X_{PHA}
$Y_{PAO, NO}$		anoxic yield coefficient (biomass/PHA)	0.47	g COD-X_{PAO} g^{-1} COD-X_{PHA}
Y_{GLY}	p(18)	PHA requirement for aerobic glycogen replenishment	1	g COD-X_{GLY} g^{-1} COD-X_{GLY}
$Y_{GLY, NO}$		PHA requirement for anoxic glycogen replenishment	1	g COD-X_{GLY} g^{-1} COD-X_{GLY}

The authors use the terminology *"aerobic/anoxic glycogen yield coefficient"*. In accordance with the terminology used for Y_{PHA} the terminology for Y_{GLY} has been altered.

Table 35 Typical values for the kinetic parameters for PAOs and GAOs (T = 20°C) (Manga et al., 2000)

			Manga	ASM2d	
Glycogen accumulating organisms: X_{GAO}					
K_{HNO3}	p(38)	saturation/inhibition coefficient for nitrate	0.50		g $N\text{-}NO_3$ m^{-3}
q_{PHA}		rate constant for storage of X_{PHA}	2.60		g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
μ_{GAO}		maximum growth rate of GAO	0.80		d^{-1}
η_{GNO3}		reduction factor for anoxic activity	0		-
b_{GAO}	p(46)	rate for lysis of X_{PAO}	0.08		d^{-1}
b_{PHA}	p(48)	rate for lysis of X_{PHA}	0.08		d^{-1}
b_{GLY}		rate for lysis of X_{GLY}	0.08		d^{-1}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.20		g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	3.63		g COD m^{-3}
K_{PNH4}	p(51)	saturation coefficient for ammonium (nutrient)	0.05		g N m^{-3}
K_{PN}	p(53)	saturation coefficient for phosphate (nutrient)	0.01		g P m^{-3}
K_{PALK}	p(54)	saturation coefficient for alkalinity (HCO_3^-)	0.10		mol HCO_3^- m^{-3}
$K_{PHA}^{GAO,growth}$	p(58)	saturation coefficient for PHA for growth	0.03		g COD-X_{PHA} g^{-1} COD-X_{PAO}
$K_{PHA}^{GAO,GLY}$		saturation coefficient for PHA for glycogen storage	0.008		g COD-X_{PHA} g^{-1} COD-X_{PAO}
K_{GLY}		saturation coefficient for PHA storage	0.01		g COD-X_{GLY} g^{-1} COD-X_{PAO}
q_{GLY}		rate constant for storage of glycogen	1.2		g COD-X_{GLY} g^{-1} COD-X_{PAO} d^{-1}
$K_{MAX,GLY}^{GAO}$		maximum ratio of X_{GLY}/X_{GAO}	0.40		g COD-X_{GLY} g^{-1} COD-X_{PAO}
K_{IGLY}		inhibition coefficient for glycogen storage	0.015		g COD-X_{GLY} g^{-1} COD-X_{PAO}
Phosphorus accumulating organisms: X_{PAO}					
K_{HNO3}	p(38)	saturation/inhibition coefficient for nitrate	0.50	0.50	g $N\text{-}NO_3$ m^{-3}
q_{PHA}	p(42)	rate constant for storage of X_{PHA} (base X_{PP})	3.60	3.00	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for storage of X_{PP}	2.80	1.50	g X_{PP} g^{-1} X_{PAO} d^{-1}
μ_{PAO}	p(44)	maximum growth rate of PAO	0.84	1.00	d^{-1}
η_{PNO3}	p(45)	reduction factor for anoxic activity	0.48	0.60	-
b_{PAO}	p(46)	rate for lysis of X_{PAO}	0.08	0.20	d^{-1}
b_{PP}	p(47)	rate for lysis of X_{PP}	0.08	0.20	d^{-1}
b_{PHA}	p(48)	rate for lysis of X_{PHA}	0.08	0.20	d^{-1}
b_{GLY}		rate for lysis of X_{GLY}	0.08	-	d^{-1}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.20	0.20	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	3.63	4.00	g COD m^{-3}
K_{PNH4}	p(51)	saturation coefficient for ammonium (nutrient)	0.05	0.05	g N m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage	0.20	0.20	g P m^{-3}
K_{PN}	p(53)	saturation coefficient for phosphate (nutrient)	0.01	0.01	g P m^{-3}
K_{PALK}	p(54)	saturation coefficient for alkalinity (HCO_3^-)	0.10	0.10	mol HCO_3^- m^{-3}
K_{PP}	p(55)	saturation coefficient for poly-phosphate	0.01	0.01	g P-X_{PP} g^{-1} COD-X_{PAO}
K_{MAX}	p(56)	maximum ratio of X_{PP}/X_{PAO}	0.28	0.34	g P-X_{PP} g^{-1} COD-X_{PAO}
K_{IPP}	p(57)	inhibition coefficient for PP storage	0.001	0.02	g P-X_{PP} g^{-1} COD-X_{PAO}
$K_{PHA}^{PAO,growth}$	p(58)	saturation coefficient for PHA for growth	0.03	0.01	g COD-X_{PHA} g^{-1} COD-X_{PAO}
$K_{PHA}^{PAO,PP}$	p(58)	saturation coefficient for PHA for PP storage	0.07	0.01	g COD-X_{PHA} g^{-1} COD-X_{PAO}
$K_{PHA}^{PAO,GLY}$		saturation coefficient for PHA for glycogen storage	0.12	-	g COD-X_{PHA} g^{-1} COD-X_{PAO}
K_{GLY}		saturation coefficient for PHA storage	0.001		g COD-X_{GLY} g^{-1} COD-X_{PAO}
q_{GLY}		rate constant for storage of glycogen	3.8	-	g COD-X_{GLY} g^{-1} COD-X_{PAO} d^{-1}
$K_{MAX,GLY}^{PAO}$		maximum ratio of X_{GLY}/X_{PAO}	0.25	-	g COD-X_{GLY} g^{-1} COD-X_{PAO}
K_{IGLY}		inhibition coefficient for glycogen storage	0.015	-	g COD-X_{GLY} g^{-1} COD-X_{PAO}

Some remarks should be considered when evaluating the model proposed by Manga *et al.* (2000)

- Although the authors hypothesised that a fraction of the GAOs would be able to denitrify, the calibration results indicated that GAOs are unable to carry out this process. This result is based on only one observation with a pilot plant. Further research needs to elucidate whether or not GAOs are capable of denitrification.
- Many of the kinetic and stoichiometric parameters for PAOs are different from the ones proposed in ASM2d (Table 35). The parameter set needs to be determined again, using the default ASM2d parameters and evaluating if calibration results and operating results can be obtained in accordance and if not, to pinpoint the differences.
- The necessity for introducing different saturation coefficients for PHA usage (growth, PP storage, glycogen storage) needs to be evaluated.
- In ASM2d glycogen and PHA are lumped together into X_{PHA}. Since PHA increases when glycogen decreases and vice versa it is advisable to consider both components as separate components. However, the difficulties associated with the glycogen analysis have favoured the implementation of lump sums. Manga *et al.* (2000) do not address this issue, neither do they mention experimental glycogen profiles.

4.3.5. Metabolic modelling of EBPR processes

To limit the number of parameters to be estimated, researchers at the TU Delft in the Netherlands initiated work on metabolic modelling of EBPR processes. In such a metabolic model, the conversion of components observed on the outside of the organisms are linked to a number of internal characteristic metabolic reactions of the metabolism. In general, these reactions have been studied extensively by biochemists and have, for the most, a fixed stoichiometry determined by chemical elemental balances. Only a minimal number of independent reaction rates is then obtained, leading to a minimal number of necessary kinetic expressions (Murnleitner *et al.*, 1997).

4.3.5.1. ***Metabolic model for non-denitrifying PAOs (Smolders, 1995)***

In the framework of his Ph.D. study Smolders (1995) developed a metabolic model for the processes occurring in EBPR processes. Several papers published by Smolders *et al.* (1994a, 1995a, 1995b) are dedicated to different aspects of the kinetic model based on the internal metabolic reactions (3.4.3.1 and 3.4.3.3). Although it is stated that the biochemical reactions have been studied extensively, the publication of the first metabolic model by Smolders (1994a), created again important controversy regarding these underlying biochemistry.

For the development of the model some important differences, with ASM2d can be listed:

- No denitrification by PAOs is considered.
- In ASM2d, all internal carbon sources are lumped into one component, i.e. X_{PHA}. In this metabolic model, glycogen and PHA are accounted for separately.
- Endogenous respiration of biomass is not considered, but anaerobic and aerobic maintenance processes are accounted for.
- For this model fewer components are considered compared to ASM2d, one of the reasons being that only EBPR is considered, another reason being that growth limitation factors caused by nutrients or alkalinity limitation are not considered in the model

The model was developed for PAOs assuming:

- Only acetate was available as carbon source. Hydrolysis reactions therefore did not have to be included. Moreover, since no endogenous respiration with concomitant release of SBCOD is accounted for, acetate is indeed the only COD fraction considered.
- Acetate is fully consumed under anaerobic conditions.

The experimental set-up was:

- operated as a fully anaerobic/aerobic system, i.e. possible growth of denitrifying PAOs was prevented. Moreover, also nitrification was prevented, dosing ATU (Vanrolleghem, personal communication)
- operated under phosphorus limiting conditions (phosphorus in the effluent being zero), instead of carbon limiting conditions (phosphorus removal limited due to limited availability of anaerobic carbon source) (see discussion 4.3.5.2). Whereas Liu *et al.* (1994, 1996b) and Manga *et al.* (2000) observed growth of PAOs and/or GAOs under phosphorus limiting conditions, Smolders (1995) does not account for possible competition between PAOs and GAOs.

In Table 36 the process rate equations suggested by Smolders *et al.* (1995b) are presented. For reasons of comparison with ASM2d the notations used by Smolders *et al.* (1995b) have been altered and adapted to the ASM2d notation. Similar reactions are given the same number as for ASM2d, new ones have been left without number.

Table 36 Process rate equations for PAOs in the metabolic model of Smolders et al. (1995b)

10 bis	Uptake of S_A (Storage of X_{PHA})	$q_{Ac} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot X_{PAO}$	(1)
11	Aerobic storage of X_{PP}	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \left(\frac{X_{PHA}}{X_{PAO}}\right)^{1/3} \cdot \frac{K_{MAX}^{PP} - X_{PP}/X_{PAO}}{K_{MAX}^{PP}} \cdot X_{PAO}$	(2)
13	Aerobic growth on X_{PHA}	$\mu_{PAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{X_{PHA}}{X_{PAO}} \cdot X_{PAO}$	(2)
	Aerobic formation of X_{GL}	$q_{GL} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \left[K_{MAX}^{GL} \cdot \Delta\left(\frac{X_{PHA}^{AN}}{X_{PAO}}\right) - \frac{X_{GL}}{X_{PAO}}\right] \cdot X_{PAO}$	(2)
	Anaerobic maintenance	$m_{anaer} \cdot \frac{K_{PO2}}{S_{O2}+K_{PO2}} \cdot X_{PAO}$	
	Aerobic maintenance	$m_{aer} \cdot \frac{S_{O2}}{S_{O2}+K_{PO2}} \cdot X_{PAO}$	(2)

(1) Polyphosphate switching function not explicitly mentioned in Smolders *et al.* (1995b) but mentioned in Smolders (1995)
(2) Oxygen switching functions not explicitly mentioned in Smolders *et al.* (1995b) but mentioned in Smolders (1995)

For the reactions proposed by Smolders *et al.* (1995b) the following remarks can be made:

- In ASM2d the *uptake of acetate* is directly linked with PHA storage, i.e. 1 g COD-S_A is supposed to result in 1 g COD-X_{PHA}. Smolders *et al.* (1995b) however take into account consumption of internal glycogen concomitant with acetate uptake for production of PHA (cf. Manga *et al.*, 2000). Anaerobic consumption is thus now referred to as "uptake of acetate" by the authors. To indicate this difference, this reaction is referred to as 10 bis. Glycogen limitation as proposed by Manga *et al.* (2000) is not accounted for in the rate equation proposed by Smolders *et al.* (1995b). The proposed rate constant for the anaerobic acetate uptake (Table 37) has a much higher value than the one proposed in ASM2d (Henze *et al.*, 1999). However, the value proposed by Smolders *et al.* (1995b) is more in accordance with the ones proposed by Wentzel *et al.* (1989b) (see Table 21) and by Çinar *et al.* (1998). The latter had to increase the ASM2d proposed value to 8 d^{-1} to calibrate the model for a full scale WWTP.
- The *aerobic storage of polyphosphate* is modelled in a different way than ASM2d by replacing the surface reaction for PHA with a power function. It needs to be stressed that the shape of both functions is similar. The reaction term for polyphosphate is different too.

- The *aerobic growth* is modelled proportional with the PHA content of the cells compared to a surface reaction in ASM2d. Here a distinct difference will occur for high internal PHA contents.
- For the *aerobic formation of glycogen* the assumption is put forward that the glycogen replenishment is stopped when the glycogen content consumed anaerobically has been replenished (Δ-term in rate equation). Initially (Smolders *et al*., 1995a), the term was made proportional to the PHA content at the end of the anaerobic period, whereas when taking into account different SRTs (3.4.2.5) the term was made proportional to the additional PHA content taken up anaerobically.
- *Anaerobic maintenance* comprises breakdown of poly-P with concomitant release of ortho-phosphate. Already by Wentzel *et al.* (1989b) anaerobic cleavage of poly-P for maintenance purposes was considered. Wentzel *et al.* (1989b) modelled this process as being proportional to X_{PP} whereas Smolders (1995) used a process rate equation proportional to the biomass concentration X_{PAO}. It is thus expected that the value proposed by Wentzel *et al.* (1989b) for the anaerobic maintenance rate coefficient would be higher than the one proposed by Smolders (1995). However, the opposite is observed (0.03 d^{-1} and 0.083 g P-X_{PP} g COD-X_{PAO} d^{-1} respectively) (Table 20 and Table 37).
- *Aerobic maintenance* comprises breakdown of PHA. No inert material is being released. In ASM3 aerobic maintenance process is modelled proportional with the PHA-concentration rather than proportional with X_{PAO} as proposed by Smolders (1995). However, the aerobic maintenance process proposed by Smolders (1995b) is modelled as respiration of the internal storage product as it its proposed in ASM3, i.e. no endogenous respiration of the micro-organisms themselves occurs. In later models originating from the same group (Brdjanovic *et al.*, 1998a) confusion is created (see 4.3.5.6). The aerobic decay rate measured by Wentzel *et al.* (1989b) equalled 0.04 d^{-1}. Since they observed that 45% of the decayed COD accumulated in solution either as particulate or soluble matter (Wentzel *et al.* (1989a,b), the remainder can be considered being oxidised for maintenance purposes, i.e. an aerobic maintenance coefficient of 0.022 d^{-1}, or 0.03 g COD-X_{PHB} g^{-1} COD-X_{PAO} d^{-1} is obtained (Filipe and Daigger, 1998). This value is considerably lower than the value proposed by Smolders *et al.* (1995b).

Table 37 Values for the kinetic parameters of the metabolic model by Smolders et al. (1995b)

q_{Ac}	p(42bis)	rate constant for acetate uptake	8.53	g COD-S_A g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for storage X_{PP}	4.13	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
μ_{PAO}	p(44)	maximum growth rate of PAO	3.36	g COD-X_{PAO} g^{-1} COD d^{-1}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.001	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	32.0	g COD-S_A m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage	3.10	g P-S_{PO4} m^{-3}
K_{PP}	p(55)	saturation coefficient for poly-phosphate	0.001	g P-X_{PP} g^{-1} COD-X_{PAO}
K^{PP}_{MAX}	p(56)	maximum ratio of X_{PP}/X_{PAO} [1]	0.26	g P-X_{PP} g^{-1} COD-X_{PAO}
q_{GL}		rate constant for glycogen production [1]	17.1	g COD-X_{GL} g^{-1} COD d^{-1}
m_{anaer}		anaerobic maintenance coefficient	$8.3\ 10^{-2}$	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
m_{aer}		aerobic maintenance coefficient	$9.6\ 10^{-2}$	g COD-X_{PHB} g^{-1} COD-X_{PAO} d^{-1}
K^{GL}_{MAX}		saturation coefficient for glycogen production	1.2	g COD-X_{GL} g^{-1} COD-X_{PAO}

Values are presented in Smolders *et al.* (1995b) in molar units and per unit of time in hour. Recalculated to mass units and to unit of time in days.

[1] Values in Smolders (1995) are different (K^{PP}_{MAX} = 0.34 g P-X_{PP} g^{-1} COD-X_{PAO}; k_{GL} = 19.2 g COD-X_{GL} g COD d^{-1})

The stoichiometric coefficients considered in this model are given in Table 38. Although one of the important results obtained in the work of Smolders (1995) is the study of the pH influence on the anaerobic EBPR process, this influence on the stoichiometric parameters is not explicitly accounted for in the model. The values mentioned in the tables are valid for a pH value of 7.

In Table 38 distinction has been made between the coefficient for poly-P requirement per PHA amount stored or poly-P requirement per acetate uptake. The value of 0.40 g P-X_{PP} g^{-1} COD-X_{PHA} used in ASM2d should be compared with a value of 0.45 g P-X_{PP} g^{-1} COD-S_A (indeed, since for ASM2d all PHA is formed out of S_A the value of 0.4 mentioned in the model can be interpreted as based on S_A or on X_{PHA}. The first interpretation is the correct one when also accounting for glycogen breakdown for PHA storage). A stoichiometric coefficient has also been introduced to account for anaerobic glycogen consumption. In this case, a distinction has been made between the glycogen consumption related to stored PHA or related to acetate taken up.

Table 38 Stoichiometric coefficients for the metabolic model of Smolders (1995)

Y_{PAO}	p(18)	yield coefficient (biomass/PHA)	0.73	g COD-X_{PAO} g^{-1} COD-X_{PHA}
$Y_{PO4, PHA}$ (**)	p(19)	PP requirement (PO_4 release) per PHA stored	0.30	g P-X_{PP} g^{-1} COD-X_{PHA}
$Y_{PO4, SA}$ (**)		PP requirement (PO_4 release) per S_A stored	0.44	g P-X_{PP} g^{-1} COD-S_A
$Y_{GL, PHA}$		Glycogen requirement per PHA stored	0.33	g COD-X_{GL} g^{-1} COD-X_{PHA}
$Y_{GL, SA}$ (*)		Glycogen requirement per S_A stored	0.50	g COD-X_{GL} g^{-1} COD-S_A
Y_{PHA}	p(20)	PHA requirement for PP storage	0.31	g COD-X_{PHA} g^{-1} P

(*) To be compared with [1-Y_{SA}] used by Manga *et al.* (2000) with proposed value: Y_{SA}= 0.75 g COD-S_A g^{-1} COD-X_{PHA}

(**) Value mentioned by Smolders (1995) in the final table are wrong. Values in this review are deduced from the equations

4.3.5.2. ***Revised metabolic model for non-denitrifying PAOs (Filipe and Daigger, 1998)***

According to Filipe and Daigger (1998) applying the model developed by Smolders *et al.* (1995b) to continuous flow data, revealed inconsistencies. The treatment system considered by Filipe and Daigger (1998) was operated under carbon limiting conditions instead of phosphorus limiting conditions as was the case for the experimental set-up used by Smolders (1995).

Table 39 Process rate equations for PAOs in the revised metabolic model of Filipe and Daigger (1998)

10 bis	Uptake of S_A (Storage of X_{PHA}) (*)	$q_{PHA} \cdot \frac{S_A}{K_{PA} + S_A} \cdot (\text{Glycogen switch}) \cdot (\text{Poly} - \text{P switch}) \cdot \frac{K_{PO2}}{K_{PO2} + S_{O2}} \cdot X_{PAO}$
11	Aerobic storage of X_{PP}	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2} + S_{O2}} \cdot \frac{S_{PO4}}{K_P + S_{PO4}} \cdot \frac{X_{PHA} / X_{PAO}}{K_{PHA} + X_{PHA} / X_{PAO}} \cdot \frac{K^{PAO}_{MAX,PP} - X_{PP} / X_{PAO}}{1.05 \cdot K^{PAO}_{MAX,PP} - X_{PP} / X_{PAO}} \cdot X_{PAO}$
13	Aerobic growth on X_{PHA}	$\mu_{PAO} \cdot \frac{S_{O2}}{K_{PO2} + S_{O2}} \cdot \frac{X_{PHA}}{X_{PAO}} \cdot X_{PAO}$
	Aerobic formation of X_{GL} (**)	$q_{GL} \cdot \frac{S_{O2}}{K_{PO2} + S_{O2}} \cdot \frac{X_{PHA}}{X_{PAO}} \cdot \frac{K_{GL} + X_{GL} / X_{PAO}}{X_{GL} / X_{PAO}} \cdot X_{PAO}$
	Anaerobic maintenance	$m_{anaer} \cdot \frac{K_{PO2}}{S_{O2} + K_{PO2}} \cdot X_{PAO}$
	Aerobic maintenance	$m_{aer} \cdot \frac{S_{O2}}{K_{PO2} + S_{O2}} \cdot X_{PAO}$

(*) Glycogen switch : if $X_{GL} > 0$, then 1 otherwise 0 ;Poly-P switch : if $X_{PP} > 0$, then 1 otherwise 0

(**) Possibly the surface rate expression for X_{GL} should be $\frac{K_{GL} - X_{GL} / X_{PAO}}{X_{GL} / X_{PAO}}$

Comparing the revised model (Table 39) with the model proposed by Smolders *et al.* (1995b) reveals the following changes:

- *Storage of acetate* is stopped when glycogen or poly-P would become 0, whereas Smolders *et al.* (1995b) only took into account poly-P limitation. Glycogen limitation was introduced by Filipe and Daigger (1998) after indirect evidence obtained by Randall and Chapin (1995). In 1997 Murnleitner *et al.* also accounted for possible glycogen limitations. In their model Manga *et al*. (2000) accounted for glycogen limitations as well.
- For the *aerobic storage of poly-P* the power function for the PHA concentration is changed to the expression used in ASM2d, i.e. a surface reaction. Again another function is introduced for the dependency on the poly-P content.
- The main difference between the models is found in the rate equation for *aerobic formation of glycogen* : the limitation of glycogen formation on the previous anaerobic phase is no longer considered, i.e. the amount of glycogen was consumed anaerobically does not influence the glycogen replenishment. It is stressed that in any steady state system, the system will be balanced with replenishment of the glycogen under aerobic conditions to an amount equal to the initial anaerobic concentration. The change introduced by Filipe and Daigger (1998), however, allows for a better prediction of the system under non-steady state conditions, where for example the anaerobic glycogen content is increasing each cycle until steady state is reached. The expression used for the glycogen dependency is different from the one proposed by Manga *et al.* (2000). The rate of aerobic glycogen storage is also made proportional to the PHA content. Since glycogen is formed out of PHA this term is to be expected. In their model, Manga *et al.* (2000) considered a surface reaction for PHA and used a Monod-like term in the rate equation.

Table 40 Values for the kinetic parameters in the revised metabolic model of Filipe and Daigger (1998)

q_{Ac}	p(42bis)	rate constant for acetate uptake (*)	8.64	g COD-S_A g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for storage X_{PP}	3.07	g P-X_{PP} g^{-1} X_{PAO} d^{-1}
μ_{PAO}	p(44)	maximum growth rate of PAO (*)	3.34	g COD-X_{PAO} g^{-1} COD d^{-1}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.001	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	32.0	g COD m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage	4.266	g P-S_{PO4} m^{-3}
$K^{PAO}_{MAX,PP}$	p(56)	maximum ratio of X_{PP}/X_{PAO}	0.36	g P-X_{PP} g^{-1} COD-X_{PAO}
K_{PHA}	p(58)	saturation coefficient for PHA	0.136	g COD-X_{PHA} g^{-1} COD-X_{PAO}
q_{GL}		rate constant for glycogen production	4.32	g COD-X_{GL} g^{-1} COD d^{-1}
m_{anaer}		anaerobic maintenance coefficient (*)	$8.2\ 10^{-2}$	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
m_{aer}		aerobic maintenance coefficient	$9.6\ 10^{-2}$	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
K^{MAX}_{GL}		saturation coefficient for glycogen production	$1.56\ 10^{-2}$	g COD-X_{GL} g^{-1} COD-X_{PAO}

(*) values correspond nearly exactly to the ones proposed by Smolders *et al.* (1995b). Possibly Filipe and Daigger (1998) made a mistake recalculating the values from Smolders *et al.* (1995b) expressed as molar values to their own units. In their later model Filipe and Daigger (1999) used "the correct" values for q_{Ac} and μ_{PAO} (for m_{anaer} the authors later use the value proposed by Kuba *et al.* (1996a)).

Comparing the values of the kinetic parameters and the stoichiometric coefficients proposed by Filipe and Daigger (1998) and those proposed by Smolders *et al.* (1995b) reveals the following (Table 40):

- The maximum ratio of X_{PP}/X_{PAO} is set at 0.36 g P-X_{PP} g^{-1} COD-X_{PAO} as it was originally suggested by Smolders (1995) in thes final table of his Ph.D.
- Due to the different rate equations used by the different authors, the rate constants for glycogen production are different.

- The values for the Poly-P requirement per PHA stored and per S_A stored respectively are higher than the values proposed by Smolders *et al.* (1995b). The system considered by Filipe and Daigger (1998) was operated at pH 7.5, indicating that pH differences can be used to explain for the difference observed.

Table 41 Stoichiometric coefficients for the revised metabolic model (after Filipe and Daigger, 1998)

Y_{PAO}	p(18)	yield coefficient (biomass/PHA)	0.73	g COD-X_{PAO} g^{-1} COD-X_{PHA}
$Y_{PO4, PHA}$	p(19)	PP requirement (PO_4 release) per PHA stored [1]	0.38	g P g^{-1} COD-X_{PHA}
$Y_{PO4, SA}$		PP requirement (PO_4 release) per S_A stored [1]	0.56	g P g^{-1} COD-S_A
$Y_{GL, PHA}$		Glycogen requirement per PHA stored	0.33	g COD-X_{GL} g^{-1} COD-X_{PHA}
$Y_{GL, SA}$		Glycogen requirement per S_A stored	0.50	g COD-X_{GL} g^{-1} COD-S_A
Y_{PHA}	p(20)	PHA requirement for PP storage	0.31	g COD-X_{PHA} g^{-1} P

[1] Valid at pH=7.5, value is pH dependent.

4.3.5.3. ***Metabolic model for denitrifying PAOs (Kuba et al., 1996a)***

For the development of their model Kuba *et al.* (1996a) relied on the metabolic model developed by Smolders (1995). To establish a model for denitrifying PAOs (DPAOs), they subjected the biomass to anaerobic-anoxic conditions. Aerobic conditions never occurred. As such, Kuba *et al.* (1996a) were not only able to prove the denitrifying capacity of PAOs, but they were also able to determine the parameters for the anoxic reactions without interference with strictly aerobic PAOs.

The metabolic model structure developed by Smolders (1995) could easily be tuned into a model for DPAOs. However, several model parameters differ (Table 43). From a physiological point of view, the kinetic parameters could be expected to be similar, since the metabolism is essentially thought to be the same. However, the micro-organisms involved can be different, in which case the stoichiometry and the kinetic structure should not be affected, but the kinetic parameters can change strongly. Although Murnleitner *et al.* (1997) (4.3.5.5) and Brdjanovic (1998a) (4.3.5.6) later presented and used a unified model using only one biomass component, the problem will surely be readressed when EBPR will be incorporated in ASM3.

Table 42 Process rate equations for DPAOs in the metabolic model of Kuba et al. (1996a)

10 bis	Uptake of S_A (Storage of X_{PHA})	$q_{Ac} \cdot \frac{S_A}{K_{PA}+S_A} \cdot \frac{K_{HNO3}}{S_{NO3}+K_{HNO3}} \cdot X_{DPAO}$
12	Anoxic storage of X_{PP}	$q_{DPP} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{K^{PP}_{MAX} - X_{PP}/X_{DPAO}}{K^{PP}_{MAX}} \cdot \left(\frac{X_{PHA}}{X_{DPAO}}\right)^{\frac{1}{3}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot X_{DPAO}$
14	Anoxic growth on X_{PHA}	$\mu_{DPAO} \cdot \frac{X_{PHA}}{X_{DPAO}} \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{DPAO}$
	Anoxic formation of X_{GL}	$q_{DGL} \cdot \left(K^{GL}_{MAX} - \frac{X_{GL}}{X_{DPAO}}\right) \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{DPAO}$
	Anaerobic maintenance	$m_{anaer} \cdot \frac{K_{HNO3}}{K_{HNO3}+S_{NO3}} \cdot X_{DPAO}$
	Anoxic (ATP) maintenance	$m_{anox} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{DPAO}$

Comparing the model for DPAOs proposed by Kuba *et al.* (1996a) (Table 43) with the model for PAOs proposed by Smolders *et al.* (1995b) reveals the following changes:

- *Storage of acetate* is presented without a poly-P switching function and without a glycogen switching function as it was presented by Smolders *et al.* (1995b), Murnleitner *et al.* (1997) and Manga *et al.* (2000). It is thought in general that this switching function should be introduced to prevent anaerobic storage of acetate when the poly-P pool is exhausted.
- *Anoxic formation of glycogen* is modelled without the glycogen replenishment being limited by the anaerobic amount of glycogen used as was suggested by Smolders *et al.* (1995b) and contested by Filipe and Daigger (1998). Smolders *et al.* (1995b) introduced this limitation when evaluating the results obtained under different SRTs. Apparently, Kuba *et al.* (1996a) were able to model their experimental results for different SRTs without necessitating this limitation.

Although Kuba *et al.* (1994) observed acetate uptake under anoxic conditions, i.e. they observed acetate uptake under anaerobic conditions when nitrate was present using DPAO sludge, this process was not modelled by the same group of researchers in their model (Kuba *et al.*, 1996a). Filipe and Daigger (1999) will use this observation to present their revised model (4.3.5.4).

Table 43 Definition of values for the kinetic parameters of the metabolic model by Kuba et al. (1996a)

K_{HNO3}	p(38)	saturation coefficient for nitrate	0.035	g N-S_{NO3} m^{-3}
q_{Ac}	p(42bis)	rate constant for acetate uptake	4.27	g COD-S_A g^{-1} COD-X_{PAO} d^{-1}
q_{DPP}	p(43)	rate constant for storage X_{PP}	2.07	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
μ_{DPAO}	p(44)	maximum growth rate of PAO	1.20	g COD-X_{PAO} g^{-1} COD d^{-1}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	32.0	g COD-S_A m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage	3.10	g P-S_{PO4} m^{-3}
K^{PP}_{MAX}	p(56)	maximum ratio of X_{PP}/X_{PAO}	0.36	g P-X_{PP} g^{-1} COD-X_{PAO}
q_{GL}		rate constant for glycogen production	19.9	g COD-X_{GL} g^{-1} COD-X_{PAO} d^{-1}
m_{anaer}		anaerobic maintenance coefficient	$5.2\ 10^{-3}$	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
m_{anox}		anoxic maintenance coefficient	$8.6\ 10^{-2}$	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
K^{GL}_{MAX}		maximum ratio of X_{GL}/X_{PAO}	0.27	g COD-X_{GL} g^{-1} COD-X_{PAO}

Comparing the kinetic parameters in the model for DPAOs proposed by Kuba *et al.* (1996a) (Table 42) with the kinetic parameters in the model for PAOs proposed by Smolders *et al.* (1995b) reveals the following important changes:

- the *rate constant for anaerobic acetate uptake* is only half the value mentioned by Smolders *et al.* (1995b). When combining DPAOs and PAOs this needs to be accounted for properly, i.e. the fraction of DPAOs needs to be considered, plus on top of that the reduced rate coefficient. Kuba *et al.* (1996c) demonstrated that PAOs that are only able to denitrify do not exist, i.e. the population is able to use both oxygen and nitrate as terminal electron acceptor.
- the *rate constant for anoxic poly-P uptake* is only half the value mentioned by Smolders *et al.* (1995b). This is in accordance with the observation by Kuba *et al.* (1996a) that the energy production efficiency with nitrate compared to oxygen is about 40%. When combining DPAOs and PAOs this needs to be accounted for properly, i.e. the fraction of DPAOs needs to be considered, plus on top of that the reduced rate coefficient.
- the *maximum growth rate* was observed to be much smaller for DPAOs than for PAOs and was thus modelled accordingly.
- the *rate constant for anoxic glycogen production* has a completely different value compared to the one proposed by Smolders *et al.* (1995b), which is caused by the different rate equation proposed by the different authors.

- the *anaerobic maintenance coefficient* is smaller than the value reported by Smolders *et al.* (1995b). Kuba *et al.* (1996a) observed that the anaerobic phosphate release for maintenance was 50% of the value determined by Smolders *et al.* (1995b).
- the *anoxic maintenance coefficient* has ± the same value as the one presented by Smolders *et al.* (1995b)

4.3.5.4. *Coupled metabolic model for non-denitrifying PAOs and denitrifying PAOs (Filipe and Daigger, 1999)*

In this literature review two subparagraphs deal with non-PAOs and DPAOs. Because of the important difference between both models presented by different authors, these models are presented separately. Moreover, since Filipe and Daigger (1999) mainly followed the ideas proposed by Kuba *et al.* (1996a), their model is presented prior to the one presented by Murnleitner *et al.* (1997), although the latter was published earlier.

Some of the kinetic expressions presented in their 1998 paper are altered and resemble more the kinetic expressions presented below by Murnleitner *et al.* (1997). As an important difference, it is observed that whereas in ASM2d anoxic reactions for DPAOs are modelled using the same basic rate expression as the corresponding aerobic reactor multiplying the latter with a reduction factor for anoxic activity, Filipe and Daigger (1999) made explicit distinction between rate reduction due to a lower activity under anoxic conditions (Table 46) and between the rate reduction caused by the fraction of PAOs capable of also using nitrate as final electron acceptor. The authors did not mention the fraction of DPAOs observed.

Table 44 Process rate equations for PAOs and DPAOs in the integrated model of Filipe and Daigger (1999)

Denitrifying phosphorus accumulating organisms (DPAOs) and non-denitrifying phosphorus accumulating organisms (PAOs): X_{DPAO} and X_{PAO} (combined reactions)		
10 bis	Uptake of S_A (Storage of X_{PHA})	$q_{PHA} \cdot \frac{S_A}{K_{PA}+S_A} \cdot (\text{Glycogen switch}) \cdot (\text{Poly}-\text{P switch}) \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot X_{(D)PAO}$
11	Aerobic storage of X_{PP}	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \left(\frac{X_{PHA}}{X_{(D)PAO}}\right)^{1/3} \cdot \frac{K_{MAX}^{PP} - X_{PP}/X_{(D)PAO}}{K_{MAX}^{PP}} \cdot X_{(D)PAO}$
13	Aerobic growth on X_{PHA}	$\mu_{PAO} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{X_{PHA}}{X_{(D)PAO}} \cdot X_{(D)PAO}$
	Aerobic formation of X_{GL}	$q_{GL} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot (K_{MAX}^{GL,aer} - \frac{X_{GL}}{X_{(D)PAO}}) \cdot X_{(D)PAO}$
	Anaerobic maintenance	$m_{anaer} \cdot \frac{K_{PO2}}{S_{O2}+K_{PO2}} \cdot X_{(D)PAO}$
	Aerobic maintenance	$m_{aer} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot X_{(D)PAO}$
Denitrifying phosphorus accumulating organisms (DPAOs): X_{DPAO} (specific reactions)		
	Anoxic uptake of S_A	$q_{PHA}^{anox} \cdot \frac{S_A}{K_{PA}+S_A} \cdot (\text{Poly}-\text{P switch}) \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{DPAO}$
	Anoxic growth on X_{PHA}	$\mu_{PAO}^{anox} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{X_{PHA}}{X_{PAO}} \cdot X_{(D)PAO}$
	Anoxic storage of X_{GL}	$q_{Gl}^{anox} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot (K_{MAX}^{GL,anox} - \frac{X_{GL}}{X_{DPAO}}) \cdot X_{DPAO}$
	Anoxic maintenance	$m_{anox} \cdot \frac{K_{PO2}}{S_{O2}+K_{PO2}} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{DPAO}$

Evaluating the integrated model for PAOs and DPAOs proposed by Filipe and Daigger (1999) and presented in Table 44, the following observations are made:

- The *aerobic storage of polyphosphate* is modelled differently from the authors' own prior model (1998), i.e. instead of using a surface rate reaction for PHA, the PHA dependency is now modelled as a power function as proposed by Smolders *et al.* (1995b). It is stressed again that the shape of both functions is alike.
- The *aerobic formation of glycogen* is modelled according to the rate expression proposed by Kuba *et al.* (1996a). Filipe and Daigger's own prior model (1998) has been abandoned.
- Additionally to the model presented by Kuba *et al.* (1996a), Filipe and Daigger (1999) introduced in their model a kinetic expression for *acetate uptake under anoxic conditions* with concurrent PHA production.
- *Anoxic maintenance* is only considered active for DPAOs.

Table 45 Values for the kinetic parameters proposed by Filipe and Daigger (1999)

q_{Ac}	p(42bis)	anaerobic rate constant for acetate uptake	8.53	g COD-S_A g^{-1} COD-X_{PAO} d^{-1}
q_{Ac}^{Anox}		anoxic rate constant for acetate uptake	8.53	g COD-S_A g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for aerobic storage of PP	4.13	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
q_{PP}^{Anox}		rate constant for anoxic storage of PP	2.07	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
μ_{PAO}	p(44)	maximum aerobic growth rate of PAO	3.36	g COD-X_{PAO} g^{-1} COD d^{-1}
μ_{PAO}^{Anox}		maximum anoxic growth rate of PAO	1.20	g COD-X_{PAO} g^{-1} COD d^{-1}
K_{PA}	p(50)	saturation coefficient for acetate, S_A	32.0	g COD-S_A m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage	3.10	g P-S_{PO4} m^{-3}
K_{MAX}^{PP}	p(56)	maximum ratio of X_{PP}/X_{PAO}	0.26	g P-X_{PP} g^{-1} COD-X_{PAO}
q_{GL}		rate constant for aerobic glycogen production	17.07	g COD-X_{GL} g^{-1} COD-X_{PAO} d^{-1}
q_{GL}^{Anox}		rate constant for anoxic glycogen production	17.07	g COD-X_{GL} g^{-1} COD-X_{PAO} d^{-1}
m_{anaer}		anaerobic maintenance coefficient	$5.2\ 10^{-3}$	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
m_{anox}		anoxic maintenance coefficient	$8.64\ 10^{-2}$	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
m_{aer}		aerobic maintenance coefficient	$9.6\ 10^{-2}$	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
$K_{MAX}^{GL,aer}$		aerobic maximum ratio of X_{GL}/X_{PAO}	0.24	g COD-X_{GL} g^{-1} COD-X_{PAO}
$K_{MAX}^{GL,anox}$		anoxic maximum ratio of X_{GL}/X_{PAO}	0.27	g COD-X_{GL} g^{-1} COD-X_{PAO}

Evaluating the kinetic parameters (Table 45) proposed by Filipe and Daigger (1999) the following observations are made:

- The *rate constant for anaerobic acetate uptake* is equal to the value proposed by Smolders *et al.* (1995b), thus double the value proposed by Kuba *et al.* (1996a).
- The *rate constant for anoxic poly-P uptake* is half the rate constant for aerobic poly-P uptake, in accordance with the findings of Kuba *et al.* (1996a).
- The *anaerobic maintenance coefficient* is taken according to Kuba *et al.* (1996a), thus half the value in Filipe and Daigger's own model (1998) where the coefficient was taken following Smolders *et al.* (1995b). The *aerobic maintenance coefficient* remained unchanged.
- The *rate constants for anoxic and aerobic glycogen production* are considered equal to one another and are nearly equal to the anoxic rate constant proposed by Kuba *et al.* (1996a) (17.07 and 19.9 g COD-X_{GL} g^{-1} COD-X_{PHA} d^{-1} respectively).

- The maximum ratio of X_{PP}/X_{PAO} is taken according to Smolders *et al.* (1995b), which is lower than the value proposed in Filipe and Daigger's own prior model and lower than the value proposed by Kuba *et al.* (1996a).

In Table 46, an overview is provided of the rate reduction coefficients for the anoxic processes for DPAOs. Indeed, using their model structure, Filipe and Daigger (1999), as Kuba *et al.* (1996a), had to introduce a reduction coefficient to model the anoxic process properly.

Table 46 Rate reduction coefficients for anoxic processes for DPAOs

Process	Expression	Anoxic rate reduction factor
Storage of polyphosphate	q_{PP}^{Anox}/q_{PP}	0.50
Formation of glycogen	q_{GL}^{Anox}/q_{GL}	1.00
Growth of DPAOs	$\mu_{DPAO}^{Anox}/\mu_{DPAO}$	0.35
Maintenance	m_{anox}/m_{aer}	0.90

4.3.5.5. ***Integrated metabolic model for non-denitrifying PAOs and denitrifying PAOs (Murnleitner et al., 1997)***

Based on the results obtained by Smolders *et al.* (1995b) and by Kuba *et al.* (1996a), Murnleitner *et al.* (1997) combined the existing knowledge into one model. A kinetic structure was proposed, assuming a fraction of the phosphorus removing population has denitrifying capacities but also assuming that essentially the same rate equations can be used. Therefore, Murnleitner *et al.* (1997) introduced an important conceptual change. Whereas Smolders *et al.* (1995b) and Kuba *et al.* (1996a) used aerobic product formation rates (i.e. production of poly-P, glycogen and biomass) as starting point for the kinetic expressions necessary to describe the process and thus obtained substrate (PHA) conversion as a result, in the model presented by Murnleitner *et al.* (1997) biomass growth is taken as a result and kinetic expressions are proposed for PHA consumption, polyphosphate and glycogen production and maintenance (see also 3.4.3.4). Whereas in the kinetic expressions this difference is not always very easily observed, the difference becomes much more apparent when considering the stoichiometric matrix, where the growth of biomass is now stoichiometrically coupled with the consumption of PHA. Moreover, from Table 48, it is obvious rate reduction coefficients for the anoxic phase do not have to used anymore.

Murnleitner *et al.* (1997) introduced quite a number of changes compared to the models presented by Smolders *et al.* (1995b) and by Kuba *et al.* (1996a) (Table 47). These differences are:

- *Aerobic storage of poly-P* was observed to be negatively influenced by the poly-P content of the biomass, but to be independent of the PHA content of the cells. The latter is a clear difference between the observations made by the authors compared to the work of their prior colleagues, which was not explained. Whereas most other authors generally introduce a Monod-inhibition term or a maximum fraction term, Murnleitner *et al.* (1997), introduced a simple inverted first order reaction, which is inconvenient for easy comparison between models. This model is chosen for because it only required one parameter and because it was capable of describing the experimental observations (van Loosdrecht, personal communication). Numerical problems, possibly occurring when the poly-P content becomes nearly zero, are not reported. Later research performed by the same research group recognised the numerical problem and re-introduced the Monod-type equations (van Loosdrecht, not yet published results, personal communication).
- the *aerobic consumption of X_{PHA}* is modelled to be proportional to the PHA content to the power 2/3. The authors state that when the PHA content increases, e.g. doubles, the specific surface area for

reaction only increases with $2^{2/3}$. In prior models, aerobic growth on PHA was the starting point and aerobic consumption of PHA was a result (3.4.3.4.)

- the *glycogen formation rate* is assumed to depend positively on the PHA and negatively on the glycogen content. The dependency on the PHA content is again modelled as a surface rate reaction, introducing the power function, whereas for glycogen a simple inverted first order reaction is introduced.
- For the *anoxic storage of poly-P and glycogen and the anoxic consumption of PHA* a reduction factor is introduced to account for the fraction of PAOs capable of using nitrate as final electron acceptor.
- for the *anaerobic maintenance* a poly-P switching function is introduced slowing down anaerobic breakdown of poly-P for maintenance when the concentration of this component becomes lower.

The authors reported that the kinetic equations for anaerobic acetate uptake could not always accurately describe the behaviour experimentally observed.

Table 47 Integrated process rate equations for PAOs and DPAOs in the metabolic model of Murnleitner et al. (1997)

10 bis	Uptake of S_A (Storage of X_{PHA})	$q_{Ac} \cdot \frac{S_A}{K_{PA}+S_A} \cdot (\text{Glycogen switch}) \cdot (\text{Poly - P switch}) \cdot X_{(D)PAO}$
11	Aerobic storage of X_{PP}	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{1}{X_{PP}/X_{(D)PAO}} \cdot X_{(D)PAO}$
12	Anoxic storage of X_{PP}	$\rho_{12} = \rho_{11} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K^{PP}_{HNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}}$
13 bis	Aerobic consumption of X_{PHA}	$k_{PHA} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \left(\frac{X_{PHA}}{X_{(D)PAO}}\right)^{2/3} \cdot (\text{Poly - P switch}) \cdot X_{(D)PAO}$
14 bis	Anoxic consumption of X_{PHA}	$\rho_{14} = \rho_{13} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K^{PHB,GL}_{HNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}}$
	Aerobic formation of X_{GL}	$q_{GL} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \left(\frac{X_{PHA}}{X_{(D)PAO}}\right)^{2/3} \frac{1}{X_{Gl}/X_{(D)PAO}} \cdot (\text{Poly - P switch}) \cdot X_{(D)PAO}$
	Anoxic formation of X_{GL}	$\rho_x = \rho_{x-1} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K^{PHB,GL}_{HNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}}$
	Anaerobic maintenance	$m_{anaer} \cdot (\text{poly - P switch}) \cdot X_{(D)PAO}$
	Aerobic maintenance	$m_{aer} \cdot \frac{S_{O2}}{K_{PO2}+S_{PO2}} \cdot X_{(D)PAO}$
	Anoxic maintenance	$m_{anox} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot X_{(D)PAO}$

All switches, i.e. glycogen, poly-P and oxygen and nitrate are defined as $S_?/(K_? + S_?)$ with $K_? = 0.0001$

Comparing the kinetic parameters in the integrated model proposed by Murnleitner *et al.* (1997) (Table 48) with the model for DPAOs proposed by Kuba *et al.* (1996a) and with the kinetic parameters in the model for PAOs proposed by Smolders *et al.* (1995b) reveals the following important changes:

- the *rate constant for acetate uptake* is taken as the average value from Smolders *et al.* (1995b) and Kuba *et al.* (1996a).
- the *rate constant for poly-P storage* is significantly smaller than the values obtained by Smolders *et al.* (1995b) and Kuba *et al.* (1996a). The difference can easily be attributed to the different rate expressions used.
- the *rate constant for PHB consumption* is new introduced. This parameter should not be confused with the maximum growth rate which has a different value but also an inversed unit (cf. Smolders *et al.*, 1995b and Kuba *et al.* 1996a).
- the *rate constant for glycogen formation* is significantly smaller than the values obtained by Smolders *et al.* (1995b) and Kuba *et al.* (1996a). No remark is made to this respect by the authors.

Table 48 Definition and values for the kinetic parameters of the metabolic model by Murnleitner et al. (1997)

K_{HNO3}	p(38)	saturation coefficient for nitrate	0.0001	g N m^{-3}
q_{Ac}	p(42bis)	rate constant for acetate uptake	6.4	g COD-S_A g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for storage of PP	1.03 10^{-1}	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen	0.0001	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A [1]	32.0	g COD m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage [1]	3.10	g P m^{-3}
K_{PP}	p(55)	saturation coefficient for poly-phosphate	0.0001	g P-X_{PP} g^{-1} COD-X_{PAO}
q_{GL}		rate constant for glycogen production	0.43	g COD-X_{GL} g^{-1} COD-X_{PAO} d^{-1}
k_{PHA}		rate constant for PHB consumption	7.20	g COD-X_{PHB} g^{-1} COD-X_{PAO} d^{-1}
m_{anaer}		anaerobic maintenance coefficient [2]	5.16 10^{-2}	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
m_{aer}		aerobic (ATP) maintenance coefficient [2]	8.6 10^{-2}	g COD g^{-1} COD-X_{PAO} d^{-1}
m_{anox}		anoxic maintenance coefficient [2]	8.6 10^{-2}	g COD g^{-1} COD-X_{PAO} d^{-1}
$K_{HNO3}^{PHB,GL}$		saturation coefficient for nitrate for growth and glycogen formation	1.4	g N-NO_3 m^{-3}
K_{HNO3}^{PP}		saturation coefficient for nitrate for poly-P formation	0.14	g N-NO_3 m^{-3}

Remark: all values are presented in the paper as molar values and per hour. They were recalculated to mass and per day for reasons of comparison with other models.

[1] By Murnleitner *et al.* (1997) taken from Smolders *et al.* (1995a)

[2] By Murnleitner *et al.* (1997) taken from Kuba *et al.* (1993)

4.3.5.6. *ASM2 + Delft BPR Model (Brdjanovic, 1998)*

The model proposed by Brdjanovic (1998) is a combination of the integrated model for denitrifying and non-denitrifying biological phosphorus removal proposed by Murnleitner *et al.* (1997) (4.3.5.4) and ASM2, the latter not taking into account reactions accounting for EBPR. However, in his model Brdjanovic made several changes to the original model suggested by Murnleitner *et al.* (1997) and therefore the model is listed below (Table 49). For the non-EBPR related reactions Brdjanovic (1998) mainly used the values suggested in ASM2d (except for the maximum fermentation rate). For the EBPR related reactions, parameters were taken from Henze *et al.* (1995), Smolders (1995), Murnleitner *et al.* (1997) and from own experimental evidence (Table 50).

Table 49 Process rate equations for PAOs and DPAOs in the metabolic model of Brdjanovic (1998)

10 bis	Uptake of S_A (*) (Storage of X_{PHA})	$q_{Ac} \cdot \frac{S_A}{K_{PA}+S_A} \cdot (\text{Glycogen swith}) \cdot (\text{Poly - P switch}) \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot \frac{K_{PNO3}}{K_{PNO3}+S_{NO3}} X_{PAO}$
11	Aerobic storage of X_{PP}	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2} \cdot g_{PP}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{1}{X_{PP}/X_{PAO}} \cdot X_{PAO}$
12	Anoxic storage of X_{PP}	$q_{PP} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K_{PNO3} \cdot g_{PP}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{1}{X_{PP}/X_{PAO}} \cdot X_{PAO}$
13 bis	Aerobic consumption of X_{PHA}	$k_{PHA} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \left(\frac{X_{PHA}}{X_{PAO}}\right)^{\frac{2}{3}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot X_{PAO}$
14 bis	Anoxic consumption of X_{PHA}	$k_{PHA} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K_{PNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{PNH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_{PN}+S_{PO4}} \cdot \left(\frac{X_{PHA}}{X_{PAO}}\right)^{\frac{2}{3}} \cdot \frac{S_{ALK}}{K_{PALK}+S_{ALK}} \cdot X_{PAO}$
X1	Aerobic formation of X_{GL}	$q_{GL} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \left(\frac{X_{PHA}}{X_{PAO}}\right)^{2/3} \frac{1}{X_{Gl}/X_{PAO}} \cdot X_{PAO}$
X2	Anoxic formation of X_{GL}	$\rho_x = \rho_{x-1} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K_{PNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}}$
	Anaerobic maintenance	$m_{anaer} \cdot (\text{poly - P switch}) \cdot X_{PAO}$
	Aerobic (ATP) maintenance	$m_{aer} \cdot \frac{S_{O2}}{K_{PO2}+S_{PO2}} \cdot \frac{X_{PHA}}{K_{PHA}+X_{PHA}} \cdot X_{PAO}$
	Anoxic (ATP) maintenance	$m_{anox} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{X_{PHA}}{K_{PHA}+X_{PHA}} X_{PAO}$

(*) In his model Brdjanovic again uses the terminology anaerobic storage of PHA. However, the rate constant and the reaction is to be interpreted as uptake of S_A. For reasons of clarity, in this table the terminology "uptake of S_A" is used.

Comparing the rate equations proposed by Brdjanovic (1998) with author references, the following observation can be made:

- For *uptake of S_A*, Brdjanovic (1998) introduced switches for oxygen and nitrate to prevent any anoxic or aerobic uptake of acetate. Whereas the models developed by Smolders (1995) and by Murnleitner *et al.* (1997) were developed for ideal situations where acetate breakthrough to the anoxic or aerobic phases did not occur, Brdjanovic (1998) needed a model applicable for full scale installations. He assumed that acetate can only be consumed anaerobically by PAOs, and that any acetate remaining in the other phases will be utilised for growth by heterotrophic bacteria. This statement is in contradiction with the observations reported by Kuba *et al.* (1994) and by Chuang *et al.* (1996).

- For the *aerobic (and anoxic) storage of X_{PP}* Brdjanovic (1998) used the assumption made by Murnleitner *et al.* (1997) suggesting storage of X_{PP} is not dependent on the X_{PHA} content of the micro-organisms. In addition to Murnleitner *et al.* (1997) a nitrate sensitivity factor (g_{PP}) was introduced in the denominator of the oxygen and nitrate switching functions. This sensitivity function in the rate expression for anoxic storage is also used by Murnleitner *et al.* (1997) but not explicitly. Indeed, in his anoxic rate expressions for PHA consumption, storage of X_{PP} and glycogen formation, a saturation coefficient is introduced which is made dependent of the phase, using a 10 fold lower value for the poly-P formation reaction. Brdjanovic (1998) used the same value for the saturation coefficient for all three reactions, but introduces a sensitivity factor which has a value of 0.1. For clarity reasons, the latter approach is to be preferred. It is, however, strange that for aerobic poly-P formation the sensitivity factor for nitrate is multiplied with the saturation coefficient for oxygen.

- Looking through the model proposed by Brdjanovic (1998), apparently no reaction is defined for the *consumption of PHA*. However, the reaction described as "*lysis of X_{PHA}*" apparently should be interpreted as the consumption of PHA as defined by Murnleitner *et al.* (1997). However, Brdjanovic (1998a) eliminated the poly-P switch and included alkalinity. The latter is justified since his model was applied for full scale modelling purposes where alkalinity limitation might prevent reactions to occur.
- For the *aerobic and anoxic maintenance* functions Brdjanovic (1998) introduced a PHA-switching function, not used by Murnleitner *et al.* (1997). This switching function is not considered for *anaerobic maintenance*. The latter is to be explained by the fact that PHA is stored anaerobically and thus inhibition due to lack of PHA is not to be expected. Moreover, anaerobic maintenance is modelled only as breakdown of poly-P for energy requirements.
- For the *aerobic (and anoxic) glycogen formation* Brdjanovic (1998) omitted the poly-P switch introduced by Murnleitner *et al.* (1997). He also neglected possible rate reduction due to ammonia limitation.

*Table 50 Definition of values for the kinetic and stoichiometric parameters of the model proposed by Brdjanovic (1998)**

q_{Ac}	p(42bis)	rate constant for acetate uptake [2]	9.67	g COD g^{-1} COD-X_{PAO} d^{-1}
q_{PP}	p(43)	rate constant for storage X_{PP} [4]	0.11	g P-X_{PP} g^{-1} X_{PAO} d^{-1}
η_{PNO3}	p(45)	reduction factor for anoxic activity	0.8	
K_{PO2}	p(49)	saturation/inhibition coefficient for oxygen [1]	0.02	g O_2 m^{-3}
K_{PA}	p(50)	saturation coefficient for acetate, S_A [1]	4	g COD m^{-3}
K_{PNH4}	p(51)	saturation coefficient for ammonium (nutrient) [1]	0.05	g N m^{-3}
K_P	p(52)	saturation coefficient for phosphorus in PP storage [1&]	0.01	g P m^{-3}
K_{PN}	p(53)	saturation coefficient for phosphate (nutrient) [3]	3.1	g P m^{-3}
K_{PALK}	p(54)	saturation coefficient for alkalinity (HCO_3^-) [1]	0.10	mol HCO_3^- m^{-3}
K_{PP}	p(55)	saturation coefficient for poly-phosphate	0.01	g X_{PP} g^{-1} COD-X_{PAO}
k_{PHA}		rate constant for PHB consumption	7.55	g COD-X_{PHA} g^{-1} COD-X_{PAO} d^{-1}
k_{GL}		rate constant for glycogen production	0.15	g COD-X_{GL} g^{-1} COD d^{-1}
m_{anaer}		anaerobic maintenance coefficient [2]	0.05	g P g^{-1} COD-X_{PAO} d^{-1}
m_{aer}		aerobic maintenance coefficient [3]	0.06	g COD g^{-1} COD-X_{PAO} d^{-1}
m_{anox}		anoxic maintenance coefficient [3]	0.02	g N g^{-1} COD-X_{PAO} d^{-11}
K^{GL}_{MAX}		saturation coefficient for glycogen production	0.01	g COD g^{-1} COD-X_{PAO}
K_{PNO3}		saturation coefficient for nitrate [3]	1.4	g N-NO_3 m^{-3}
g_{PP}		nitrate sensitivity function	0.1	

* Kinetic parameters related to phosphorus uptake are presented. Only when corrections were suggested for non-PAO related reactions, have they been presented.

[1] By Brdjanovic (1998) taken from Henze *et al.* (1995)

[2] By Brdjanovic (1998) taken from Smolders *et al.* (1995a)

[3] By Brdjanovic (1998) taken from Murnleitner *et al.* (1997)

[4] By Brdjanovic (1998) taken from van Veldhuizen *et al.* (incomplete reference mentioned by Brdjanovic, 1998)

[&] According to the author after [1], but not corresponding with papers from this author! (see corresponding paragraphs)

4.3.6. Upgrading ASM3 to account for EBPR, precipitation and the role of magnesium

Currently research groups are updating ASM3 to account for EBPR. However, research papers are still under review and results can not be published in the scope of this overview. It is expected these results will be available 2001 (Rieger *et al*, submitted a, b).

Published results from a different research group are already available. They concern a calibration study based on the effluent characteristics of a cheese factory. The company was faced with more stringent phosphorus effluent standards. Because of the high content of volatile fatty acids available it was decided to design an SBR for EBPR. In the framework of this study a model was necessary to assess different operating conditions (Ky, 1999). The authors choose to update ASM3 to account for EBPR and for the additional reactions observed in the system.

A mathematical model was proposed, based on ASM3 for carbon an nitrogen removal (Henze, *et al.* 2000), on the Delft metabolic model (Murnleitner *et al.*, 1997) (see remarks Table 51) and on the model proposed by Maurer *et al.* (1999) for precipitation of phosphorus with calcium. Additionally the role of magnesium in EBPR is accounted for (Ky *et al.*, 2000).

The effluent of the cheese factory contained relatively high ortho-phosphate concentrations (66 mg P/ℓ). For these high concentrations it was observed that low magnesium concentrations limit EBPR. Magnesium limitations were accounted for by multiplying the aerobic and anoxic poly-P synthesis rates proposed by Murnleitner *et al.* (1997) with a switching function with the half saturation coefficient having the value of 10 mg Mg/ℓ. For the stoichiometric relationship taken into account between magnesium and phosphate a value of 0.157 g Mg/g P (0.2 mol Mg/mol P) is proposed. In Table 51 the additional and/or modified rate equations proposed by Ky (1999) are presented. Besides the inclusion of magnesium limitations and accounting for precipitation reactions, Ky (1999) also added aerobic and anoxic storage of acetate and fermentation of S_F since ASM3 does not comprise S_A as a model component.

In the work of Ky (1999) and Ky *et al*. (2000) a clear overview is lacking concerning the exact rate equations, kinetic and stoichiometric coefficients used, e.g. it is observed that values and rate equations reported by Filipe and Daigger (1998) (i.e. for aerobic and anoxic storage of X_{PP}) and reported by Brdjanovic (1998a) (i.e. for the aerobic maintenance) are used. However, in her work, Ky (1999) mentioned the complete listing used for simulating the SBR processes using Matlab 5.3 and GPS-X-2.4.1. All information is provided in this form. Table 51 is drawn based on this listing.

Calibrating the proposed model, it was observed that only a limited number of parameters had to be changed. In Table 52 and Table 53 the modified and additional values for the kinetic parameters and stoichiometric coefficients proposed by Ky (1999) and Ky *et al.* (2000) are presented.

- As for the value for $Y_{PO4,SA}$ the authors remark that adjusting the value from 0.44 g P g^{-1} COD-S_A (Smolders *et al.* 1994a and adjusted by Ky (1999) to account for the actual pH) to 0.22 g P g^{-1} COD-S_A is difficult to justify on a theoretical basis. The discrepancy therefore was attributed to the presence of GAOs, utilising VFAs without concurrent release of ortho-phosphate. The model proposed by Manga *et al.* (2000) could eventually be combined with Ky's model to verify this hypothesis. Additionally, Ky *et al.* (2000) attributed the observed difference to the presence of propionate and butyrate.
- The value for Y_{PHA} was lowered to stabilise the accumulation of X_{PAO}. At too high X_{PAO} concentration the Monod saturation function for magnesium had little affect.

*Table 51 Additional and/or modified process rate equations proposed by Ky (1999)**

	Aerobic storage of S_A	$k_{STO} \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_A}{K_F+S_A} \cdot X_H$
	Anoxic storage of S_A	$k_{STO} \cdot \eta_{HNO3} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{S_A}{K_F+S_A} \cdot X_H$
8	Fermentation (§)	$q_{fe} \cdot \frac{K_{HO2}}{K_{HO2}+S_{O2}} \cdot \frac{K_{HNO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{S_F}{K_{fe}+S_F} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot X_H$
11 bis	Aerobic storage of X_{PP} (*)	$q_{PP} \cdot \frac{S_{O2}}{K_{PO2}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{1}{X_{PP}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{1.05 \cdot K_{MAX}-X_{PP}/X_{PAO}} \cdot \frac{S_{Mg}}{K_{Mg}+S_{Mg}} \cdot X_{PAO}$
12 bis	Anoxic storage of X_{PP} (*)	$\rho_{12,bis} = \rho_{11,bis} \cdot \eta_{PNO3} \cdot \frac{S_{NO3}}{K^{PP}_{HNO3}+S_{NO3}} \cdot \frac{K_{PO2}}{K_{PO2}+S_{O2}}$
	Aerobic maintenance (**)	$m_{aer} \cdot \frac{S_{O2}}{K_{PO2}+S_{PO2}} \cdot \frac{X_{PHA}}{K_{PHA}+X_{PHA}} \cdot X_{PAO}$
	Anoxic (ATP) maintenance (**)	$m_{anox} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot \frac{X_{PHA}}{K_{PHA}+X_{PHA}} X_{PAO}$
	Precipitation of Hydroxydicalcium phosphate (HDP)	$k_{HDP} \cdot 0.46 \cdot S_{PO4} \cdot 0.66 \cdot S_{Ca} \cdot \frac{K_{HDP1}}{K_{HDP1}+X_{HDP}/X_{TSS}}$
	Redissolution of HDP	$k_{HDP} \cdot \frac{10^{-22.6}}{1.94E-14} \cdot \frac{10^{2.(14-pH)}}{0.66 \cdot S_{Ca}} \frac{X_{HDP}/X_{TSS}}{K_{HDP2}+X_{HDP}/X_{TSS}}$
	Precipitation of HAP	$k_{HAP} \cdot \frac{X_{HDP}/X_{TSS}}{K_{HDP2}+X_{HDP}/X_{TSS}}$
20	Precipitation of MeP (§)	$k_{PRE} \cdot S_{PO_4} \cdot X_{MeOH}$
21	Redissolution of MeP (§)	$k_{RED} \cdot X_{MeP} \cdot S_{ALK}/(K_{ALK}+S_{ALK})$

(*) rate equation is combination of the one proposed by Murnleitner *et al.* (1997) with on top of it the poly-P limitation proposed by Filipe and Daigger (1997). Dependency on X_{PP}/X_{PAO} thus appears twice in the rate equation.
(**) Rate equations proposed by Brdjanovic (1998a) are used.
(§) In accordance with ASM2d (Henze *et al.*, 1999)

Table 52 Additional and/or modified values for the kinetic parameters proposed by Ky (1999) and by Ky et al. (2000)

q_{PP}	p(43)	rate constant for storage X_{PP}	0.07	g P-X_{PP} g^{-1} COD-X_{PAO} d^{-1}
K_{Mg}		saturation coefficient for magnesium	10.0	g Mg m^{-3}
k_{HDP}		rate constant for HDP precipitation	0.29	g Ca m^{-3} d^{-1}
k_{HAP}		rate constant for HAP precipitation	15.5	g P m^{-3} d^{-1}
K_{HDP1}		saturation coefficient for HDP (precipitation of HDP)	3E-2	g T g^{-1} X_{TSS}
K_{HDP2}		saturation coefficient for HDP (redissolution of HDP & precipitation of HAP)	3E-5	g T g^{-1} X_{TSS}

Table 53 Additional and/or modified values for the stoichiometric coefficients proposed by Ky et al. (2000)

$Y_{PO4, SA}$		PP requirement (PO_4 release) per S_A stored [1]	0.22	g P-X_{PP} g^{-1} COD-S_A
$Y_{PHA, aer}$		Aerobic yield coefficient (PHA/biomass)	1.80	g COD-X_{PHA} g^{-1} COD-X_{PAO}
$Y_{PHA, anox}$		Anoxic yield coefficient (PHA/biomass)	2.20	g COD-X_{PHA} g^{-1} COD-X_{PAO}

[1] Valid at pH=6.8, value is pH dependent. According to Ky *et al.* (2000) Smolders *et al.* (1994a) would propose a value of 0.44 g P g^{-1} COD-S_A.

Whereas the model proposed by Ky (1999) and Ky *et al.* (2000) indicates the necessity to account for magnesium limitation when calibrating data of treatment units dealing with high phosphorus influent concentrations, it also needs to be stressed that this limitation in general will not occur when calibrating municipal WWTP. Indeed, with an average magnesium content of 5-30 mg Mg/ℓ in municipal wastewater (Arvin, 1985) and an average ortho-phosphate concentration of 10 mg P/ℓ for moderate municipal wastewater (Henze *et al.*, 1996) or a concentration of 3.6 mg P/ℓ as suggested in ASM2d (Henze *et al.*, 1999) or even concentrations ranging between 2 and 20 mg P/ℓ as it was suggested in ASM2 (Henze *et al.*, 1995) only a maximum of 4 mg Mg/ℓ is necessary. For municipal wastewater treatment magnesium therefore does not seem to be a limiting factor. This statement is confirmed by a study performed by Pattarkine (1991) who stated that the requirements for both magnesium and potassium are such that they are unlikely to be limiting in municipal wastewater, but need to be considered when treating industrial wastewater or wastewaters with high fractions of industrial wastewater mixed with domestic wastewater.

4.3.7. Model developments: conclusions

In this paragraph the reader will not encounter a unified model based on the observations from the different researchers, rather this section aims at highlighting the strengths and weaknesses of the models developed. Moreover, this paragraphs also aims at pinpointing the necessity to increase trust in the mathematical models yet developed.

Since the initiative was taken to develop a standard activated sludge model (1985), researchers gained an ever increasing interest in improving the proposed models. At the beginning, getting access to the latest publications was still difficult. Moreover, due to the long period between submission and final publication of the articles, information reached interested researchers much later then the first submission to the journal. Additionally, communication ways at that time were still difficult and expensive, limiting the number of personal contacts between researchers in different countries. With the number of congresses increasing, the direct access to information getting much easier via electronic media and because of the increased efforts done by the international water associations, the number of published articles has drastically increased. As such, nowadays we see more and more researchers applying AS models for full scale plant upgrading, to evaluate plant performance for future compliance with stricter discharge values, to develop new process design in combination with pilot plant testing (Nolasco *et al.*, 1998), …

Although still only a limited number of published articles is yet available, ASM2d has proven a trustworthy simulation tool for full scale municipal WWTPs. Calibration revealed that with a limited number of parameter adjustments, plant operating conditions could be modelled satisfactorily (Çinar *et al.*, 1998). However, an important observation is that no standard strategy is applied concerning parameter estimation procedures. Mathematical packages comprise nowadays sophisticated computing algorithms to allow any scientist to perform sensitivity analysis and parameter estimation. With models of the size of ASM2d or ASM2 + Delft EBPR model, it needs to be stressed that the number of parameters allows many authors to end up with their set of parameters, all giving rise to approximately the same predictive capacity of the model. Indeed, the larger number of parameters, the easier it becomes to find a set of parameters capable of predicting the measured values correctly. However, is this really the best, the most robust solution? Taking into account the metabolic models proposed nowadays, it is obvious that an important number of parameters does not need calibration, but are on the contrary predetermined by the biochemical laws. If these parameters need alteration, one has to realise that probably other reactions and or micro-organisms are governing the system (e.g. Ky *et al.*, 2000, Manga *et al.*, 2000), indicating the overall model needs alteration to obtain again a robust model that can stand the test of time.

For engineering purposes, the need for a standard model, including guidance on parameter estimation is getting more and more important. Information is necessary which parameters need alteration under certain conditions and to which extent these parameters can be altered. Too limited information is yet

available and published articles (Wanner *et al.*, 1992) did not receive the appropriate attention nor were they yet updated to the latest model developments. However, it is thought that currently so much data has been gathered, published and unpublished, and used for calibration purposes by so many researchers, each implementing their own procedure(s), that taking these input data and applying different methodologies on it, would not only prove to be useful from a research point of view, but would contribute to a general methodology for parameter estimation. Moreover, this exercise would also provide a necessary platform to prove the usefulness of Activated Sludge Models from an engineering point of view. Additionally, the exercise would allow the establishment of a database comprising sets of kinetic and stoichiometric parameters to be used for different operating conditions.

As a side remark its is mentioned that many models have been developed since the publication of ASM1 and especially since ASM2, accounting for EBPR. Unfortunately, researchers feel the need of writing their models using their terminology, their units, etc. Comparing models therefore becomes a difficult task. The effort done in this work to compare different models, led to the conclusion that probably it would be a good idea to urge scientists to use the same notations and units.

Using the same input data from a full scale installation, and using different models with their respective kinetic and stoichiometric parameter values, would reveal the predictive capacity of the models more clearly. This exercise should be performed using data from several full scale plants. Changing parameters should be based on a biochemical basis rather than on a mathematical/statistical basis. Parameters resulting from such a study will probably reveal to be more robust.

From personal communication it is known that ASM3 is currently being updated to account for EBPR by different research groups. No published literature references are yet available and results are therefore not yet included in this literature review.

CHAPTER 2

Gas Chromatographic analysis of PolyHydroxyButyrate in activated sludge: A Round-Robin test

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Chapter 2

Gas Chromatographic analysis of PolyHydroxyButyrate in activated sludge: A Round-Robin test

Abstract - PolyHydroxyAlkanoates (PHA) and Poly-β-HydroxyButyrate (PHB) in particular has become a compound which is routinely investigated in wastewater research. The PHB analysis method is only recently applied to activated sludge samples where PHA contents might be relatively low. This urges the need to investigate the reproducibility of the gas chromatographic method for PHB analysis. This was evaluated in a Round-Robin test in 5 European laboratories with samples from lab-scale and full-scale enhanced biological phosphorus removal systems. The basic method was direct solvent extraction of lyophilised cells, depolymerisation (hydrolysis) of the polymer and alkylation of the alkanoates. All steps take place in one tube with the necessary incubation time generally being 2 hours. The concentration of the (propyl)ester is then analysed by gas chromatography. Each lab used slightly modified methods. It was observed that the use of an internal standard improved the reproducibility of the method, that using dichloro(m)ethane as solvent instead of chloroform improves the GC analysis and that shaking the reaction tubes during heating improves the esterification reaction. It was shown that the standard deviation of measurements in each lab and the reproducibility between the labs was very good. Experimental results obtained by different laboratories using this analysis method can be compared. Sludge samples with PHB contents varying between 0.3 and 22.5 mg PHB/mg sludge were analysed. The gas chromatographic method allows for PHV, PH2MB and PH2MV analysis as well. The gas chromatographic analysis of PHA is important for kinetic modelling of EBPR processes. Moreover, with the publication of the Activated Sludge Model No3 and the general acceptance that storage polymers are of prime importance for non polyphosphate accumulating organisms as well, analysis of internal storage components becomes a prerequisite for modelling the activated sludge processes.

1. INTRODUCTION

Bacterial cells may contain inclusions of storage products serving as a source of energy or building blocks. Amongst others, carbon storage can take the form of glycogen, lipids, starch and poly-β-hydroxybutyric acid (PHB). The latter is mainly encountered in prokaryotic micro-organisms. Glycogen and starch are the most encountered storage polymers in bacterial cells, and are often referred to as polysaccharides (Schlegel, 1993). According to van Loosdrecht *et al.* (1997b), PHB is thought to be the most dominant polymer in general as it is directly formed out of the central metabolite acetyl-CoA. Also lipid-like substances, i.e. intracellular fat granules and droplets occur in many organisms (Zevenhuizen and Ebbink, 1974; Schlegel, 1993).

In 1926, Lemoigne was the first to describe PHB (Figure 47) as cell internal component. It would take until 1960 before a renewed interest was created because of the presumed metabolic significance of PHBs. The storage polymer was particularly encountered under certain growth conditions such as limiting nutrient conditions (low N/COD ratio) (Braunegg *et al.*, 1978). According to Schlegel (1993) PHB is accumulated by aerobic and facultative bacteria when the cells are faced with oxygen limiting conditions, or restricted growth by a lack of nutrients like nitrogen and phosphorus as referred to by Anderson and Dawes (1990). On return to aerobic conditions the storage polymer can then be used as an energy and carbon source and incorporated in the oxidative metabolism. Under suitable culture conditions

Alcaligenes autrophus, now called *Ralstonia eutropha* (Madison and Huisman, 1999), could reach a PHB content of more than 90% (w/w) of its dry weight (Schlegel, 1993).

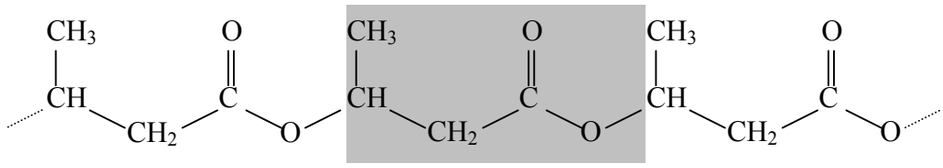

Figure 47 Poly-β-hydroxybutyric acid

With the discovery of the Enhanced Biological Phosphorus Removal (EBPR) process (Srinath et al., 1959; Barnard, 1975) and especially with the elucidation of the biochemical mechanisms underlying this process, it became clear that PHB and glycogen are the most important storage polymers in EBPR processes (van Loosdrecht et al., 1997a,b). Intracellular carbon storage other than PHB in EBPR processes was first reported by Comeau et al. (1987), indicating the existence of co-polymers composed of 3HB and 3HV (3-Hydroxy-Valerate). In 1992, Matsuo et al. added 3H2MV (3-Hydroxy-2-MethylValerate) and 3H2MV (3-Hydroxy-2-MethylValerate) to the list of possible co-polymers. Inoue et al. (1996) indicated that PolyHydroxyAlkanoate (PHA) co-polymers in EBPR processes are composed of random co-polymers with different monomer compositions. According to current knowledge specialised bacteria, so-called Polyphosphate Accumulating Organisms (PAOs) are able to internally store poly-Phosphate (poly-P) granules under aerobic or anoxic conditions. A prerequisite for the excess phosphorus to occur is the fact that the biomass first needs to pass through an elector acceptor free phase. In this anaerobic phase (different from an anoxic phase where nitrate is available as oxidant) PAOs are able to store certain carbon source as PHAs, using energy from breakdown of intracellular poly-phosphate and glycogen (Smolders et al., 1994a; Pereira et al., 1996; Hesselmann et al., 2000).

In the 1990's, Cech and Hartman (1990, 1993) indicated proliferation of glycogen accumulating organisms with concurrent EBPR breakdown. Experimental evidence indicated that one of the possible pathways involves anaerobic PHA storage (Liu *et al.*, 1996b), again pointing out the importance of storage polymers.

Apart from EBPR processes, it becomes more and generally accepted that storage polymers are a key intermediate in biological wastewater treatment. In general, activated sludge systems are highly dynamic with respect to the feed regime and thus micro-organisms are subjected to feast-famine regimes. Only for a relatively short period micro-organisms have external substrate available. Without internally stored substrate, organisms undergo rapid growth and starvation periods (van Loosdrecht *et al.*, 1997b). In these mixed microbial populations a feast-famine regime enriches for organisms which can use storage polymers to balance their growth. Experimental evidence, using respirometry to elucidate the underlying mechanisms and the kinetics of carbon utilisation (Dircks *et al.*, 1999; Petersen *et al.*, 2000), point strongly to the occurrence of storage polymers. Additional experimental evidence is gained from the ratio between oxygen use and substrate removed, which is much lower in mixed culture than in pure culture studies, providing more evidence that storage products are clearly involved. One of the practical examples of storage polymers involved in wastewater treatment is the development of selectors to prevent bulking sludge (Chudoba *et al.*, 1973). At that time the internal metabolism of the system was unknown but selectors made use of the storage principle. For modelling purposes the latest version of the activated sludge model (ASM3) now comprises storage of readily biodegradable substrate prior to growth of heterotrophic and nitrifying organisms (Henze *et al.*, 2000).

Microbial production of PHA, both under aerobic and anaerobic conditions, has gained considerable interest because of the potential applications of these polyesters as biodegradable plastics (Brock and Madigan, 1991). PHAs can be thermoplastically moulded and can be used as new plastics which have the

advantage over polypropylene and polyethylene of being biodegradable. They can be transformed into sheets, fibres and hollow bodies like bottles and mugs (Schlegel, 1993). Besides being the only linear biodegradable polyester, they are also biocompatible with numerous applications in medicine, pharmacy and packaging (Steinbüchel and Wiese, 1992). More than 40 different hydroxyalkanoic acids (HAs) have been detected as constituents of PHA, but only few homopolyesters besides poly(3-hydroxybutyrate) (PHB) are available from bacteria (Steinbüchel and Wiese, 1992).

Initially, PHA analysis techniques were only used for determination of the PHB content in pure culture with high internal storage capacity. Analysis procedures comprised tedious purification steps and could not detect PHV or distinguish between PHB and PHV (Hesselmann, 1999). Lemoigne (1926) optimised a gravimetric determination method, based upon the fact that PHB is soluble only in boiling chloroform. All contaminants may thus be removed by extraction with other solvents. To compensate for large errors when the PHB content is low, large volumes had to be used. In 1958, Williamson and Wilkinson worked out a method of residual optical density after complete dissolution of the cells in a NaOCl-solution. The result of this method revealed to be dependent on the size, form and number of the PHB granules. Preferably this method is only used for routine checking. In the 60-ies a rapid spectrophotometric method was developed (Slepecky and Law, 1960; Law and Slepecky, 1961). First PHB is depolymerised and dehydrated by sulphuric acid. Crotonic acid is then analysed spectrophotometrically. Although this analysis procedure is apparently simple, the method is not reliable for accurate analysis since it only gives satisfactory results with an undue amount of effort (Hesselman *et al.*, 1999). In 1975, Jüttner *et al.* designed an infrared spectrographic determination method. However, lipids falsify the PHB analysis and have to be removed from the sample prior to analysis, rendering this method a laborious and difficult one to obtain reproducible results. Finally, in 1978, Braunegg developed a PHB analysis method for pure cultures, which is still used today after slight modifications to adapt for the inherent characteristic of activated sludges (Apostolides and Potgieter, 1981; Comeau *et al.*, 1988; Huijberts *et al.*, 1994). This method allows extraction, depolymerisation and esterification to proceed concurrently in one mixture within a reasonable amount of time. At the time of its development, high accuracy, excellent reproducibility (max standard deviations of ± 0.5%), lower detection limit at 10^{-5} g/ℓ were reported. The procedure depended neither on the volume of the cell suspension nor on the PHB content of the cells. The method was only dependent on the initial volume of the cell suspension (0.5 and 5 mℓ). The method was tested using cells of *Alcaligenes eutrophus* H16 and M7 and validated on *Mycoplana rubra*. The method was later optimised for activated sludge samples by weighing precise amounts of lyophilised biomass instead of taking mixed liquor samples with an observed error greater than 10% (Apostolides and Potgieter, 1981). With the development of capillary columns for gas chromatographic analysis of the methylesters produced, the method was optimised further (Comeau *et al.*, 1988).

Despite all the promising results mentioned in the previous paragraph, and the wide spread use of this gas chromatographic analysis, much doubt remained, especially amongst scientists when comparing results from different laboratories for modelling purposes. Indeed, to explain experimental observations a reliable and accurate generally applied analysis method for all components involved is necessary. It was thought the analysis technique mentioned in the previous paragraph still depended largely on external factors and did not fulfil the requirements of a standard measurement procedure. As such, it was observed that scientists continued seeking for new, more reliable analysis procedures.

In the course of the European COST action 682 (Dochain *et al.*, 2000), a Round-Robin test was set up between five laboratories to verify the analysis method for PHB from biomass. Biomass samples from phosphorus removing activated sludge treatment plants have been analysed for their PHB content. Lyophilised biomass was analysed using different, but resembling sample preparation and analysis procedures. Results from this analysis are presented here. This article provides information for a reliable and accurate PHB analysis using a gas chromatographic method and evidence for its reproducibility when

different laboratories are performing similar, but different, analysis methods. It is indicated the analysis method can be used for PHV, PH2MV and PH2MB analysis as well.

2. MATERIALS AND METHODS

2.1.1. Sample preparation

Biomass samples were collected from different types of pilot and full scale plants treating either artificial or municipal wastewater and showing a wide range of P-removal capacities. Each participating laboratory prepared one sample. Samples were freeze-dried, shipped to the co-ordinating partners and from there distributed to the participating laboratories for analysis.

The lyophilised samples were subjected to an esterification reaction using an acidified alcohol/organic solvent mixture (Table 54). Methyl- or propylesters were formed during a 2–4 h incubation period at 100°C in closed cap tubes. Tubes were shaken every 15 minutes to allow good contact between biomass and products. After cooling down to room temperature (eventually putting the tubes in an ice bath for several minutes), the organic phase was extracted with 1-3 ml distilled water by shaking vigorously to remove the cell debris. The organic phase was separated from the water phase or by simple vigorous shaking of the tubes or by applying vortex or by centrifugation. The two liquids were allowed to separate, during which cell debris gather at the interface. Finally, the organic phase was dried on Na_2SO_4. To this end, small tubes can be filled with some glass wool as bottom layer and Na_2SO_4 as the top layer. The organic phase is allowed to run through this filled tube. Of the dried organic phase 0.5 or 1 $\mu\ell$, depending on the laboratory, was injected on GC.

Possible factors influencing the analysis procedure are the accuracy the reagents are prepared with and the time reagents are stored before complete usage. Indeed, for instance the acidified alcohol solution is prepared on a regular basis and the solvent containing internal standard might evaporate if not sealed properly. Other factors influencing the analysis procedure are wearing of the GC column and contamination of the carrier gasses. It was observed these parameters did not influence the measurement if proper actions were taken. As for the solvent containing the internal standard, it is advised to make a new calibration line whenever this product is renewed. Large quantities of solvent with internal standards can be prepared and when stored properly sealed this product remained stable for at least 2.5 years. With every set of samples analysed it is advised to take along one calibration sample (see below) to check the accuracy of the analysis.

From Table 54 it is observed that especially the parameters used by the Italian laboratory differ from the others. For quantitative extraction of PHB from cells in non-gas chromatographic methods, the biomass was put in boiling chloroform. Initially chloroform was used as the preferred solvent. However, dichloroethane and -methane have higher polar character than chloroform, giving rise to sharper gas chromatographic elution of the polar methyl/propylester and thus better detection of the component. Extraction of the PHB from the sludge did not deteriorate using dichloroethane/methane instead of chloroform. The initial method proposed by Braunegg *et al.* (1978) suggested the use of sulphuric acid rather than hydrochloric acid to obtain complete depolymerisation of PHB. Fukui *et al.* (1976) already suggested using mildly alkaline conditions resulting in complete depolymerisation as well. Later, sulphuric acid was preferred above hydrochloric acid since formation of the by-product crotonic acid, can be better guaranteed. Initially, methylation of the monomer was proposed. Later, propylation became the more generally applied technique because improved separation of the different HA units could be obtained. When only the measurement of PHB is aimed for, little impact is expected using propanol or methanol for the esterification reaction.

Table 54 Sample preparation procedures

Country	Sample amount [mg]	solvent	Internal standard in solvent [mg/sample]
Belgium	8 - 60	1.5 mℓ 1,2-dichloroethane	1 mg benzoic acid
Denmark	50	1.5 mℓ dichloromethane	1.5 mg benzoic acid
Finland	50 – 70	1.5 mℓ chloroform	1 mg benzoic acid
Italy	5 - 20	1 mℓ chloroform	no internal standard
The Netherlands	20	1.5 mℓ 1,2-dichloroethane	1 mg benzoic acid

Country	Alcohol	acid in alcohol	incubation at 100°C
Belgium	1.5 ml 1-propanol	25 % HCl	2 h
Denmark	1.5 ml 1-propanol	25 % HCl	2 h
Finland	1.5 ml 1-propanol	25 % HCl	2 h
Italy	2 ml methanol	3 % H_2SO_4	4 h
The Netherlands	1.5 ml 1-propanol	25 % HCl	2 h

2.1.2. Gas chromatographic analysis

The organic phase was analysed using a Gas Chromatograph (GC). The participating laboratories used different GC brands, columns, injector and detector temperatures (Table 55) and temperature profiles (Table 56). All GCs were equipped with a Flame Ionisation Detector (FID) and helium or nitrogen was used as a carrier gas.

Table 55 GC configuration

Country	Sample [µl]	Injector Temp. [°C]	Detector Temp. [°C]	GC	Column
Belgium	0,5	200	250	Chrompack CP9001	HP Innowax
Denmark	1	240	240	VEGA 6000, serie 2	Chrompack WCOT
Finland	0,5	200	250	Hewlet Packard 7590A	HP Innowax
Italy	1	200	250	Perkin Elmer 8500	Reoplex
The Netherlands	0,5	200	250	Chrompack 238A	Resteck Stabilwax

Table 56 GC Temperature profiles

Belgium		Denmark		Finland		Italy		The Netherlands	
Time [min]	*Temp (rise) °C (/min)*	*Time [min]*	*Temp (rise) °C (/min)*	*Time [min]*	*Temp (rise) °C (/min)*	*Time [min]*	*Temp (rise) °C (/min)*	*Time [min]*	*Temp (rise) °C (/min)*
0-0.1	80°C	0-8	140°C	0-0.1	90°C	0-1	100°C	0-0.1	80°C
0.1-2.15	35°C/min			0.1-18.4	6°C/min	1-6	10°C/min	0.1-2.15	35°C/min
2.15-5.55	4°C/min			18.4-19.4	200°C	6-9	150°C	2.15-5.55	4°C/min
5.55-9.19	15°C/min							5.55-9.19	15°C/min
9.19-22.19	220°C							9.19-22.19	220°C

2.1.3. Calibration procedure

By means of example the calibration procedure used in the Laboratory in Ghent is elaborated on. As calibration products polyβ-hydroxybutyric acid from *Alcaligenes* (Sigma, P-8150; MW 535), poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) with 14% PHV (Sigma-Aldrich, 34,749-6) and poly(3-hydroxybutyric acid-co-97% 3-hydroxyvaleric acid) (LMB Ghent, batch no F15-31) were used. PHB is a white fluffy product stable over long periods of time. From personal communication with Sigma-Aldrich it was assured the product does not degrade in time when stored at room temperature. However, the product was kept in the refrigerator sealed to avoid moisture introduction. Using a microbalance, a mass ranging between 0.5 and 2.5 mg (with approximate intervals of 0.25 mg) of this calibration standard is sampled. During weighing attention needs to be paid to avoid attraction by static electricity that would lead to loss of product. The sample is then treated as normal lyophilised biomass to obtain the ester ready for injection on GC (see sample preparation).

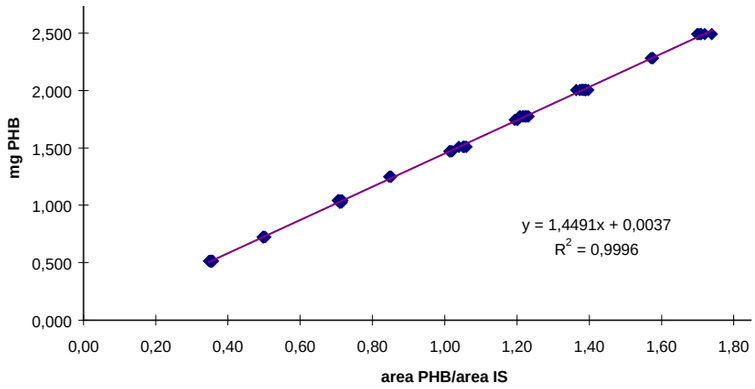

Figure 48 Calibration line for PHB analysis

Every sample was then injected at least three times to check the accuracy of the results. Figure 48 shows the calibration line that was obtained taking into account 21 calibration samples. For determination of the PHV content the calibration line used had a smaller slope (y =1.3426 x -0.0088) but a similar regression coefficient (R^2= 0.9999). The calibration lines obtained are only used within the boundary limits of the highest and lowest concentrations used for calibration.

3. RESULTS

All biomass samples were subject to the complete above-mentioned procedure in at least 4 replicates by each of the 5 laboratories involved All results obtained by the involved laboratories using their analysis procedure on the different sludge sample are given in Table 61 The average PHB content ranged between 0.3 and 22.7 mg PHB/g sludge. This difference can be easily explained by the difference in origin of the samples. Whereas the Finish sample originated from a pilot scale UCT wastewater treatment plant exhibiting minor phosphorus removal, the Italian sample originated from a sequencing batch reactor (SBR) operated to obtain pure cultures of PHB accumulating sludge.

From Table 61 it is observed that the standard deviation expressed in terms of percentage obtained by the Belgian laboratory remains nearly constant and very low for all PHB contents. The Dutch laboratory obtained similar results except for the very low PHB contents of the Finish sludge. This result indicates that increased amounts of freeze dried sludge are to be preferred when analysing sample with very low polymer contents. The standard deviations expressed in terms of percentage from the Danish laboratory are consistently higher than values obtained by the other laboratories which is explained by the lack of shaking. The Round-Robin test provided proof for the Danish laboratory that shaking during esterification reaction, i.e. during the heating period, improves the reproducibility of the analysis. The sometimes higher standard deviation expressed in terms of percentage for the samples treated by the Italian laboratory are explained by the absence of an internal standard. From the table it is observed that in

general the differences are small and thus forms an illustration that very accurate and reproducible results can be obtained by different laboratories, despite small variations in the methods used.

An overview of the average concentrations is presented in Table 57. To verify for meaningful differences between the averages obtained, a parametric statistical method, ANalysis Of Variance (ANOVA) with two criteria of classifications, was used (Table 58). Samples from one wastewater treatment unit are considered as blocks, whereas the different analysis procedures are considered as treatments. The grouping is done to have the units in each block as uniform as possible so that the observed differences will be largely due to the treatments (Steel and Torrie, 1980). Mathematically, the optimal procedure is to take all individual analysis results into account. However, from Table 61 it is observed that different numbers of samples were analysed by the different laboratories and that the variances obtained by the different laboratories differed. The statistical analysis can be performed both using the average values or accounting for all the measurements performed. Both statistical analysis revealed the same result. Only the one using the average values is presented in Table 58.

For a reliable and general analysis procedure it is expected that analysis procedures, regarded alike, deliver the same result, regardless the PHB concentration of the lyophilised sludge sample. Statistically this is expressed as the H_0 hypothesis. From the analysis of the ANOVA results, it is clear that the calculated F-value is significantly lower than the critical F-value (4 and 16 degrees of freedom) at a 95% confidence level. It is therefore clear that there is no reason to reject the H_0 hypothesis.

Table 57 Average PHB concentrations (mg PHB/g sludge)

	Block Italy	Block Finland	Block Delft	Block Denmark	Block Ghent
Treatment Italy	22.437	0.314	5.577	1.262	2.108
Treatment Finland	22.509	0.377	5.742	1.224	1.739
Treatment Delft	21.721	0.331	5.701	1.303	1.836
Treatment Denmark	22.766	0.372	5.007	1.645	1.699
Treatment Ghent	21.336	0.340	5.335	1.267	1.827

Table 58 ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments (laboratory method)	0.3534	4	0.0884	0.8412	0.5191	3.007
Samples	1659.6123	4	414.9031	3950.24	9.86E-24	3.007
Error	1.6805	16	0.1050			
Total	1661.6463	24				

4. DISCUSSION

When the sample preparation methods and GC operating conditions evaluated in this study are compared to other methods mentioned in literature (Table 59 and Table 60 respectively), a few differences are observed. Sample amounts were often not mentioned. In the past and especially when analysing PHB contents of pure culture with high polymer contents, exact volumes of mixed liquor were taken instead of weighing freeze dried sludge amounts. However, in the case of activated sludge samples, it is difficult to take small, completely mixed amounts of sludge. Therefore, Apostolides and Potgieter (1981) suggested weighing freeze dried sludge. Lemos *et al.* (1998) are still using the original method, sampling known amounts of mixed liquor. For all references cited chloroform was used as solvent and H_2SO_4 as acid. As

explained above, this results from historical developments of PHB analysis techniques. The statistical analysis of the Round Robin test indicated all analysis procedures deliver the same result. The Italian laboratory is using chloroform as extracting agent, sulphuric acid as depolymerisation agent and methanol for esterification as was mostly applied in the former methods. Clearly this analysis procedure is as valid as the newer ones. Internal standards were only occasionally used. Although the use of an internal standard is becoming more and more common when analysing samples on GC, this is still not a standard working method. The results of the Italian laboratory, working without internal standard, indicated that the use of an internal standard improves the accuracy because it eliminates for eventual non careful addition of liquids.

As for the cooling down and phase separation after esterification, only Comeau *et al.* (1988) mention a somewhat different method by adding 1 mℓ of an aqueous ammonia solution (28%).

Table 59 Sample preparation

Reference	Sample amount [mℓ or mg]	solvent	internal standard [mg/sample]
Braunegg *et al.* (1978)	0.5 - 5 mℓ	2 mℓ chloroform	benzoic acid
Lageveen *et al.* (1988) for medium chain PHAs	0.1 - 4 mℓ (max. 15 mg)	2 mℓ chloroform	benzoic acid in the alcohol
Comeau *et al.* (1988)	20 mg	2 mℓ chloroform	benzoic acid in the alcohol
Satoh *et al.* (1994, 96)	± 12 or 24 mg*	2 mℓ chloroform	y ml methylbenzoate in solvent later : benzoic acid in the alcohol
Huijberts *et al.* (1994)	?	x mℓ chloroform	no internal standard
Huijberts *et al.* (1994) for medium and long chain PHAs	?	x ml chloroform	no internal standard
Lemos *et al.* (1998)	2 mℓ	2 mℓ chloroform	0.5 mg benzoic acid in alcohol

* mixed liquor samples with fixed volumes are taken

Reference	Alcohol	acid in alcohol	incubation at 100°C
Braunegg *et al.* (1978)	2 mℓ methanol	3 % H_2SO_4	3.5 h
Apostolides *et al.* (1981)	2 mℓ methanol	3 % H_2SO_4	3.5 h
Lageveen *et al.* (1988)	2 mℓ methanol	15 % H_2SO_4	140 min
Comeau *et al.* (1988)	2 mℓ methanol	3 % H_2SO_4	3.5 h
Satoh *et al.* (1994, 96)	2 mℓ methanol	20 % later 10% H_2SO_4	7 h*
Huijberts *et al.* (1994)	x mℓ methanol	15 % H_2SO_4	2 h
Huijberts *et al.* (1994) for medium and long chain PHAs	x mℓ methanol	15 % H_2SO_4	4 h
Lemos *et al.* (1998)	1 mℓ methanol	20 % H_2SO_4	3.5 h

* after heating for 7 hours, 1 mℓ of aqueous ammonia solution (28% later 14%) was added
acid in alcohol expressed as volume percentage

According to Huijberts *et al.* (1994) the PHB concentration could be determined with an accuracy of 0.018 mg/mg sludge. Reducing the methanolysis time to 2 hours instead of 140 min. was possible. For determination of medium and long chain PHAs, however; the methanolysis time in the assay had to be increased to at least 4 hours in order to achieve complete conversion of the polyester to methyl-3-hydroxy fatty acids. Increasing the methanolysis time had no detrimental effects. Using this method, the PHA concentration could be determined with an accuracy of 0.304 mg PHB/mg sludge. The decrease in accuracy was attributed to an increased number of different monomers present in the PHA. Using

sulphuric acid too, Lemos *et al.* (1998) indicated that 3.5 hours was the best time for the recovery of the PHA polymer.

Lageveen *et al.* (1988) reported a decrease in the amount of methyl-3-hydroxy fatty acids during prolonged methanolysis. Huijberts *et al.* (1994) attributed this decrease to formation of crotonic acid methylester as a result of the acid catalysed reaction on the 3-hydroxyl group. However ^{13}C-NMR analysis of the reaction products provided evidence that the crotonic acid methylester was not present in the samples prepared by Lageveen *et al.* (1988).

Table 60 GC configurations and temperature profiles

GC configuration						Temp. profile	
Reference	**Sample [µl]**	**Injector Temp. [°C]**	**Detector Temp.**	**GC**	**Column**	**Time [min]**	**Temp (rise) [°C (/min)]**
Braunegg *et al.* (1978)				Hewlett-Packard 5840A	packed column 1 L=8 ft 2% Reoplex 400 on chromosorb GAW	0 - 1 min 1 - 8.5 min 8.5 - 13.5 min	90°C 8°C/min 150°C
					packed column 2 L=6 ft Carbowax M20 TPA on chromosorb GAW	0 - 1 min 1 - 6.25 min 6.3 - 11.3 min	100°C 8°C/min 150°C
Apostolides and Potgieter (1981)			FID	Beckman GC 4	packed column L=1.5 m 5% Carbowax M20 TPA on chromosorb GAW	0 - 1 min 1 - 6.25 min 6.3 - 11.3 min	100°C 8°C/min 150°C
Lageveen *et al.* (1988)				Packard	capillary column L= 25m CPSIL-5CB	0 - 2 min 2 - 10 min	80°C 5°C:min (to 120°C)
Comeau *et al.* (1988)	1	210	220 (FID)	Hewlett-Packard 5880A	capillary column L=15 m; ϕ=0.52 mm DB-Wax (1.0 µm)	0 - 1 min 1 - 14.75 min 14.8 - 19.8 min	50°C 8°C/min (to 160°C) 160 °C
Satoh *et al.* (1994)	0.5	230	250 (FID)	Shimadzu GC-14A	capillary column L=30 m; ϕ=0.25 mm Neutrabond-1 (0.4 µm)	0 - 4 min 4 - 9 min 9 - 11.67 min	80°C 8°C/min (to 120°C) 30°C/min (to 200°C)
Satoh *et al.* (1996)	0.5	225	280 (FID)	Hewlett-Packard 5880A	capillary column L=30 m; ϕ=0.32 mm DB-1 (0.25 µm)	0 - 4 min 4 - 10 min 10 - 12.7 min 12.7 - 15.7 min	70°C 8°C/min (to 118°C 30°C/min (to 200°C) 200°C
Lemos *et al.* (1998)	1	200	220	Chrompack	capillary column L= 25m CPSIL-5CB (0.25µm)	0 - 1 1 - 1.33 1.33 – 6.33 6.33 – 20.08 20.08 – 21.08	40°C 30°C/min 50°C 8°C/min 160°C

In literature pre-treatment of the activated sludge sample prior to freeze drying is not elaborated on. The Belgian laboratory used a method optimised and validated for the EBPR sludge grown in the SBR. Extrapolation of these results to other experimental circumstances has not been validated yet. Mixed liquor samples were taken from the reactor and immediately filtered under vacuum. The remaining

biomass on the glass fibre (Whatman GF/C) was then transferred to a small tube, put in glycol with CO_2-snow (-40°C) and finally kept at -18°C. After freezing, this biomass was then lyophilised. No products were added to the mixed liquor to prevent breakage of cells. Other methods used by the other laboratories are centrifugation of sludge mixed liquor, decantation of the supernatant and then lyophilisation of the pellet. Louie *et al.* (2000) added hypochloric acid to inhibit enzymatic activity and to enhance recovery of PHA.

Hesselmann *et al.* (1999) developed a method for PHB analysis in activated sludge by ion chromatography and enzymatic methods. It is beyond the scope of this article to evaluate the applicability of this method. However, with the main idea of interchangeability of research results in mind, this new method, if not laborious and if in future proven more reliable than the gaschromatographic method, needs to be applied by many researchers currently using the gas chromatographic method. Moreover, with the results obtained in our research, it is esteemed the high accuracy and reliability obtained with the gaschromatographic method allows for continued application of this analysis technique by the research groups already implementing it. The enzymatic method still has to proof its general applicability.

5. CONCLUSIONS

The Round-Robin test clearly demonstrates that PHB analysis on activated sludge sample performed by different laboratories results in statistically equal observations. It was shown that the standard deviation of measurements in each lab and the reproducibility between the labs was very good. It was observed that during reaction it is necessary to shake the vials regularly as to allow optimal reaction conditions. To increase accuracy of the gas chromatographic analysis it is advised to use an internal standard and to use dichloro(m)ethane as solvent instead of chloroform. To eliminate for eventual external factors influencing the analysis procedure, it is advised to analyse with every set of sample one standard. Sludge samples with PHB contents varying between 0.3 and 22.5 mg PHB/g sludge were analysed. The gas chromatographic method allows for PHV, PH2MB and PH2MV analysis as well.

Experimental results obtained by different laboratories using this analysis method can be compared. The gas chromatographic analysis of PHA is important for kinetic modelling of EBPR processes. Moreover, with the publication of the Activated Sludge Model No3 and the general acceptance that storage polymers are of prime importance for non polyphosphate accumulating organisms as well, analysis of internal storage components becomes a prerequisite for modelling the activated sludge processes.

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Table 61 Results obtained by the different laboratories on the different samples

	Italian sample				
	Italy	Denmark	Belgium	Holland	Finland
	23.780	23.614	21.243	22.000	22.367
	21.821	24.371	21.580	22.120	22.790
	22.800	23.919	21.044	21.910	22.740
	23.223	23.437	21.231	21.650	22.140
	21.572	26.454	21.607	21.657	
	22.003	24.935	21.570	21.480	
	21.862	19.976	20.945	21.510	
		22.189	20.755	21.763	
		22.148	21.640	21.397	
		22.166	21.489		
		20.983	21.588		
		21.435			
		20.325			
average	22.437	22.766	21.336	21.721	22.509
variance	0.700	3.596	0.096	0.061	0.096
stdev	0.837	1.896	0.310	0.248	0.310
%stdev	3.729	8.329	1.454	1.141	1.378

	Finish sample				
	Italy	Denmark	Belgium	Holland	Finland
	0.257	0.302	0.343	0.313	0.398
	0.266	0.339	0.341	0.310	0.379
	0.407	0.385	0.335	0.307	0.360
	0.365	0.362	0.340	0.337	0.370
	0.274	0.354	0.339	0.330	
		0.373		0.410	
		0.514		0.323	
		0.345		0.323	
				0.330	
average	0.314	0.372	0.340	0.331	0.377
variance	0.005	0.004	0.000	0.001	0.000
stdev	0.068	0.063	0.003	0.031	0.016
%stdev	21.606	16.853	0.904	9.388	4.287

	Dutch sample				
	Italy	Denmark	Belgium	Holland	Finland
	5.669	5.588	5.438	5.793	5.722
	6.026	5.450	5.220	5.677	5.756
	5.636	5.156	5.281	5.760	5.770
	5.445	5.030	5.312	5.657	5.720
	5.852	4.988	5.311	5.730	
	5.503	4.910	5.336	5.727	
	5.088	4.979	5.448	5.610	
	5.395	4.829	5.323	5.640	
		5.027	5.243	5.716	
		4.962	5.417		
		4.810	5.357		
		4.427			
		4.931			
average	5.577	5.007	5.335	5.701	5.742
variance	0.083	0.082	0.006	0.004	0.001
stdev	0.289	0.286	0.075	0.059	0.025
%stdev	5.179	5.714	1.409	1.041	0.434

	Danish sample				
	Italy	Denmark	Belgium	Holland	Finland
	1.303	1.849	1.221	1.293	1.251
	1.295	1.500	1.284	1.303	1.163
	1.253	1.691	1.265	1.310	1.280
	1.386	1.701	1.287	1.303	1.200
	1.112	1.611	1.244	1.287	
	1.253	1.601	1.282	1.303	
	1.228	1.679	1.276	1.357	
		1.662	1.277	1.267	
		1.642		1.303	
		1.513			
		1.661			
		1.600			
		1.671			
average	1.262	1.645	1.267	1.303	1.224
variance	0.007	0.008	0.001	0.001	0.003
stdev	0.084	0.088	0.023	0.024	0.052
%stdev	6.623	5.347	1.830	1.844	4.263

	Belgian sample				
	Italy	Denmark	Belgium	Holland	Finland
	2.100	1.581	1.842	1.823	1.615
	2.150	1.793	1.822	1.810	1.699
	2.108	1.793	1.839	1.813	1.790
	2.158	1.666	1.829	1.833	1.850
	2.092	1.715	1.832	1.853	
	2.117	1.734	1.849	1.860	
	2.034	1.937	1.779	1.853	
		1.678	1.821	1.840	
		1.574	1.815	1.837	
		1.647	1.827		
		1.612	1.843		
		1.655			
average	2.108	1.699	1.827	1.836	1.739
variance	0.002	0.011	0.000	0.000	0.011
stdev	0.041	0.104	0.019	0.018	0.103
%stdev	1.955	6.124	1.043	0.973	5.931

CHAPTER 3

Enhanced Biological Phosphorus removal from Wastewater: SBR versus Phoredox and UCT

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Chapter 3

Enhanced Biological Phosphorus removal from Wastewater: SBR versus Phoredox and UCT

Abstract - Enhanced Biological Phosphorus Removal (EBPR) is achieved when activated sludge and wastewater are subjected to subsequent anaerobic-aerobic/anoxic conditions. Different process configurations, continuous processes as well as batch operated processes, combined with different carbon sources were evaluated for their capacity to select for Phosphorus Accumulating Organisms (PAOs). For the continuous processes, i.e. the Phoredox and University of Cape Town (UCT) installations, EBPR activity was observed but stable process operation was difficult to achieve. With both UCT and Phoredox configurations comparable EBPR activity was observed. Using acetate as sole carbon source for the continuous installation complete phosphorus removal could be observed. However, the competition for organic carbon between different microbial populations dominated the process. Production of extracellular polymers prevented stable operating conditions. Therefore, to study quantitatively the different processes, a sequencing batch reactor (SBR) was operated where acetate is used as sole carbon source to promote growth of (PAOs). Good EBPR activity was observed and stable process operation could be maintained. High internal carbon storage polymer contents, i.e. PolyHydroxyAlkanoates (PHAs), were observed probably when the biomass was grown under nutrient limiting conditions or external conditions preventing normal EBPR activity. The kinetics of the process involved were studied performing batch experiments where concentration profiles are recorded during the anaerobic and aerobic period. Results are compared with concentration profiles obtained using sludge originating from a conventional Phoredox installation and a UCT installation. Oxygen penetration in the anaerobic phase due to sampling caused anaerobic uptake of phosphorus and consumption of PHA.

1. INTRODUCTION

Biological phosphorus removal from waste water is preferred above chemical phosphate precipitation because of its lesser environmental impact. A conventional waste water treatment plant can easily be converted to a biological phosphorus removal plant by providing an anaerobic tank ahead of the aerobic reactor.

In wastewater phosphates are encountered as ortho-phosphate (PO_4^{3-}), polyphosphate (P_2O_7) and organically bound phosphorus. The two latter components make up 70% of the phosphorus concentration encountered in the influent of a wastewater treatment plant (WWTP) (Metcalf and Eddy, 1991). Micro-organisms use phosphorus for cell synthesis and energy transport. Due to these processes 10 to 30% of the phosphorus content in the wastewater is eliminated during the secondary phase of conventional wastewater treatment plants.

Since the 1990's research is being conducted at the Laboratory for Technical Chemistry and Environmental Engineering (Ghent University) on the kinetics of several processes occurring in Enhanced Biological Phosphorus Removal (EBPR) from wastewater. Initially, bacteria were cultured in a continuous Phoredox installation. In a later stage, two new installations have been taken into operation: a continuous University of Cape Town (UCT) installation and a discontinuous Sequencing Batch Reactor (SBR). Batch experiments were performed using sludge originating from one of the reactors of the continuous installations or directly on the SBR.

1.1. *DESCRIPTION OF THE ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL PROCESS*[1]

Enhanced biological phosphorus removal, with removal efficiencies of over 80% of the influent phosphorus content, is obtained when the activated sludge is subjected to changing conditions, i.e. anaerobic and aerobic conditions. The micro-organisms capable of storing high amounts of phosphorus as intracellular polyphosphate granules are called hereafter *Phosphorus Accumulating Organisms (PAOs).* These micro-organisms are also capable of storing volatile fatty acids (VFAs), with as most important component acetate, under anaerobic conditions. The energy needed for this storage is obtained from the breakdown of an internal polyphosphate chain. VFAs are stored as PolyHydroxyAlkanoates (PHAs) with as most cited constituent Poly-β-HydroxyButyrate (PHB). Under optimal conditions no carbon source (BOD) remains in the aerobic phase following the anaerobic one. The PAOs will then use the carbon source internally stored during the anaerobic phase. With the energy released from utilisation (some researchers use the terminology "sequestration") of PHA, the PAOs are capable growing and at the same time storing ortho-phosphate present in the wastewater as polyphosphate granules. Thus phosphorus is not only used for cell maintenance, cell synthesis and energy transport, but is also stored as energy reserve for anaerobic consumption. Since PAOs are obligatory aerobic bacteria, they can only grow under aerobic conditions. The sludge produced aerobically is purged and as such the phosphorus leaves the installation. In so-called side stream processes, the sludge is subjected to anaerobic conditions and releases its phosphorus content again. To this concentrated side stream, precipitants are added to remove the phosphorus from the liquid phase.

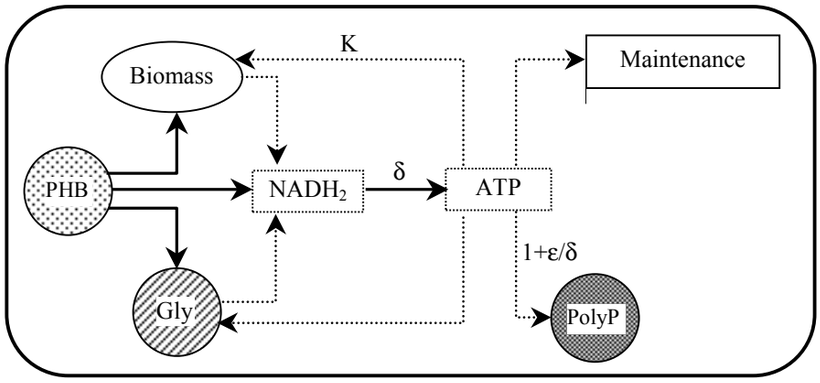

Figure 49 Aerobic mechanism according to Smolders et al. (1995a)

In Figure 49 and Figure 50 possible aerobic and anaerobic mechanisms are presented. In literature two metabolic pathways are presented to explain the anaerobic production of $NADH_2$, i.e. production via the TriCarboxilic Acid (TCA)-cycle (not active according to some authors under anaerobic conditions), or via the glycolysis cycle.

Enhanced biological phosphorus removal thus needs the sludge to pass through different phases, i.e. at least an anaerobic phase followed by an aerobic phase. In many reactor configurations also an anoxic phase is included to remove the nitrogen in the wastewater as nitrogen gas through denitrification of the nitrate formed during the aerobic phase. In wastewater terminology, anoxic conditions are characterised by the absence of free oxygen, but presence of nitrate. Combined denitrification/dephosphatation needs to be operated carefully since introduction of nitrate into the anaerobic phase decreases the phosphorus removal capacity of the unit. Since denitrification uses part of the available carbon source, and since utilisation of carbon sources for denitrification is faster than uptake by PAOs, denitrifiers compete with PAOs for the carbon source. Many reactor configurations have been suggested to avoid penetration of nitrate in the anaerobic phase.

[1] This paper was presented at a Flemish conference (KViV Studiedag "Nutriëntenverwijdering over de grens: laatste ontwikkelingen") March 1996. Since then many article have been published with new explanations on possible metabolic pathways and involved micro-organisms. The content of this paper has not been changed, therefore also the state of the art, as it was known from published articles anno 1996, has not been altered in this paper.

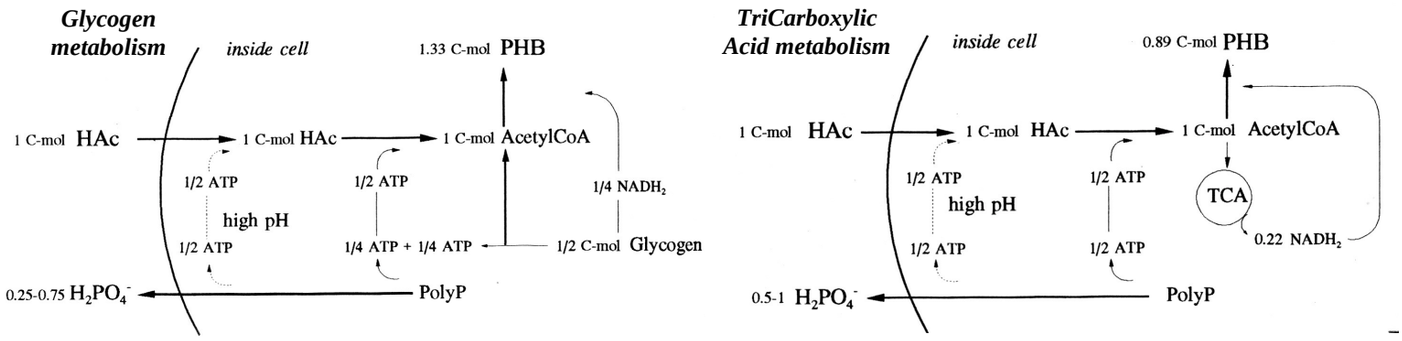

Figure 50 Possible anaerobic mechanisms (Smolders et al., 1994a)

Reactor configurations where only phosphorus removal is aimed at are the A/O and the PhoStrip-process. Typical examples of combined phosphorus/nitrogen removal processes are the A^2/O, the Bardenpho, the UCT and the VIP process. Operating parameters and indicative values for optimal operation of these processes can be found in several handbooks some of which are mentioned in the reference list.

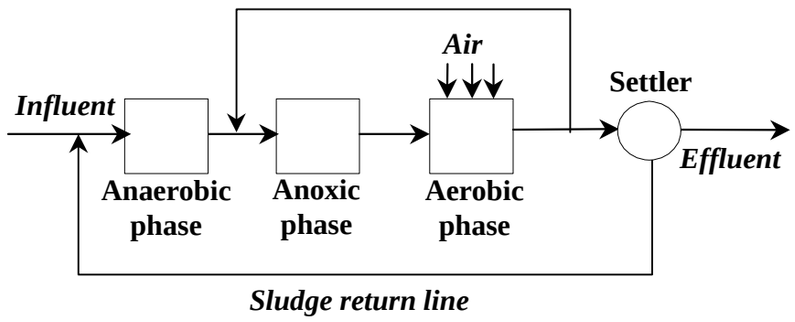

Figure 51 Schematic layout of a Phoredox installation

A schematic layout of a Phoredox installation is presented in Figure 51. As can be seen the sludge return line is directly connected to the anaerobic phase. Remaining nitrate is thus introduced in this phase and competition between PAOs and denitrifiers will occur. To obtain low nitrate concentration in general, an internal recycle (with high flow rate) from the aerobic to the anoxic phase is included.

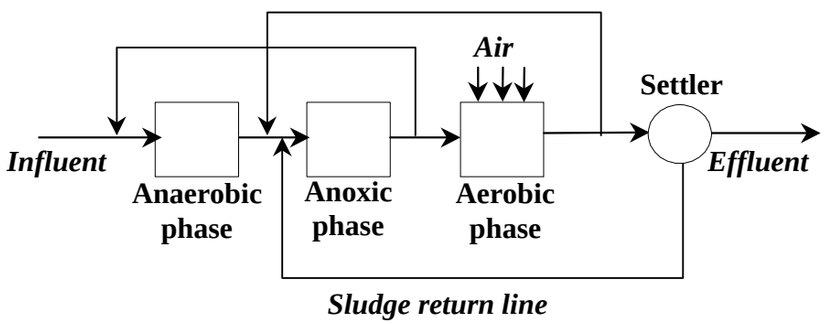

Figure 52 Schematic layout of a UCT installation

For the UCT installation, the sludge return line is connected to the anoxic phase (Figure 52). A second sludge return line then connects the anoxic phase to the anaerobic phase. With this configuration, remaining nitrates first pass through the anoxic phase were denitrification eliminates this component. The sludge return to the anaerobic phase now is nitrate-free and PAOs don't have to compete with denitrifiers for the available substrate.

When the EBPR research started, it was often thought that the causal microbial population consisted of *Acinetobacter spp.* However later research provides evidence that, although *Acinetobacter* has high phosphorus storage capacities, probably it is not the main, and certainly not the only, micro-organism

responsible for enhanced biological phosphorus removal. Micro-organisms belonging to the genus *Aeromonas*, *Pseudomonas*, *Moraxella* and *Nocardia* are attributed phosphorus removal capacities as well.

Nitrification is a two step reaction, i.e. first ammonium is converted to nitrite by *Nitrosomonas* and subsequently this nitrite is converted to nitrate by *Nitrobacter*. As for denitrification causal organisms encountered in literature mainly belong to the genus *Pseudomonas*. *Bacillus licheniformis, Paracoccus denitrificans* and *Thiobacillus denitrificans* are also able to denitrify under well defined conditions (Schlegel, 1993).

2. MATERIALS AND METHODS

2.1. *INSTALLATIONS*

2.1.1. Phoredox installation

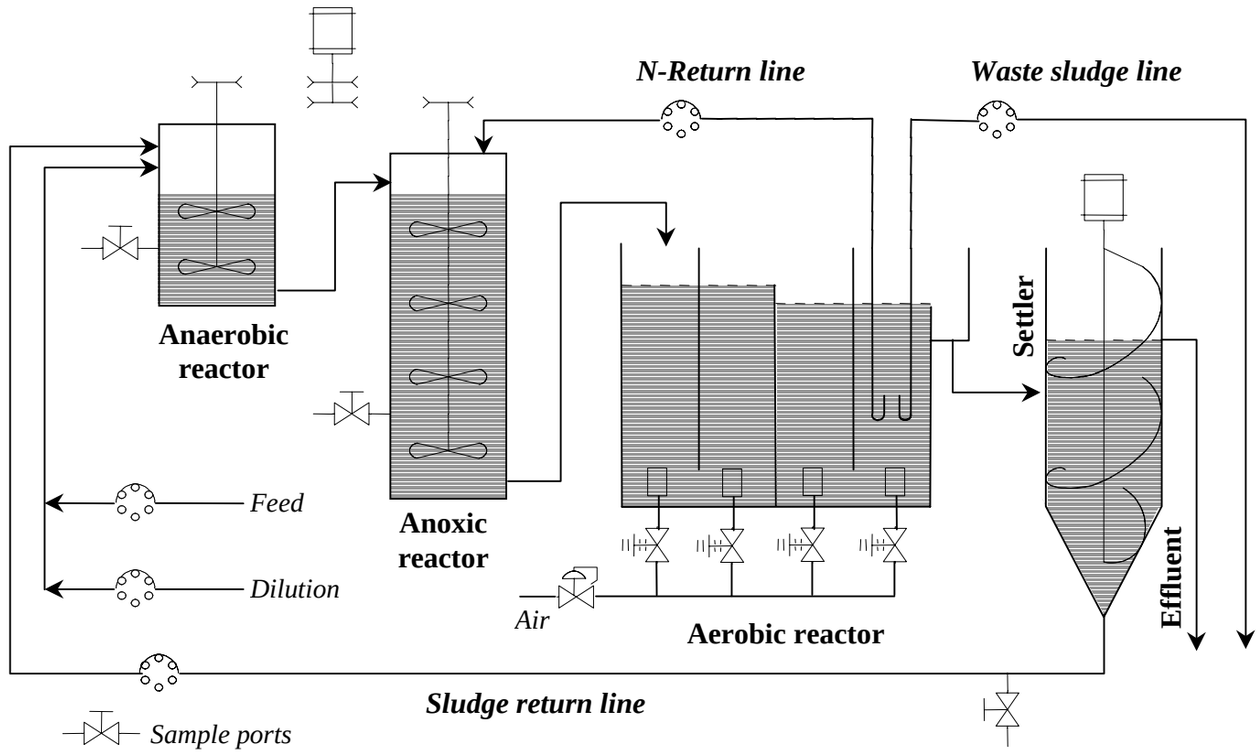

Figure 53 Phoredox installation, practical implementation in laboratory

The Phoredox installation (Figure 53) consists of a cylindrical anaerobic reactor with an active volume of 1.74 ℓ, an anoxic reactor with an active volume of 5.54 ℓ, a rectangular aerobic reactor consisting of 4 compartments with a total active volume of 14.24 ℓ and a clarifier. In the anaerobic and anoxic reactor complete mixing is obtained by stirring by means of aluminium blades. The aerobic reactor is divided in 4 compartments to obtain partial plug flow behaviour and to avoid short circuiting. Each of the aerobic compartments contains a porous block through which compressed air flows to obtain fine bubbles in the liquid phase. The settler has an active volume of 5 litres and consists of a cylindrical and a conical part. The installation was constructed in the laboratory. The sludge age is maintained at 10 days. The residence times and flow rates in and to the compartments are presented in Table 62.

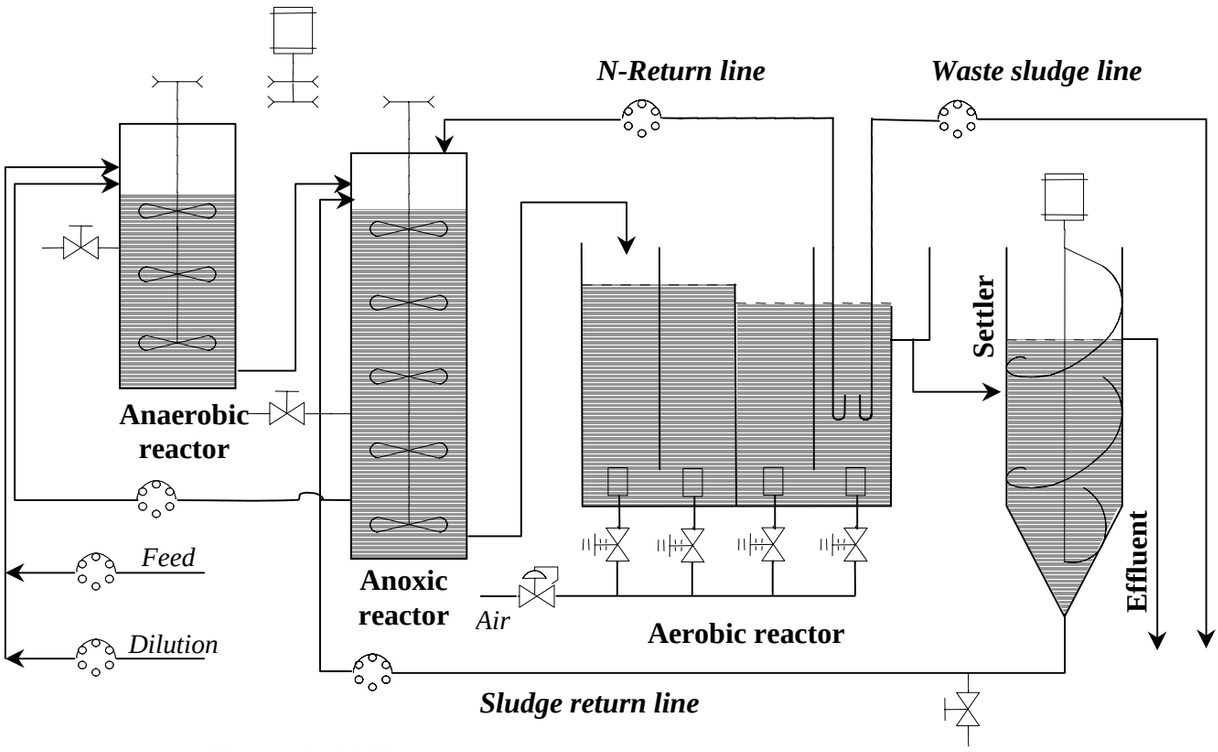

Figure 54 UCT installation, practical implementation in laboratory

2.1.2. University of Cape Town (UCT) installation

The installation is constructed using essentially the same vessels as described for the Phoredox installation. In the UCT configuration return sludge from the settler is sent to the anoxic reactor instead of the anaerobic reactor in the Phoredox configuration. The UCT installation also has an additional sludge return line from the anoxic to the anaerobic reactor, which is not present in the Phoredox configuration. The sludge age is maintained at 10 days as for the Phoredox installation. The residence times and flow rates are presented in Table 62.

Both the UCT and the Phoredox installation were inoculated using sludge from a full scale activated sludge treatment plant (Nevele, Belgium) exhibiting carbon removal and nitrification/denitrification, but not exhibiting phosphorus removal.

2.1.3. Sequencing Batch Reactor (SBR)

The equipment consists of a lab-scale fermentor with a useful volume of 11 litre filled with 8 litre of mixed liquor. The total cycle length is 6 hours: 15 minutes for filling up of the reactor (i.e. adding 4 litres of feeding), 1.5 hours for the anaerobic phase, 3.25 hours for the aerobic phase, an anaerobic period of at most 10 minutes followed by a settling phase of at least 33 minutes. During 17 minutes 3.8 litre of clear liquid is removed. At the end of the aerobic period 200 mℓ of mixed liquor is removed to obtain a sludge age of 10 days. The hydraulic residence time is 12 hours. The installation is fully automated.

Nitrogen gas is bubbled through the reactor during the anaerobic phase when the dissolved oxygen (DO) concentration is above 0.05 mg O_2/ℓ. During the aerobic phase the DO concentration was controlled to obtain a constant value of 2 mg/ℓ. The band width was set at 0.2 mg/ℓ. The real band width was up to 1 mg/ℓ due to overshoot in both directions. Gas flows were controlled using mass flow controllers (Bronkhorst). The flow rates were fixed for nitrogen and air and can, during the course of the cycle, only

be changed manually. A constant stirrer speed of 350 rpm was maintained except for the settling and effluent purge period. A pH and temperature control system was installed in a later stage of the research programme.

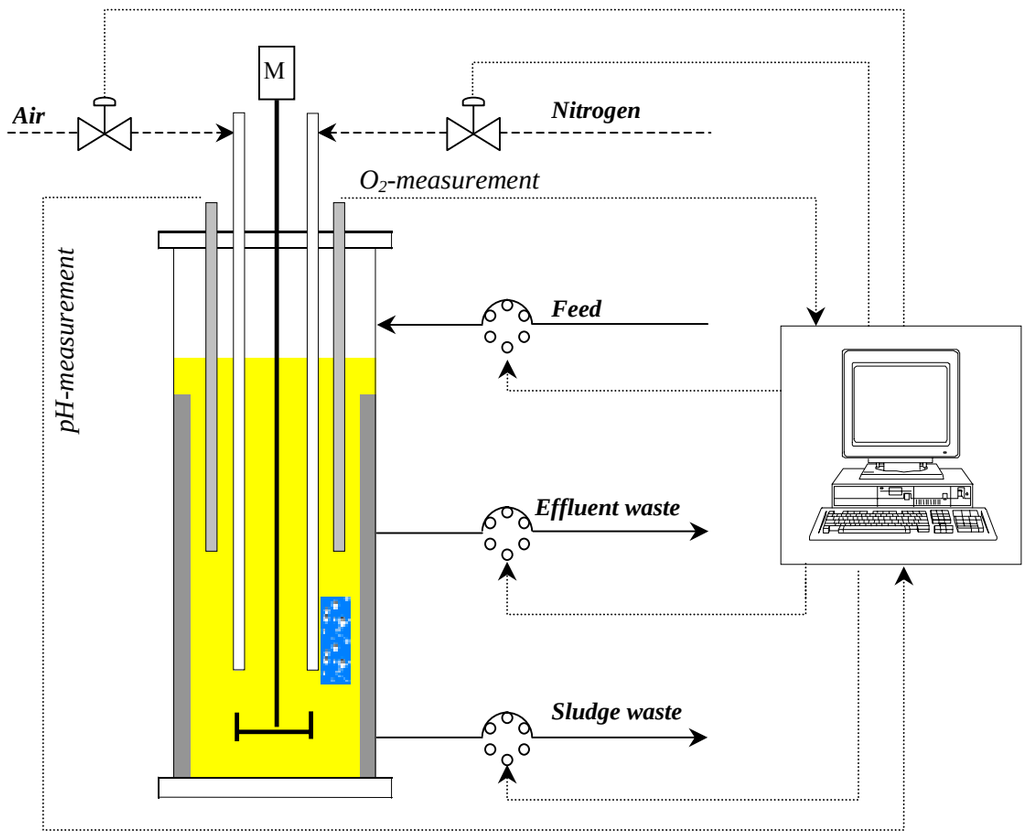

Figure 55 Practical implementation of the sequencing batch reactor

Nitrate is present at the end of aerobic period due to nitrification reactions. The anaerobic in-between period, when nitrogen gas is bubbled through the liquid phase, is introduced to prevent any oxygen remaining in the mixed liquor when new feed is added at the beginning of the next cycle. During this in between anaerobic period (to use correct terminology it should be called in between anoxic period since nitrate is present) no remaining or freshly provided carbon is available, so denitrification is almost not occurring and. When feed is added to the SBR at the beginning of the cycle, anoxic conditions prevail and denitrification reactions consume part of the carbon source provided. The length of this anoxic period depends on the nitrate concentration. With the operating conditions used in this study, the anoxic period takes about 2 minutes.

The SBR was inoculated with sludge from the Phoredox installation after this had been in operation for several months.

2.1.4. Flow rates and residence times for the installations

Table 62 presents an overview of the flow rates to and the residence times in the different zones/phases of the installations used.

Table 62 Flow rates and residence times for the different installations

	Phoredox	UCT	Sequencing Batch
Flow rates			
Influent (feed + dilution)	0.96 ℓ/h	0.96 ℓ/h	4ℓ/6h
Anaerobic reactor → Anoxic reactor	1.21 ℓ/h	3.46 ℓ/h	-
Anoxic reactor → Anaerobic reactor	-	2.5 ℓ/h	-
Anoxic reactor → Aerobic reactor	3.71 ℓ/h	3.71 ℓ/h	-
Aerobic reactor → Settler	0.835 ℓ/h	0.835 ℓ/h	-
Nitrogen return line (Aerobic→Anoxic)	2.5 ℓ/h	2.5 ℓ/h	-
Sludge return line	0.25 ℓ/h	0.25 ℓ/h	-
Waste sludge line	0.125 ℓ/h	0.125 ℓ/h	200 ml/6h
Effluent	0.835 ℓ/h	0.835 ℓ/h	3.8 ℓ/6h
Residence times			
Anaerobic phase	1 hour 21 min	1 hour 18 min	1 hour 30 min
Anoxic phase	1 hour 36 min	1 hour 27 min	
Aerobic phase	3 hour 50 min	3 hour 50 min	3 hour 15 min
Settler	5 hour 12 min	5 hour 12 min	+/- 30 minutes

2.1.5. Feed compositions

Since the first start-up of the Phoredox installation different feed compositions were tested. From 1994 till 1996 meat extract, meat extract/acetate and pure acetate feeds were used. The SBR was only fed with acetate as carbon source.

The meat extract feed composition consists of 82.26 mg KH_2PO_4 (18.7 mg P/ℓ) and 3.125 ml meat extract (Liebox) per litre. The feed then contains 1200 mg COD/ℓ and 29 mg P/ℓ. Ortho-phosphates originates both from the KH_2PO_4 and from the meat extract.

The meat extract/acetate feed mixture consists of 0.85g $NaAc.3H_2O$ (400 mg COD/ℓ), 2.1 mℓ meat extract (Liebox), 82.26 mg KH_2PO_4 (18.7 mg P/ℓ), 0.275 g $NaHCO_3$ and 0.2875 mℓ nutrient solution (see further). The feed then contains 1200 mg COD/ℓ as for the pure meat extract feed but only 25.6 mg P/ℓ.

For the acetate feed a non sterilised medium was used containing 0.85 g $NaAc.3H_2O$ or 0.375 g HAc (400 mg COD/ℓ), 65.81 mg KH_2PO_4 (15 mg P/ℓ), 90 mg $MgSO_4.7H_2O$ (12 mg S/ℓ), 14 mg $CaCl_2.2H_2O$, 36 mg KCl, 107 mg NH_4Cl (28 mg N/ℓ), 1 mg yeast extract, 275.4 mg $NaHCO_3$ and 0.3 ml nutrient solution per litre.

The nutrient solution contained per litre: 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5\ H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO_4.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$ and 10 g EDTA. Only reagent grade products were used.

When batch experiments were performed using sludge from the UCT or Phoredox installations and using "acetate feed", the sodiumacetate content was increased to 1.275 g $NaAc.3H_2O$ (600 mg COD/ℓ), which yields a COD values in between the normal feed composition for UCT/Phoredox and the SBR.

2.2. *ANALYSIS METHODS*

Ortho-phosphate analyses, using the ascorbic acid method, were performed with a colorimetric auto-analyser (TrAAcs 800) with a measurement range between 0.5 and 5 mg P/ℓ. *Nitrate measurements*, using the hydrazine reduction method, were also performed on TrAAcs, with a measurement range between 0.2 and 2 mg N/ℓ. Colorimetric *COD analyses* were performed according to the Standard Methods using Hach tubes and a Nanocolor colorimeter. *Potassium analyses* were performed using a flame photometer. For *PHA determination* lyophilised biomass was subjected to a propylation reaction. 1 mg benzoic acid in n-propanol was added to 25 à 120 mg lyophilised biomass in 15 mℓ tubes. One and a half millilitres of a n-propanol/HCl mixture (4:1) and 1.5 mℓ of 1,2 dichloroethane were added to the lyophilised sample. The mixture was heated for 2 hours at 100°C and shaken every 15 minutes. After cooling, the organic phase was extracted with 3 mℓ of water. Two millilitres of the organic phase was dried on Na_2SO_4. About 0.5 µℓ was injected on a gas chromatograph with an FID detector at 250°C and a splitter/split-less injector at 200°C (Chrompack). An Innowax column (Hewlett Packard) was used. The temperature programme started at 80°C and rose to 225°C in 9.2 minutes, remaining at this temperature during 13 minutes. Replicated analyses showed an average procentual standard deviation of 1.3 %.. For *MLSS measurements* 25 ml mixed liquor was filtered on Whatman glass microfibre filters (GF/C). Before and after filtration, the filters were dried at 105°C and weighed.

2.3. *BATCH EXPERIMENTS*

Batch experiments were performed using sludge sampled from the aerobic reactor of the Phoredox and UCT configurations. This sludge is transferred to a mini SBR. Therefore 3 litres of mixed liquor is taken and after settling of the sludge during 30 minutes, 1.5 litres of clear liquid is removed. To the remaining 1.5 litres the same volume of acetate feed is added at the beginning of the experiment. The control system of the SBR installation is used to control the oxygen concentration in the different phases of the experiment.

2.4. *PROFILE RECORDING AND KINETIC EXPERIMENTS*

On an average basis, the SBR unit was sampled twice a week to verify the phosphorus removal capacity of the installation. For this purpose, samples were taken at the end of the previous cycle in the settling phase, at the end of the feeding phase, at the end of the anaerobic phase and at the end of the aerobic phase . Samples were then analysed at least for ortho-phosphate, nitrate and MLSS. COD was analysed less frequently. These data are plotted versus cycle number and will be termed “profile recordings” hereafter.

When experiments were performed to elucidate the kinetics of the different reactions, in total 30 samples were taken during the anaerobic and aerobic phase of one cycle. Samples were then analysed for ortho-phosphate, COD, nitrate and PHB.

3. RESULTS

3.1. *RESULTS OBTAINED WITH THE CONTINUOUS PHOREDOX AND UCT INSTALLATIONS*

3.1.1. Phosphorus removing characteristics of sludge cultivated with meat extract

When the Phoredox installation was inoculated with sludge from a wastewater treatment plant not exhibiting phosphorus removing capacities, about 4 to 6 weeks were needed to obtain a typical phosphorus removing profile, i.e. anaerobic release followed by aerobic uptake with complete phosphorus removal and thus a zero phosphorus concentration in the effluent.

From Table 63 it can be seen that, an increase in phosphorus concentration is observed in the anaerobic phase followed by a net phosphorus removal in the aerobic phase. Stable operating conditions were not observed. The reason for this can be attributed to several causes: changing composition of the meat extract, especially due to settling of the particulate constituents in the feed reservoir, adsorption of phosphorus components to the components of the meat extract, a shortage of available SCFAs, growth of micro-organisms at the surface of the plexi reactors and at the aluminium blades of the rotors, etc… As for the growth of micro-organisms on the walls and blades, this will cause increased sludge ages and growth of higher trophic level organisms, amongst them predators. In Table 63 a typical COD profile is presented too. It is observed that the main part of the available COD is utilised in the anaerobic phase. Apparently enough COD remains in the anoxic phase, since no nitrate was observed in this phase although the aerobic nitrate concentration was about 9 mg/ℓ.

Table 63 Ortho-phosphate and COD profiles recorded in the Phoredox installation fed with meat extract

Days since start-up	Influent	Anaerobic phase	Anoxic phase	Aerobic phase	Effluent
Ortho-phosphate [mg P/ℓ]					
31	16.4	29.8	40.7	11.8	7.3
38	26.7	29.4	32.1	12.6	8.7
45	24.9	33.5	-	10.2	6.4
52	29.3	25.1	39.3	16.7	10.6
COD [mg COD/ℓ]					
31	n.m.	n.m.	n.m.	n.m.	n.m.
38	1097	756	174	117	n.m.
45	1197	661	121	136	n.m.
52	1315	577	167	128	n.m.

n.m.: not measured

3.1.2. Phosphorus removing characteristics of sludge cultivated with meat extract/acetate feed

To achieve a higher net phosphorus removal efficiency it was decided to feed both the Phoredox and UCT installations with a mixture of meat extract and acetate. The total COD concentration in the influent remained the same. By addition of acetate it was thought the PAO fraction in the sludge would increase, since these organisms would be less dependent on fermentative bacteria producing acetate. It was also expected that less competition would exist with other heterotrophic bacteria. From the ortho-phosphate profiles recorded (Table 64), however, it is clear that still no complete phosphorus removal was achieved.

Table 64 Ortho-phosphate and PHB profiles recorded in the Phoredox installation fed with meat extract/acetate

Days since start-up	Influent	Anaerobic phase	Anoxic phase	Aerobic phase	Effluent
Ortho-phosphate [mg P/ℓ]					
30	28.2	40.0	40.2	24.5	18.6
41	28.5	37.9	38.4	15.5	13.9
69	21.0	43.8	39.6	11.1	11.5
80	29.5	44.3	42.5	20.9	10.4
COD [mg COD/ℓ]					
41	537	110	47	102	n.m.
PHB [%]					
41	-	2.771	-	1.132	-

Table 65 Ortho-phosphate and PHB profiles recorded in the UCT installation fed with meat extract/acetate

Days since start-up	Influent	Anaerobic phase	Anoxic phase	Aerobic phase	Effluent
Ortho-phosphate [mg P/ℓ]					
36	22.6	26.7	32.5	18.8	13.8
41	24.8	56.1	24.0	23.4	14.7
69	29.3	56.0	47.4	16.9	16.9
76	28.5	53.2	45.4	15.4	16.5
COD [mg COD/ℓ]					
41	1170	242	121	129	n.m.
PHB [%]					
41	-	6.709	5.099	1.002	-

From Table 64 and Table 65 a clear PHB profile is observed, i.e. a high PHB concentration in the anaerobic and anoxic reactors and a lower concentration in the aerobic reactor. Both the anaerobic phosphate and PHB concentrations are higher in the UCT installation than in the Phoredox installation.

From the batch experiment shown in Figure 56 it is observed that complete phosphorus removal could be obtained when sludge from the Phoredox installation is subjected to anaerobic/aerobic conditions and acetate is used as sole carbon source. Subjecting sludge originating from the UCT installation to the same operating conditions did not lead to complete phosphorus removal (Figure 57). However, from these graphs it is also observed that complete phosphorus removal might have been achieved by increasing the length of the aerobic phase. Indeed, for the UCT sludge a clear phosphorus profile was recorded too and a final effluent concentration of 2 mg P/ℓ only was reached.

During both experiments nearly the same COD and PHB profiles were observed. The micro-organisms originating from the Phoredox installation, however, show higher nitrification capacities than the micro-organisms originating from the UCT installation.

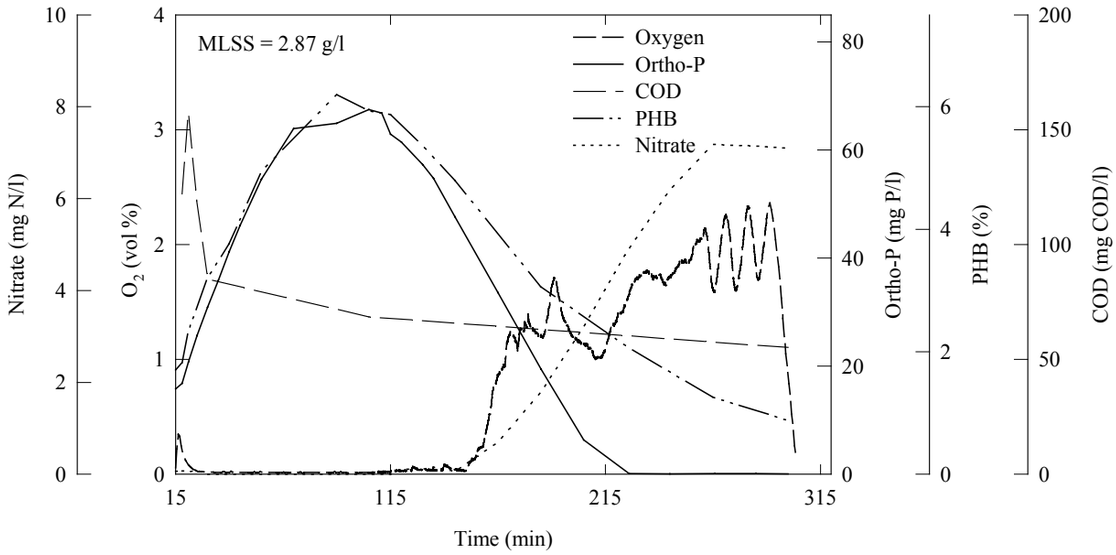

Figure 56 Anaerobic-aerobic concentration profiles recorded during a batch experiment using sludge from the Phoredox installation and using acetate as sole carbon source

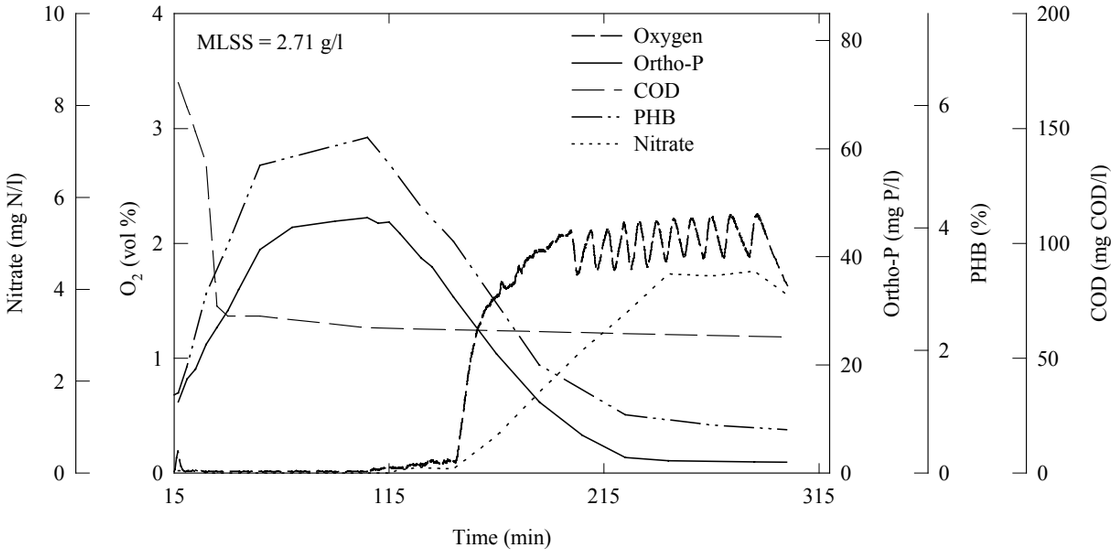

Figure 57 Anaerobic-aerobic concentration profiles recorded during a batch experiment using sludge from the UCT installation and using acetate as sole carbon source

An essential difference between both batch experiments was the pH value at which the experiments were performed. The sludge samples originating from both installations had relatively high pH values. Therefore some drops of sulphuric acid were added to bring the pH at about the same value as the one observed in the SBR. For the experiment using sludge originating from the UCT installation, in the anaerobic phase the pH started at 7.6 whereas for the Phoredox installation this value was only 7.1. In both experiments an increase in pH during the anaerobic phase was observed to a pH of 8.9 for the UCT sludge and to a pH of 8.1 using Phoredox sludge. Biologically induced phosphorus precipitation might have occurred at these high pH values.

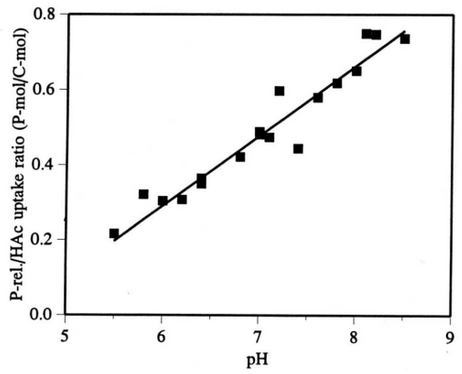

Figure 58 P-release/acetate uptake ratio as a function of pH (Smolders et al., 1994a).

A pH decrease was observed at the moment nitrification started in the aerobic phase. At the end of the anaerobic phase, sulphuric acid was added to experiments using UCT sludge to allow both experiments to be continued at the same pH during aerobic phosphorus uptake.

According to Smolders *et al.* (1994a) the anaerobic phosphorus release to acetate uptake ratio is pH dependent (Figure 58). In a pH range from 5.5 to 8.5, with increased pH an increased phosphorus release is observed with equal amounts of acetate consumed. Our observations predict the opposite pH dependency, i.e. a lower phosphorus released acetate uptake ratio at higher pH.

During the experimental runs it was observed that, although using a smaller reactor volume (3ℓ) for these experiments (compared to the experiments run in the SBR (8ℓ)), it was not possible to achieve the desired oxygen concentration of 2 mg O_2/ℓ during the aerobic phase using the same compressed air flow rate as for the SBR. After half an hour of aerobic conditions at low oxygen concentration the compressed air flow rate was increased to obtain a concentration of 2 mg O_2/ℓ. Although it was initially thought that the low oxygen concentration during the initial aerobic period would have a repercussion on the phosphorus uptake, PHB utilisation and phosphorus uptake started as soon as the compressed air was allowed to flow through the reactor. Nitrification, however, only occurred when the oxygen concentration started increasing. Further explanation is provided in a later section (SBR system) where it was observed that oxygen penetration during the anaerobic phase induced a phosphorus uptake, even though no oxygen concentration was measured.

3.1.3. Phosphorus removing characteristics of sludge cultivated with acetate feed

Due to the promising results obtained during the batch experiments and since complete phosphorus removal was still not achieved in the Phoredox or UCT installation, these installations were fed with acetate as the sole carbon source. A total concentration of 600 mg COD/ℓ was used, which is an average of the value used in the SBR installation and in the Phoredox/UCT installations. The phosphorus concentration in the influent was set at 30 mg P/ℓ.

Table 66 Ortho-phosphate profiles recorded in the Phoredox and UCT installation fed with acetate solution

Days since start-up	Influent	Anaerobic phase	Anoxic phase	Aerobic phase	Effluent
Ortho-phosphate [mg P/ℓ]					
Phoredox installation					
22	31.4	69.5	28.7	8.1	3.5
25	25.0	55.0	44.9	17.9	10.3
40	22.5	69.8	60.7	12.6	1.5
43	26.5	68.7	68.6	8.4	3.6
UCT installation					
22	35.6	47.0	44.0	7.6	2.6
25	28.0	48.3	44.1	22.3	10.5
40	23.6	31.7	33.4	1.7	0.9
43	23.9	44.8	38.7	14.2	3.2

At the moment of publication of these results, the switch had only recently been performed and provided mainly proof that the effluent phosphorus concentration in both Phoredox and UCT configurations nearly instantly reached a value close to zero (Table 66). Noteworthy is that the shift created favourable conditions for slime forming bacteria. Due to this phenomenon many cloggings of the tubing occurred, especially for the UCT installation. It was observed that the sludge concentration decreased due to the lower COD load.

3.2. *RESULTS OBTAINED WITH THE SEQUENCING BATCH REACTOR (SBR)*

The SBR was mainly taken into operations to obtain a sludge consisting primarily of phosphorus removing organisms, this because of the feed containing acetate as the sole carbon source. The phosphorus concentration in the influent to the SBR installation only contained 15 mg P/ℓ compared to the 30 mg/ℓ in the influent to the Phoredox and UCT installations.

Initially, the acetate feed was made using acetic acid as acetate source. The pH in the reactor varied from 6.5 at the end of the aerobic period, up to 7.5 at the end of the anaerobic phase. Only three days after inoculation of the SBR (with sludge from the Phoredox installation) complete phosphorus removal was obtained. However, an increase of the effluent phosphorus concentration was observed afterwards. A typical phosphorus removal profile, anaerobic release and aerobic uptake, was recorded but no net phosphorus removal was obtained.

The installation was inoculated three times with fresh sludge without noticeable improvement of EBPR activity. At last it was decided to change the acetic acid feed to sodium acetate. As is shown in Figure 59 only ten days after switching to the new carbon source complete phosphorus removal was achieved. A pH increase with 1 unit was observed too.

From the results it is clear that complete phosphorus removal can be achieved in SBRs. The biomass showed very good settling characteristics and disturbances in the installation did not induce the disappearance of EBPR activity. Even after long anaerobic or aerobic conditions due to malfunctioning of the installation, phosphorus removal profiles were recorded after as few as two cycles. The SBR system thus reveals very robust.

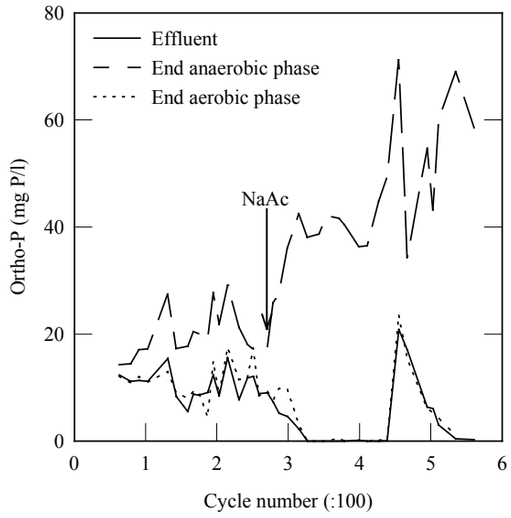

Figure 59 EBPR activity for HAc and NaAc

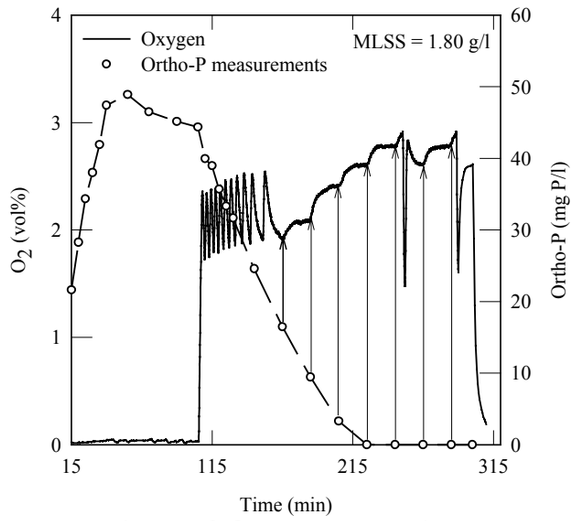

Figure 60 Oxygen penetration

For the first batch tests performed, it was observed that after an initial anaerobic increase of both ortho-phosphate and PHB concentration, a decrease was recorded before the aerobic phase was initiated. An explanation for this phenomenon was found when examining the aerobic oxygen profiles in detail (Figure 60). Indeed, at a certain moment the oxygen concentration started increasing in the aerobic phase without pressurised air being provided to the reactor. It was noticed that every systematic increase from that moment on was linked to the moment a sample was taken. This observation led to the conclusion that probably also during the anaerobic period, oxygen penetrated in the reactor although it was immediately consumed by the micro-organisms with consumption of ortho-phosphate and PHB. It was decided then for future experiments to have continuous nitrogen bubbling through the liquid and to have a continuous nitrogen blanket above the liquid. From that moment on, ortho-phosphate and/or PHB decreases during the anaerobic phase were no longer observed.

During the first test it was observed ortho-phosphate release and PHB formation occurred very fast. Using the normal feeding cycle of the installation, 4 litres of feed was added to the reactor during the first 15 minutes. In this period much information is lost. Sampling the reactor during that time period is difficult and careful interpretation of the data is necessary to account for changing volumes. A more elegant solution was applied by changing the measurement campaign. The feed liquor was allowed to flow in a separate vessel. This vessel was purged with nitrogen to eliminate all possibly present oxygen. Then the content of this vessel was manually added to the SBR. A possible aerobic utilisation of acetate during the feeding was thus also eliminated.

In Figure 61 a typical profile is shown. PHB and ortho-phosphate profiles nearly have the same profile. Nearly all acetate is consumed aerobically. The maximum anaerobic ortho-phosphate concentration for the SBR or the Phoredox. UCT experiments are comparable.

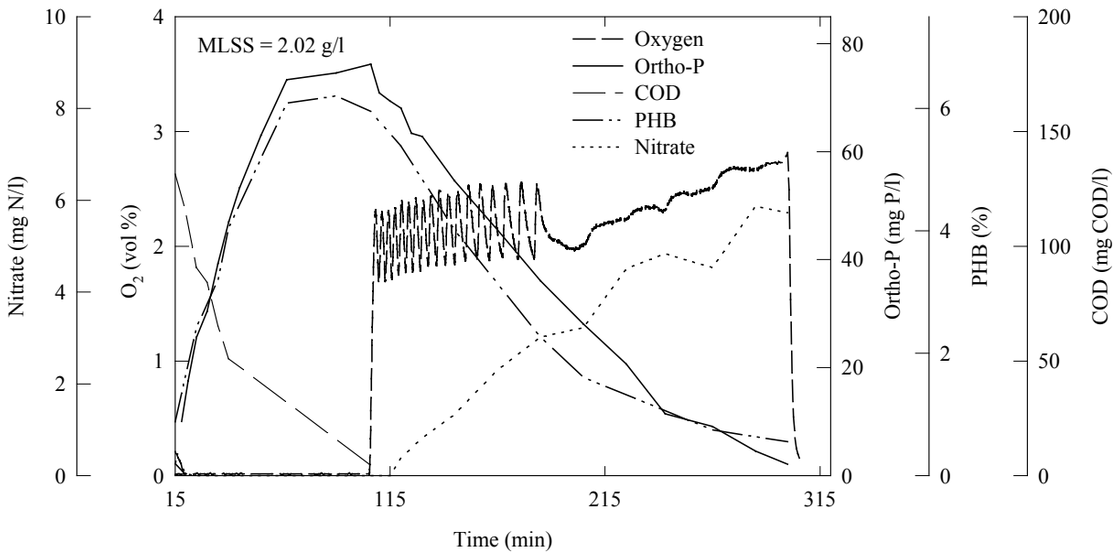

Figure 61 Anaerobic-aerobic concentration profiles recorded during SBR experiment (112 days after start-up)

To verify the denitrifying capacity of the microbial population, an experiment was performed where an additional amount of nitrate was injected at the beginning of the anaerobic phase to increase the nitrate concentration to a value of 25 mg $N\text{-}NO_3/\ell$. As can be seen in Figure 62 no significant denitrification was observed. However, the slight decrease of both ortho-phosphate and PHB coupled with a decrease in nitrate might indicate some denitrifying capacity of the microbial population.

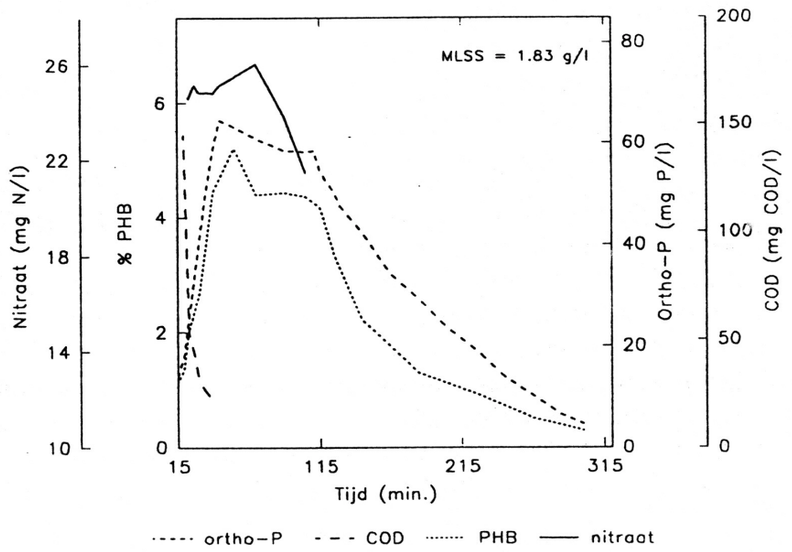

Figure 62 Anaerobic-aerobic concentration profiles recorded during SBR experiment

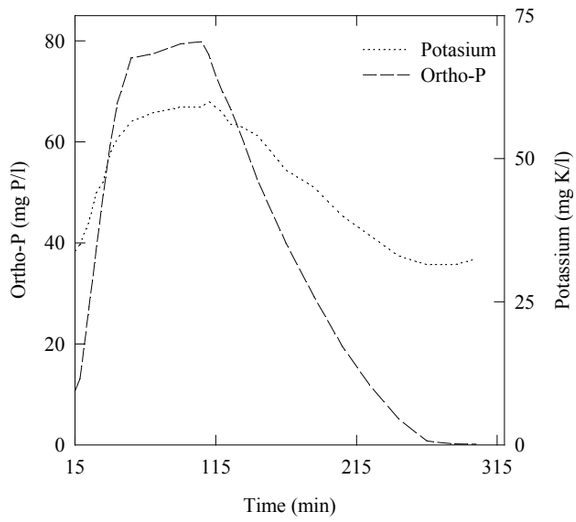

Figure 63 Ortho-phosphate and potassium profiles for a batch experiment

To gain even more proof that the profiles recorded are linked with biological phosphorus removal and not with precipitation of phosphorus compounds, potassium profiles were recorded as well during later batch experiments. Transport of phosphate over the cell membrane asks for co-transport potassium and magnesium as components for the polyphosphate structure. An ortho-phosphate profile should thus be accompanied by a potassium profile (Figure 63). According to Comeau *et al.* (1987a) a typical molar composition can be presented as $Mg_{1/3}K_{1/3}P$. For potassium a more precise value of 0.26 is mentioned. Our experimental results showed a molar ratio of K/P of 0.268 for the anaerobic period and a ratio of 0.274 for the aerobic period.

119 days after the start-up of the installations increased phosphorus effluent concentration were observed. From Figure 64 it can be seen that also a very high PHB concentration is recorded. Clearly the bacteria had stored a high amount of PHB in preceding periods, but were unable to use this feed stock for EBPR activity. Although a scattered PHB profile is obtained, anaerobically a net PHB increase occurs and aerobically PHB is net consumed. Growth of so-called G-bacteria (Cech and Hartman, 1990, 1993) who are able to use the glycolysis pathway (Satoh *et al.*, 1992) for acetate uptake and PHA production, can be a possible explanation for the observed phenomena. This experiment further indicates that in preceding experiments, the maximum amount of PHB was not yet reached. Moreover, these data provide evidence that high PHB contents can occur without net phosphorus removal. Nitrification took place, but the reaction rate is much lower than in the preceding experiments, resulting in a lower nitrate concentration at the end of the aerobic phase.[2]

[2] This information was not in the proceedings of the Flemish conference, but was presented at the 1st IAWQ specialised conference on SBR technology, Munich, 18-20 March 1996.

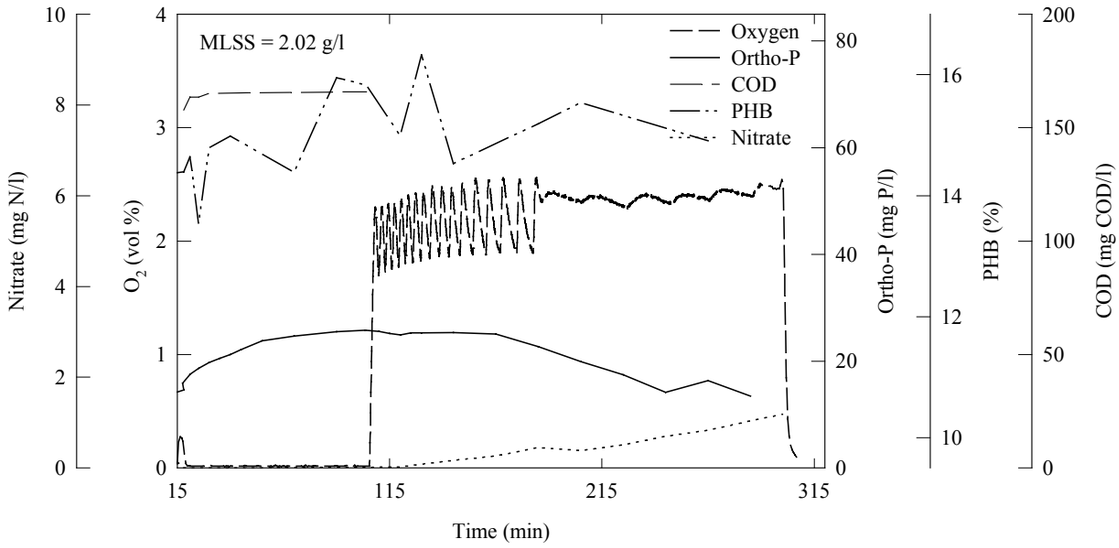

Figure 64 Anaerobic/aerobic profile without net phosphorus removal (119 days after start-up)

4. CONCLUSIONS

Different process configurations, continuous processes as well as batch operated processes, combined with different carbon sources were evaluated for their capacity to select for PAOs. For the continuous processes, i.e. the Phoredox and University of Cape Town (UCT) installations, EBPR activity was observed but stable process operation was difficult to achieve. With both UCT and Phoredox configurations comparable EBPR activity was observed. Using complex feed compositions, containing meat extract or meat extract/acetate as carbon sources, caused growth of complex microbial population competing for the available organic carbon. Using acetate as sole carbon source for the continuous installation complete phosphorus removal could be observed. Production of extracellular polymers now prevented stable operating conditions. Good EBPR activity was observed and stable process operation could be maintained operating a sequencing batch reactor (SBR) where acetate was used as sole carbon source. High internal carbon storage polymer contents, i.e. PolyHydroxyAlkanoates (PHAs), were observed probably when the biomass was grown under nutrient limiting conditions or external conditions preventing normal EBPR activity. Growth of so-called G-bacteria who are able to use the glycolysis pathway for acetate uptake and PHA production, can be a possible explanation for the observed phenomena. Oxygen penetration in the anaerobic phase due to sampling caused anaerobic uptake of phosphorus and consumption of PHA.

From the experiments obtained, no conclusive answer can be formulated which process configuration is to be preferred for full scale application. The SBR installation obviously reveals high and stable EBPR activity. The difficulties encountered for the continuous installations are typical for laboratory scale installation. Slime forming is observed in full scale application, but piping is less vulnerable for clogging. A UCT configuration is preferred over a Phoredox configuration when high nitrate concentrations are expected. However, since more recycles are necessary for the UCT installation, pumping costs will increase. A SBR installation allows for control of the effluent concentration by changing the cycle lengths. Higher operating costs are expected, but strict legislation can be better fulfilled.

Acknowledgement

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CHAPTER 4

Biological Phosphorus Removal: Composition of microbial population and kinetic modelling

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Chapter 4

Biological Phosphorus Removal: Composition of microbial population and kinetic modelling

Abstract - An SBR (Sequencing Batch Reactor) was used to study the kinetics of the processes involved in the Enhanced Biological Phosphorus Removal (EBPR) from wastewater. In addition to the batchwise operated reactor, two types of continuous plants, an A^2/O (Anaerobic-Anoxic-Oxic) system and a UCT (University of Cape Town) system, have been operated to cultivate phosphorus removing sludge. Growth of phosphorus accumulating organisms has been established. Concentration profiles indicate the possibility for complete phosphorus removal during the aerobic period. Kinetic models have been calibrated for anaerobic phosphate release and aerobic phosphate uptake. The models have been validated using experimental data from the different plants. The bacterial population of both the A^2/O and the SBR remained stable over the period tested. However, the majority of the organisms isolated from the A^2/O belonged to the Enterobacteriaceae, while the population of the SBR system was different. Representatives of Acinetobacter, regarded as a possible causal agent for biological phosphate removal, were only occasionally present in the sludge.

1. INTRODUCTION

For ecological reasons biological phosphorus removal from waste water is preferred to chemical phosphate precipitation. A conventional waste water treatment plant can easily be converted to a biological phosphorus removal plant by providing an anaerobic tank preceding the aerobic reactor.

Batch operated processes such as the SBR have the advantage that a stable operation can easily be obtained, allowing frequent sampling without disturbing the process. Continuous installations, however, are closer to real field situations. Therefore the SBR is mainly operated to record concentration profiles in time, to be used for kinetic modelling.

2. METHODS

2.1. *APPARATUS*

The study was carried out in a laboratory fermentor filled with 8 ℓ of mixed liquor. The reactor was operated as a sequencing batch with a cycle length of 6 or 8 hours: 15 min filling (anaerobic), 1.5 hours anaerobic phase, 3.5 or 5.5 hours aerobic phase, a maximum of 10 min anoxic in between phase, a minimum of 33 min settlement phase and a 17 min effluent withdrawal period. At the end of the aerobic period, 200 ml of mixed liquor was purged to obtain a sludge age of 10 or 13 days.

An amount of 3.8 litres of supernatant was removed at the end of the cycle and 4 litres of medium was fed at the beginning of the cycle. The hydraulic retention time thus was 12 or 16 hours. A time controller was used to obtain the settings for the different phases.

During the anaerobic phase, the mixed liquor was sparged with nitrogen. During the aerobic phase the dissolved oxygen (DO) concentration was controlled in an on-off strategy with a set point of 2 mg O_2/ℓ. In practice values of 2 ± 0.5 mg O_2/ℓ occurred due to overshoots in both directions. Gas flows were

controlled with mass flow controllers. Flow rates were fixed at a given value for nitrogen and air and could only be changed manually.

The A^2/O and the UCT systems consisted both of an anaerobic reactor, an anoxic reactor, an aerobic reactor and a settler. The main difference between both configurations is that the sludge recycle is directly pumped to the anaerobic zone for the A^2/O unit, and for the UCT unit to the anoxic zone with an extra internal recycle from the anoxic zone to the anaerobic zone. A sludge age of 10 days was aimed at. The reactor volumes of both systems were adjusted to obtain the same residence times. The anaerobic residence time (1h20) and the aerobic residence time (3h50) were comparable to the lengths of these phases in the SBR. The residence times in the anoxic zone and in the settler were much higher for the continuous set-ups. The influent flow rate equalled 0.96 ℓ/h.

2.2. *MEDIUM*

For the SBR, in a first experimental phase, a non sterilised medium was used containing per litre : 0.85g $NaAc.3H_2O$ (400 mg COD), 65.81 mg KH_2PO_4 (15 mg P), 90 mg $MgSO_4.7H_2O$,14 mg $CaCl_2.2H_2O$, 36 mg KCl, 107 mg NH_4Cl (28 mg N), 1 mg yeast extract, 275.4 mg $NaHCO_3$ and 0.3 ml nutrient solution. This mixture was adopted from Smolders et al. (1995a), adding $NaHCO_3$ as a buffer agent. In an second phase the COD concentration was doubled. In a third experimental phase the composition per litre was changed to : 1.7 g $NaAc.3H_2O$ (800 mg COD), 197.43 mg KH_2PO_4 (45 mg P), 180 mg $MgSO_4.7H_2O$, 28 mg $CaCl_2.2H_2O$, 72 mg KCl,214 mg NH_4Cl (56 mg N), 2 mg yeast extract, 275.4 mg $NaHCO_3$ and 0.6 ml nutrient solution.

The nutrient solution contained per litre : 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5\ H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4\ H_2O$, 0.06 g $Na_2MoO_4.2\ H_2O$, 0.12 g $ZnSO_4.7\ H_2O$, 0.15 g $CoCl_2.6\ H_2O$ and 10 g EDTA. Only reagent grade products were used.

The A^2/O and UCT were fed with a meat extract mixture consisting of 3.125 ml meat extract (Liebox) and 82.26 mg KH_2PO_4 on litre basis The influent thus contained 1450 mg COD/ℓ and 29 mg P/ℓ.

2.3. *ISOLATION PROCEDURE*

The composition of the bacterial population of the A^2/O and SBR experiments has been studied after a direct isolation procedure. Samples were plated after homogenisation and serial dilution on eight different media (general as well as specific) for colony forming units (cfu). The most abundant two or three different colony types were selected from each type of medium for further purification.

2.4. *ANALYSIS METHODS*

Orthophosphate analyses, by means of the ascorbic acid method, nitrate measurements, by means of the hydrazine reduction method, and ammonia measurements, using the Berthelot reaction, were performed with a colorimetric autoanalyser. Colorimetric COD analyses were performed according to the Standard Methods using prefabricated tubes. Potassium measurements were conducted on a flame photometer. Probably due to interference with the high sodium content, the measured potassium concentration exceeded the actual one. Since the sodium concentration remained constant, the interference was considered constant. For polyhydroxyalkanoate (PHA) analyses, lyophilised biomass was subjected to a propylation reaction. The organic phase was analysed by gas chromatography. Polyphosphate was calculated from a total phosphate analysis. Therefore, lyophilised biomass was subjected to a destruction reaction in an H_2SO_4-HNO_3-mixture (1:1). Organic phosphates were thereby converted to orthophosphate and measured as such. The polyphosphate content was calculated from this total phosphate content by subtracting the phosphorus content of the biomass (1.8%). For MLSS measurement 25 ml mixed liquor

was filtered on Whatman glass microfibre filters (GF/C). Before and after filtration, the filters were dried during two hours at 105°C and weighed.

2.5. *IDENTIFICATION OF THE MICROBIAL POPULATION*

All isolates were characterised by gas chromatographic analysis of their methylated fatty acids (FAME) (Vauterin et al., 1991).The FAME patterns were compared with the Microbial Identification Software (MIS) database (TSBA version 3.9., Microbial ID Ind.; Newark, Delaware, USA). The obtained fatty acid profiles were compared numerically and the strains were grouped in a dendrogram according to the similarities of their fatty acid profiles.

The stability of the bacterial population during the three month sampling period was studied by FAME analysis of the sludge. The obtained FAME profiles of the different samples (about 100 mg of centrifuged sludge) were compared numerically and expressed as Euclidean distances. Generally, an Euclidean distance of 13 or less is accepted as within bacterial species variability.

2.6. *PARAMETER ESTIMATION*

For modelling purposes, samples were taken every five minutes, gradually enlarging the sampling interval to 20 minutes towards the end both anaerobic and aerobic phases when limited changes were expected. The experimental data were initially confronted with basic equations, gradually taking more compounds into account, until adequate description of the experimental profiles was obtained. The parameters in the models were estimated on the basis of the generalised least squares criterion for multi-response systems, using a Levenberg-Marquardt algorithm.

$$\sum_{i=1}^{v}\sum_{j=1}^{v} w_{ij} \sum_{k=1}^{n} (y_{ki} - \hat{y}_{ki}) \,.\, (y_{kj} - \hat{y}_{kj}) \quad \rightarrow \quad \min$$

3. RESULTS AND DISCUSSION

3.1. *EXPERIMENTAL PROFILES*

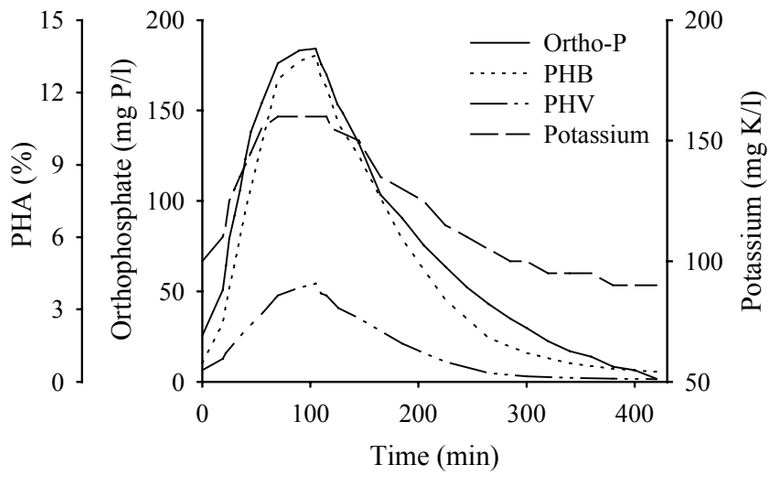

Figure 65 Typical profile recorded during an experiment

In Figure 65, a typical profile recorded during an experimental run performed on the SBR, is presented. Anaerobically orthophosphate is released, whereas acetate is consumed with concurrent formation of PHA, particularly PHB and PHV. Aerobically, phosphate is stored internally by the bacteria using PHA as internal carbon source. Potassium follows the same profile as orthophosphate, according to the formula $Mg_{1/3}K_{1/3}P$ (Comeau et al., 1987a). The feed composition from the third experimental phase was used.

3.2. *MODELLING THE AEROBIC PROCESSES*

During aerobic conditions PHA, which was stored in the preceding anaerobic phase, is oxidised by the phosphorus accumulating bacteria and orthophosphate is converted to polyphosphate. The performed experiments were designed to allow all soluble carbon sources to be converted during the anaerobic phase. Hence, aerobic growth of heterotrophic bacteria on soluble carbon sources is hindered, while concurrent nitrification, and hence chemolithotrophic growth, can take place.

The uptake of phosphorus for the synthesis of the polyphosphate chain is linked to the oxidation of PHA, especially PHB. According to the metabolic model developed by Smolders (1995a) for every mole of phosphate uptake, 0.27 C-mole of PHB is oxidised (5.34 g P/g PHB). The fraction of PHB used in the aerobic phase for biomass synthesis is also taken into account. However, the amount of orthophosphate necessary for this biomass synthesis and the biomass dynamics were neglected. According to several authors (Smolders et al., 1995a, Satoh et al., 1992) PHB is also used for glycogen formation. However, since no glycogen measurements were available, no glycogen dynamics were modelled. Hence the necessary PHB conversion is lumped into the growth term.

To account for orthophosphate limitation towards the end of the aerobic period, a switching function was introduced for orthophosphate. The affinity constant was chosen upon 0.01 mg P/ℓ. It was noticed that the uptake of orthophosphate was substantially higher when the polyphosphate content of the biomass was low. To account for this phenomena a Monod-equation, not used in the IAWQ No 2 model (Henze, 1994), was introduced for polyphosphate.

The polyphosphate content was measured only at the beginning of the aerobic period. Therefore the polyphosphate content at any time was calculated from the orthophosphate profile, according to the following mass balance:

$$X_{PP,X}(t) = X_{PP,X}(0) + \frac{C_{PO_4^{3-}}(0) - C_{PO_4^{3-}}(t)}{X_{PAO}}$$

In the following the model for polyphosphate uptake and PHB utilisation is presented. 91 orthophosphate-P measurement points and 54 PHB measurement points served the purpose of parameter estimation. Initial orthophosphate and PHB concentrations, i.e. at the beginning of the phase, were considered unknown and hence treated as parameters. Tying up the model onto the initial values for each experiment is inconsistent with the fact that they are also are subject to experimental error.

The selected model reads:

$$\frac{dC_{PHB,X}}{dt} = - k_{pp} \cdot \frac{C_{PHB,X}}{K_{PHB} + C_{PHB,X}} \cdot \frac{C_{PO_4^{3-}}}{0.01 + C_{PO_4^{3-}}} \cdot \frac{K_{pp} + X_{PP,X}}{X_{PP,X}} - k_X \cdot C_{PHB,X}$$

$$\frac{dC_{PO_4^{3-}}}{dt} = - 5.34 \cdot k_{pp} \cdot \frac{C_{PHB,X}}{K_{PHB} + C_{PHB,X}} \cdot \frac{C_{PO_4^{3-}}}{0.01 + C_{PO_4^{3-}}} \cdot \frac{K_{pp} + X_{PP,X}}{X_{PP,X}} \cdot X_{PAO}$$

Table 67 presents the statistical analysis. Estimation of all parameters together resulted in identifiability problems (Vanrolleghem et al., 1995a). The value for K_{pp} has thus been estimated separately, and hence no statistical analysis is available for this parameter. As can be seen from the statistics and from the parity plots in Figure 66 and Figure 67, the agreement between observed and calculated values is very good. Observed and calculated values for one experiment are presented in Figure 70 and Figure 71.

The model was validated using two experiments performed during a transient stage of the SBR and for batch experiments conducted on sludges taken from the A^2/O and UCT systems. Since in non of these cases it was expected that all biomass consisted of polyphosphate accumulating organisms, the fraction of these organisms was considered as a parameter. The model could also be validated on the experiments performed on the A^2/O and UCT systems, with low initial oxygen concentration, by incorporating an oxygen switching function.

Table 67 Parameters and statistical analysis for the aerobic phosphate uptake and PHB utilisation

		95%-confidence interval	
Parameter	Optimal value	lower limit	upper limit
k_{pp}	5.23	4.77	5.68
k_x	0.67	0.65	0.69
K_{PHB}	50.75	42.34	59.16
K_{pp}	2.0	-	-
Estimated error covariance matrix (PHB,phosphate)		5.97 -1.63	-1.63 5.13

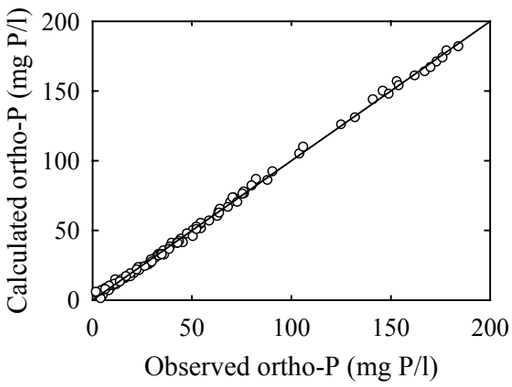

Figure 66 Parity plot for orthophosphate

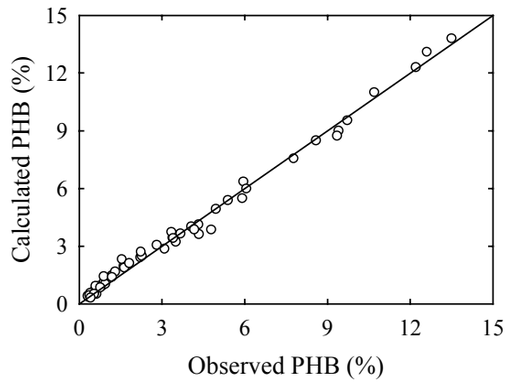

Figure 67 Parity plot for PHB

3.3. *MODELLING THE ANAEROBIC PROCESSES*

During the anaerobic phase the carbon sources from the feed are converted to PHA. The energy required for acetate uptake is provided by breakdown of polyphosphate formed during the preceding aerobic phase. Nitrate, formed also during the preceding aerobic phase, is used very rapidly as a terminal electron acceptor, so that the nitrate concentration sharply drops to zero already after a few minutes. True anaerobic conditions thus are nearly present throughout the whole "anaerobic period".

Because acetate and lactate feeds were used, mainly PHB and PHV were formed. Acetate is mainly converted into PHB, with a minor fraction into PHV. Lactate is mainly converted to PHV. Although both PHB and PHV show the same anaerobic-aerobic profiles, experiments indicated that particularly PHB is used during the aerobic period for phosphate storage. It is thought that PHV and PHB form a copolymer with varying composition according to the amount of lactate fed (Inoue et al., 1996). For modelling purposes only acetate feed were considered and model development was done on the basis that acetate is only converted to PHB. Though it is suggested that PHB is also formed from glycogen, stored in the preceding aerobic phase (Smolders et al., 1995a, Satoh et al., 1992), this process has again not been taken into account, because of a lack of glycogen data.

Beside initial orthophosphate and PHB concentrations, also the initial acetate concentrations were considered as parameters, for reasons mentioned above.

The final model selected is :

$$\frac{dC_{PHB,X}}{dt} = k_{PHB} \cdot \frac{C_{Ac}}{K_{Ac} + C_{Ac}} \cdot \frac{X_{PP,X}}{K_{PP} + X_{PP,X}}$$

$$\frac{dC_{PO_4^{3-}}}{dt} = f_{pp} \cdot k_{PHB} \cdot \frac{C_{Ac}}{K_{Ac} + C_{Ac}} \cdot \frac{X_{PP,X}}{K_{PP} + X_{PP,X}} \cdot X_{PAO}$$

$$\frac{dC_{Ac}}{dt} = -\beta \cdot k_{PHB} \cdot \frac{C_{Ac}}{K_{Ac} + C_{Ac}} \cdot \frac{X_{PP,X}}{K_{PP} + X_{PP,X}} \cdot X_{PAO}$$

Table 68 presents the statistical analysis. Again estimation of all parameters together resulted in identifiability problems. The value for K_{pp} has thus been estimated separately. Although the agreement between observed and predicted values is in this case somewhat less accurate then for the aerobic processes, the fit again is very good (Figure 68 and Figure 69).The value for K_{Ac}, however, is less precisely estimated as the other parameters. By way of example observed and calculated values for one experiment are presented in Figure 70 and Figure 71.

Table 68 Parameters and statistical analysis for the anaerobic phosphate uptake and PHB utilisation

		95%-confidence interval	
Parameter	Optimal value	lower limit	upper limit
k_{PHB}	250.33	200.74	299.91
K_{Ac}	111.77	53.25	170.28
K_{pp}	10.0	-	-
f_{pp}	0.37	0.36	0.39
β	0.92	0.88	0.96
Estimated error covariance matrix		37.84 4.32	-35.82
(phosphate, PHB, acetate)		37.84 4.31	-5.37
		4.32 7.89	1475.6

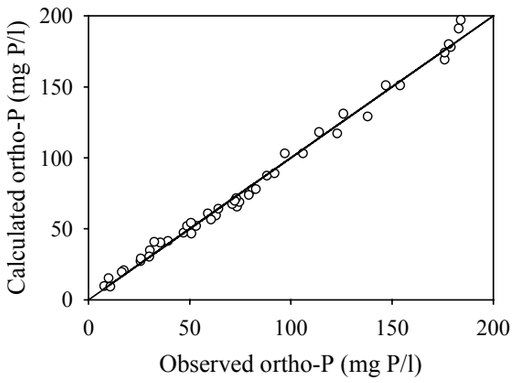

Figure 68 Parity plot for orthophosphate

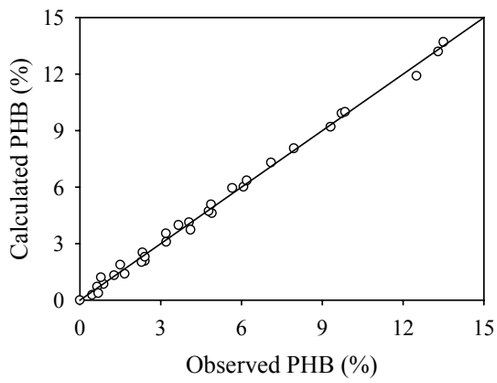

Figure 69 Parity plot for PHB

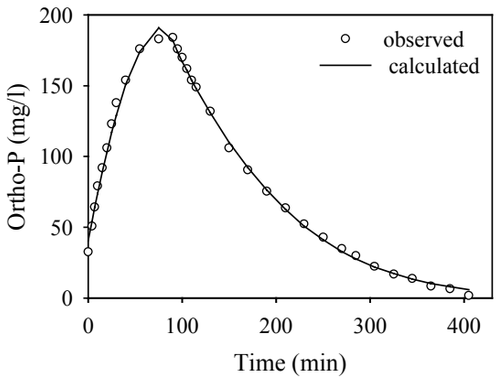

Figure 70 Observed and calculated orthophosphate profile

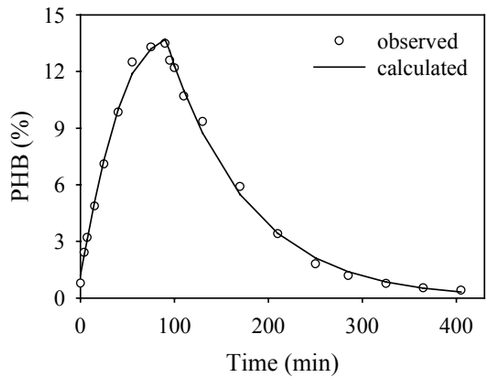

Figure 71 Observed and calculated PHB profile

Validation of the selected model using the batch experiments conducted on sludges taken from the A^2/O and UCT systems, was not yet very good. Only low acetate concentrations were used in those experiments. Calibrating the selected model using only the latter experiments, resulted in a first order for acetate and a zero order for the internal polyphosphate content. More experiments, using a broader range of feed concentrations should be performed for accurate validation of the selected model.

3.4. *APPLICABILITY RANGE OF THE SELECTED MODELS*

The models have been calibrated using feedstocks containing acetate ranging between 400 and 800 mg COD/ℓ and orthophosphate ranging between 15 and 30 mg P/ℓ. The aerobic and anaerobic orthophosphate concentrations ranged between 0 and 200 mg P/ℓ and the internal PHB contents between 0 and 14%. The selected models thus are only valid in mentioned concentration ranges.

3.5. *COMPOSITION OF THE MICROBIAL POPULATION*

More than 60% of the isolates could be identified with the MIS data base at least at the genus level. In the A^2/O installation, representatives of the *Enterobacteriaceae* formed the most dominant group (50% of all isolates). On the contrary, only 2% of the isolates of the SBR were identified as belonging to the *Enterobacteriaceae*. On the contrary, four different groups of organisms were present according to the performed FAME analysis. They represented each about 10% of the total bacterial population: two unidentified groups, a group belonging to the genus *Pseudomonas* and a group containing representatives of the lactic acid bacteria. However, experience has learned that reliable identification of lactic acid bacteria by FAME profiling is doubtful (Pot, personal communication), which means that only part of the SBR population could be identified.

This striking difference of bacterial population of the SBR and the A^2/O experimental set-ups is supported by a different FAME profile exhibited by the direct analysis of both sludges (Euclidean distance > 17). During the sampling period the fatty acid composition of the SBR and A^2/O remained fairly stable (within Euclidean distance lower than 10) which supports the stability of the system during this period.

The striking absence of *Acinetobacter* species (only one strain was isolated) confirmed again (Auling et al., 1991) that this organism is not a prerequisite for biological phosphorus removal.

Former diaminopropane analysis (DAP) of the sludges supports this observation.

4. CONCLUSIONS

The aim of this paper was to calibrate a model for the biological phosphorus removal process and to identify the causal micro-organisms. A model has been calibrated, indicating the necessity to account for low polyphosphate concentrations. The aerobic model was validated against different environmental conditions. It was confirmed that Acinetobacter is not a prerequisite for phosphorus removal. Representatives of the Enterobacteriaceae formed the most dominant group in the A²/O system, while only 2% of the isolates of the SBR belonged to this group. Further identification of unidentified groups is necessary.

Acknowledgements

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CHAPTER 5

Enhanced Biological Phosphorus Removal: Competition and symbiosis between SRBs and PAOs on lactate/acetate feed

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Chapter 5

Enhanced Biological Phosphorus Removal: Competition and symbiosis between SRBs and PAOs on lactate/acetate feed

Abstract - Sulphate reduction in activated sludge cultivated with a mixture of lactate and acetate as carbon source was investigated in a sequencing batch reactor. The system was initially operated to cultivate phosphorus removing bacteria using acetate as sole carbon source. Sulphate reduction was initially not observed when lactate was added as feed component, whereas the typical anaerobic/aerobic phosphorus profile was recorded. When an-aerobic and aerobic cycle lengths were adjusted to allow growth of sulphate reducing bacteria, sulphate reduction was observed concurrent with complete phosphorus removal. Bulking and complete deterioration of the enhanced biological phosphorus removal was observed after several months when operating problems gave the sulphate reducing population opportunity to become dominant. Complete recovery of phosphorus removal activity was obtained after only 25 days when the operating conditions were switched back to the initial conditions. Bulking even disappeared after only 2 days. Combined sulphate reduction and phosphorus removal can be possible, provided bulking conditions of the sludge and thus wash out of the EBPR active biomass can be prevented.

1. INTRODUCTION

With the discovery of Enhanced Biological Phosphorus Removal (EBPR) (Barnard, 1975), many wastewater treatment plants have been built with the aim to reduce phosphorus in the effluent by means of a biological rather than a chemical process. To enhance biological phosphorus removal, the biomass first needs to pass through an oxygen and nitrate free phase, i.e. an anaerobic phase, before entering a phase where an electron acceptor is present, i.e. an anoxic phase where nitrate is present or an aerobic phase where oxygen is present. The oxygen and nitrate free phase can be a separate reactor, the first phase of a plug flow reactor or part of a sequencing batch reactor cycle (Mino *et al.*, 1998). The EBPR process is supposed to achieve highest removal ratios when Short Chain Fatty Acids (SCFAs), with as key component acetate, are present in the influent (Fuhs and Chen, 1975; Potgieter and Evans, 1983; Malnou, 1984; Ekama *et al.*, 1984; Arvin and Kristensen, 1985 and Comeau *et al.*, 1987). Reports on the influence of non-SCFAs on the EBPR efficiency are less consistent. Using glucose or glucose/acetate as carbon sources caused in certain cases the proliferation of so called G-bacteria with a complete deterioration of EBPR activity (Cech and Hartman, 1990, 1993), whereas other authors report symbiosis between lactic acid producing bacteria and polyphosphate accumulating bacteria (PAOs) with excellent EBPR performance (Jeon and Park, 2000). Beginning of the 90'ies it was reported that anaerobic sulphate reduction caused the proliferation of filamentous bacteria with as a consequence possible hindrance of the EBPR activity (Yamamoto-Ikemoto *et al.* 1991, 1994). Wanner *et al.* (1987), however, provided evidence that inducing EBPR by enhancing growth of PAOs could suppress filamentous bulking in anaerobic-aerobic systems, even if only limited poly-P accumulation occurred.

Whereas municipal wastewaters containing significant sulphate concentrations in Europe are between 5 and 15 mg S/ℓ (Hvitved-Jacobsen *et al.*, 2000), municipal wastewater in Japan contains much higher sulphate concentrations, usually between 10 and 100 mg/ℓ. When treating industrial wastewater (alone or in combination with municipal wastewaters) originating from industrial processes that use sulphuric acid or sulphate-rich feed stocks, important sulphate concentrations can enter the anaerobic phase of the

treatment plant (Lens *et al.*, 2000). A well known indication of sulphate concentrations in sewer systems is corrosion of concrete pipes in wastewater collectors (Yamamoto-Ikemoto *et al.* 1991). The corrosion is caused by a symbiosis of several bacteria present in the sewer system: e.g. *Desulfovibrio* spp. reduce oxidised sulphur compounds to sulphide, the latter is oxidised in the atmosphere to elemental sulphur, which is deposited on the slime layer of the sewer system. *Thiobacillus* spp. presented in the slime layer can metabolise the elemental sulphur to sulphuric acid, which rapidly destroys the inner surface of the concrete system (Vincke *et al.*, 2000).

Cells need inorganic sulphur as cell constituent for growth processes. The sulphur content in living organisms is about 1 w% (Zehnder and Zinder, 1980). The organic sulphur of cysteine and methionine originates biologically from various inorganic forms, such as sulphate, thiosulphate and sulphide or even elemental sulphur. These inorganic sulphur compounds can be oxidised by some bacteria to sulphate. Various micro-organisms and plants can bring about the enzymatic reduction of sulphate and thiosulphate to yield sulphide, which may then be converted into the thiol group of cysteine (Lehninger, 1978).

In this article evidence is gathered for deterioration of EBPR activity due to induced anaerobic sulphate reduction activity when a sequencing batch reactor (SBR) is fed with a synthetic wastewater containing lactate and acetate as carbon sources. Operating conditions obviously determine the proliferation of a filamentous population with complete washout of the PAO population.

2. MATERIALS AND METHODS

2.1. *APPARATUS*

The study was carried out in a laboratory fermentor (Biostat, B. Braun, Melsinger) with a maximum volume of 11ℓ filled with 8ℓ of mixed liquor. To achieve EBPR the reactor was operated as a SBR with a cycle length of 6 hours: filling (15 minutes) (anaerobic), anaerobic phase (1.5 hours), aerobic phase (3 hours and 15 minutes), anaerobic in-between phase (maximum 10 minutes), during which nitrogen gas is flushed through the mixed liquor to prevent remaining oxygen entering the anaerobic period of the following cycle, settling phase (minimum 33 minutes) and an effluent purge (17 minutes). At the end of the aerobic phase 200 ml of mixed liquor is removed to obtain a sludge age of 10 days. Four litres of supernatant were removed at the end of the cycle and 4 litres of medium were fed at the beginning of the cycle, yielding a hydraulic retention time of 12 hours. Using lactate/acetate mixtures, the length of the aerobic phase was increased by 2 hours in order to obtain complete phosphorus removal. To promote growth of SRBs, the length of the anaerobic phase was increased to 2 hours and 45 minutes and the length of the aerobic phase was shortened to 2 hours and 15 minutes.

The SBR consisted of a Pyrex vessel with an internal diameter of 20 cm and a total height of 40 cm. Stirring occurred at 350 rpm except during the settling and effluent purge phases.

A time controller allowed the settings for the different phases. Oxygen-free nitrogen gas was flushed through the liquid during the anaerobic phase when the dissolved oxygen (DO) concentration was above 0.05 mg O_2/ℓ. During the aerobic phase the DO concentration was controlled at 2 mg/ℓ with a band width of 0.2 mg/ℓ. However, in practice a band width up to 1 mg/ℓ usually occurred due to overshoot in both directions. Gas flows were controlled with massflow controllers (Bronkhorst). However, the flow rates of nitrogen and oxygen were fixed and could only be changed manually.

The initial experiments were carried out in a research period in which pH was not controlled. The pH was stabilised around 8 by adding sodiumbicarbonate to buffer the solution. An average pH of about 8 was observed. After 242 days, the operating conditions of the SBR were changed, i.e. a pH controller was installed and operated during all phases where stirring of the liquid occurred. pH was regulated at 7.56.

2.2. *MEDIA*

Initially a non sterilised medium was used with 0.85 g $NaAc.3H_2O$ (400 mg COD/ℓ), 65.81 mg KH_2PO_4 (15 mg P/ℓ), 90 mg $MgSO_4.7H_2O$ (12 mg S/ℓ), 14 mg $CaCl_2.2H_2O$, 36 mg KCl, 107 mg NH_4Cl (28 mg N/ℓ), 1 mg yeast extract, 275.4 mg $NaHCO_3$ and 0.3 ml nutrient solution per litre de-ionised water.

The nutrient solution contained per litre de-ionised water: 1.5 g $FeCl_3.6H_2O$, 0.18 g KI, 0.03 g $CuSO_4.5H_2O$, 0.15 g H_3BO_3, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO_4.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$ and 10 g EDTA. Only reagent grade products were used.

Tests were performed with increased acetate concentration (800 mg COD/ℓ), followed by increased phos phorus concentration (45 mg P/ℓ). During the first month, the other constituents remained unchanged. Later these components were doubled in concentration (Table 69).

To stimulate sulphate reduction, half of the acetate-COD was replaced by sodium-lactate (48 ml $CH_3CHOCOONa$, i.e. 400 mg COD/ℓ). Using the lactate/acetate feed, the installation was monitored during a period of 140 days. Finally, the initial medium composition was used again to restore EBPR (Table 69).

Table 69 Feed compositions and phase lengths during the different research periods

	Acetate [g COD/ℓ]	Lactate [g COD/ℓ]	Ortho-P [mg P/ℓ]	Anaerobic phase length	Aerobic phase length	Duration [days]
1st research period	400	-	15	1h30min	3h15min	136
2nd research period	800	-	15	1h30min	3h15min	22
3rd research period	800	-	45	1h30min	3h15min	10
4th research period*	800	-	45	1h30min	5h15min	4
5th research period	400	400	45	1h30min	5h15min	24
6th research period	400	400	15	2h45min	2h15min	116
7th research period	400	-	15	1h30min	3h15min	…..

* All other nutrients were doubled in concentration

2.3. *ANALYSIS METHODS*

Ortho-phosphate analyses (ortho-P), using the ascorbic acid method (Standard Methods, APHA, 1989), were performed with a colorimetric auto-analyser (TrAAcs 800) with a measurement range between 0.5 and 5 mg P/ℓ. *Nitrate measurements*, using the hydrazine reduction method (Standard Methods), were also performed on TrAAcs, with a measurement range between 0.2 and 2 mg N/ℓ. Colorimetric *COD analyses* were performed according to the Standard Methods using Hach tubes and a Nanocolor colorimeter. *Sulphate analysis* was performed using a turbimetric method. By adding Ba^{2+}-ions to the sample, $BaSO_4$ precipitates. The transmission at 400 nm of the sample is then measured (Standard Methods). For *PHA determination* lyophilised biomass was subjected to a propylation reaction. The concentration of this ester was then analysed by gas chromatography (Baetens *et al.*, submitted). For *MLSS measurements* 25 ml mixed liquor was filtered on Whatman glass microfibre filters (GF/C). Before and after filtration, the filters were dried for two hours at 105°C and weighed.

2.4. *PROFILE RECORDING AND EXPERIMENTAL RUNS*

The unit was sampled twice a week to verify the phosphorus removal capacity of the installation. For this purpose, samples were taken at least at the end of the previous cycle in the settling phase, at the end of the feeding phase, at the end of the anaerobic phase and at the end of the aerobic phase. All samples were then analysed at least for ortho-P, nitrate and MLSS. COD, PHA and sulphate were analysed less

frequently. These data are plotted versus cycle number and will be termed "profile recordings" hereafter. When experiments were performed to elucidate the kinetics of the different reactions, at least 30 samples were taken during the anaerobic and aerobic phase of one cycle. Samples were then analysed for ortho-P, COD, nitrate, sulphate and PHA. These data are plotted versus cycle time and will be termed "experiments" hereafter.

3. RESULTS

The SBR was initially operated to enhance biological phosphorus removal with acetate as the sole carbon source (400 mg COD/ℓ) and 15 mg P/ℓortho-phosphate. The initial cycle lengths, i.e. an anaerobic phase of 1 hour and 30 minutes and an aerobic phase of 3 hours and 15 minutes, were used for low acetate concentration. In Figure 72 typical profiles recorded during an experiment are presented.

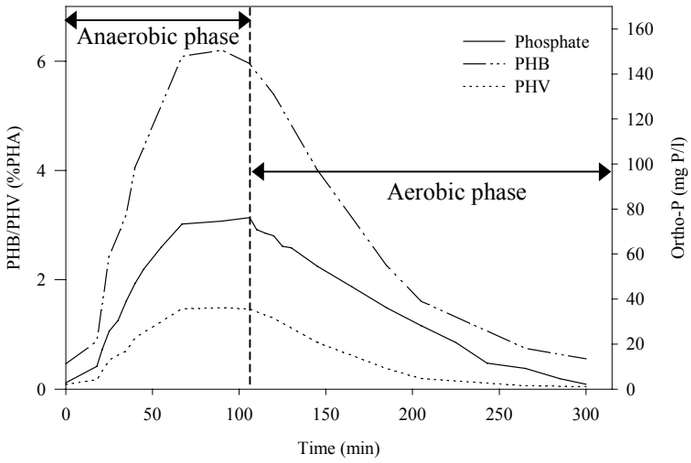

Figure 72 PHA and ortho-P profiles for low acetate feed (43 days after start-up of 1st research period) (319 mg COD/ℓ, 12.896 mg P/ℓ) (MLSS = 2.03 g/ℓ)

In order not to overcomplicate the graphs, only PHB, PHV and ortho-P profiles are shown. Anaerobically acetate was fully consumed. From the figure it can be observed that the phosphate profile and the PHB/PHV profiles follow the same trend, i.e. all concentrations increase during the anaerobic period, reaching a plateau when acetate is consumed (not visualised) and decreasing during the aerobic period. From Figure 73 it is observed that full phosphorus removal could still be achieved using a higher acetate concentration, only by increasing the length of the aerobic phase with 2 hours. No acetate breakthrough to the aerobic phase occurred. Higher phosphorus and PHA concentrations were reached at the end of the anaerobic phase compared to the first research period.

Table 70 Ortho-P release to COD uptake ratios, PHA formation to COD uptake and PHV to PHB ratios

Period	No of days elapsed	Carbon source in feed [mg COD/ℓ]	Ortho-P in feed [mg P/ℓ]	Ortho-P release to COD uptake [mg P/mg COD]	(PHB+PHV) formation to COD uptake [mg PHA/mg COD]	PHV/PHB ratio [-]	P-removal ratio [%]
1st	36	547 (acetate)	12.998	0.142	0.334 + 0.063 = 0.397	0.188	100
1st	43 (Figure 72)	319 (acetate)	12.880	0.209	0.365 + 0.089 = 0.454	0.244	84
1st	80	220 (acetate)	12.760	0.665	1.020 + 0.304 = 1.324	0.298	64
2nd	18	573 (acetate)	13.371	0.744	2.463 + 0.868 = 3.331*	0.353	100
3rd	5	751 (acetate)	39.581	0.404	0.771 + 0.241 = 1.012	0.312	72
4th	1 (Figure 73)	851 (acetate)	42.251	0.376	0.837 + 0.236 = 1.073	0.282	100
5th	16	lactate/acetate	38.50	n.m.	n.m.	1.019	47
6th	14	785 lactate/acetate	10.07	0.209	n.m.	1.17	100
6th	15	lactate/acetate	low (~ 15)	n.m.	n.m.	0.990	0

* See further, this value is too high. Most probably the COD measurement was not correct.
n.m.: not measured

From Table 70 it can be observed that the higher the acetate concentration in the feed, the higher the observed phosphorus release and PHB formation during the anaerobic phase. When increased

concentration of carbon sources are used, the PHA-formation to carbon utilisation ratio increases. The PHV/PHB ratio remains nearly constant.

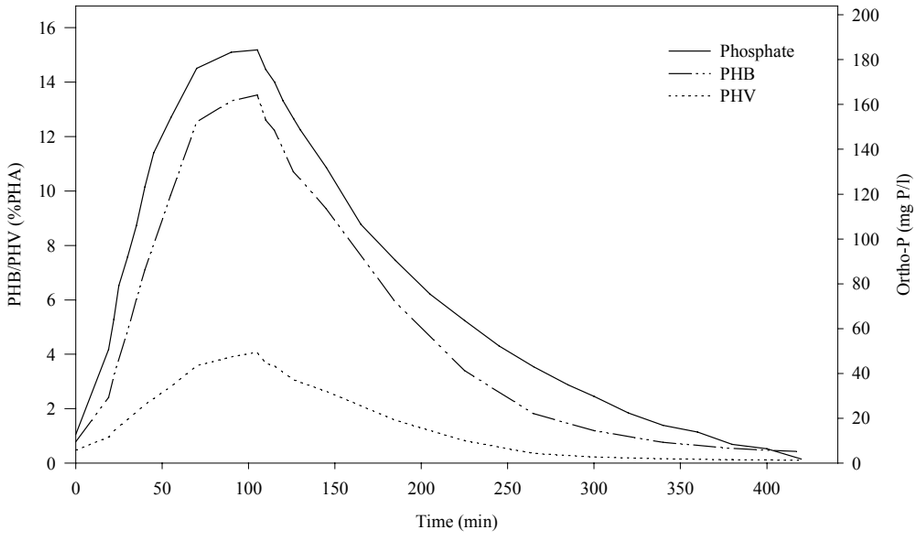

Figure 73 PHB/PHV and ortho-P for high acetate feed (beginning of 4th research period) (851 mg COD/ℓ, 42.251 mg P/ℓ) (MLSS = 2.66 g/ℓ)

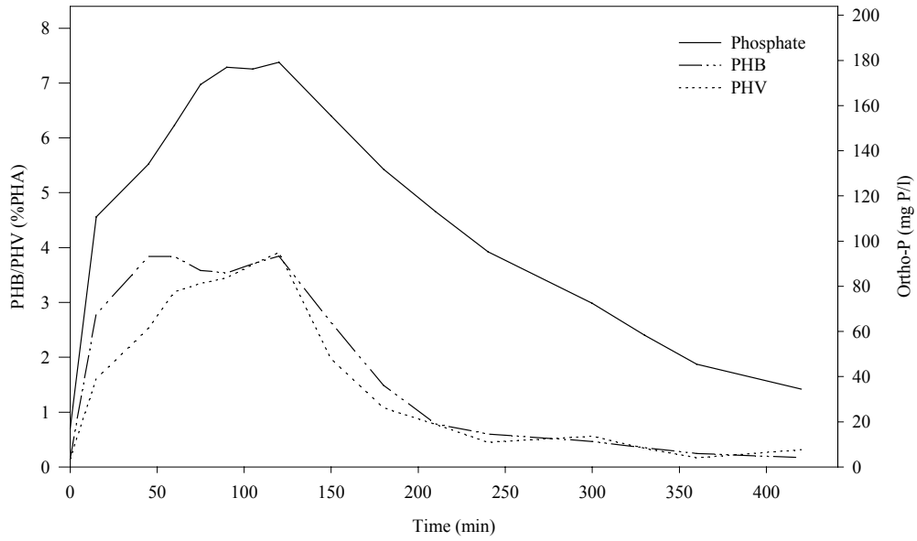

Figure 74 PHB/PHV and ortho-P profiles for lactate/acetate feed (50/50) after 16 days of acclimation to 5th research period

During the 5th research period a mixture of acetate and lactate was used as carbon source. Only after as few as 6 days, complete phosphorus removal could no longer be obtained. However, an anaerobic/aerobic phosphorus profile was still recorded (Figure 74), whereas still no sulphate profile was observed. From Figure 74 it can be deduced that using a 50/50w% lactate/acetate mixture as carbon source had almost no effect on the maximum anaerobic phosphorus concentration compared to acetate alone (Figure 73). However, the PHV/PHB ratio increased from about 0.3 for pure acetate feed to about 1 for the mixture of acetate and lactate (Table 70).

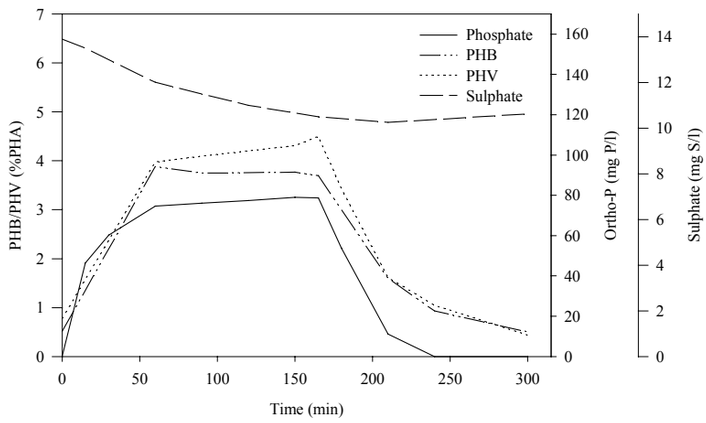

Figure 75 PHB/PHV and ortho-P profiles for lactate/acetate feed (50/50) after 13 days of acclimation to the 6th research period.

Increasing the length of the anaerobic phase to 2 hours and 45 minutes and shortening the aerobic phase to 2 hours and 15 minutes, anaerobic sulphate reduction was clearly observed (Figure 75). From the experimental results it was obvious that most of the carbon source was already used during the feeding phase (not shown). In this phase of the process, the sulphate reduction is only minor compared to the reduction in the true anaerobic phase. Sufficient carbon source thus remains for PAOs. These results indicate that sulphate reduction and phosphorus removal occur simultaneously.

One month after the operating and feeding conditions were changed to enhance sulphate reduction, the typical phosphorus profile and complete phosphorus removal were still present. However, the sludge showed very bad settling characteristics, i.e. the SVI changed from 10 ml/g to more than 500 ml/g.

Due to growth of biomass on the membrane of the pH electrode, a pH value of 8.8 occurred for a period of at maximum 2 weeks. After this period, the biomass did not settle at all and biomass was washed out with the effluent. As a result phosphorus was no longer removed. For the next 35 days the SBR was sampled regularly for ortho-phosphate, sulphate and sulphide. The phosphorus removal capacity could not be restored, whereas the sulphate profile became slightly more pronounced. Sulphide profiles could only be measured when nitrogen bubbling during the anaerobic phase was switched off.

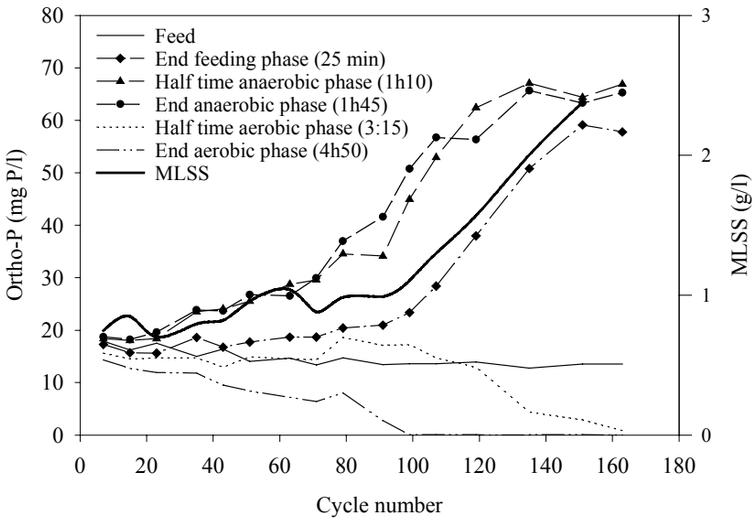

Figure 76 Evolution of phosphorus profiles during the second start-up period of the EBPR process

Finally, the feeding of lactate was ceased to enhance phosphorus removal again. The SBR was regularly monitored to verify if and how fast the phosphorus removal capacity was regained. From Figure 76 it can be concluded that only after as few as 99 cycles, i.e. 25 days (3 sludge ages), complete phosphorus removal capacity was regained. Afterwards anaerobic phosphorus release and aerobic phosphorus uptake rate kept increasing further to a plateau. The latter can be concluded from the fact that the phosphorus concentration half time of the aerobic phase decreased with time. The sulphate reduction capacity of the sludge completely disappeared after only two days and at the same moment the sludge's settling characteristics improved drastically.

During the second start-up period of the EBPR process, the biomass was observed microscopically. Starting from a sludge with nearly only filamentous bacteria a bacterial population was created with clear inclusions of intracellular storage polymers. Initially the sludge had very poor settling characteristics with a SVI of over 500 ml/g. The population consisted of filamentous bacteria belonging to *Beggiatoa* spp (Figure 77). From Figure 78 it can be seen that the biomass immediately responded to the changed operating conditions and growth started of two clearly different microbial populations. At the moment the SBR had fully restored its phosphorus removal capacity, the population containing storage polymers dominated, however, the growth of the second population continued and possible symbiosis between both occurred.

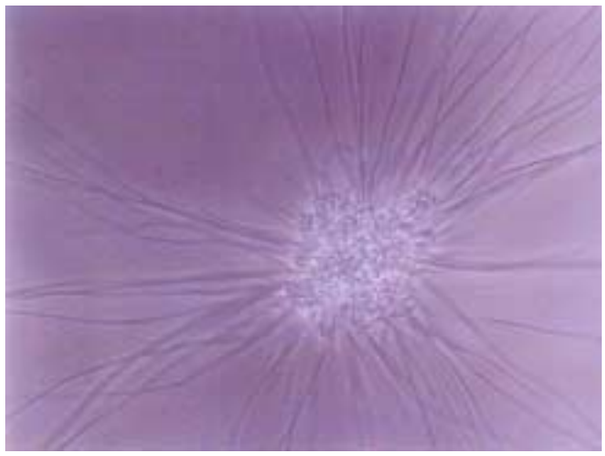

Figure 77 Filamentous biomass at cycle number 0

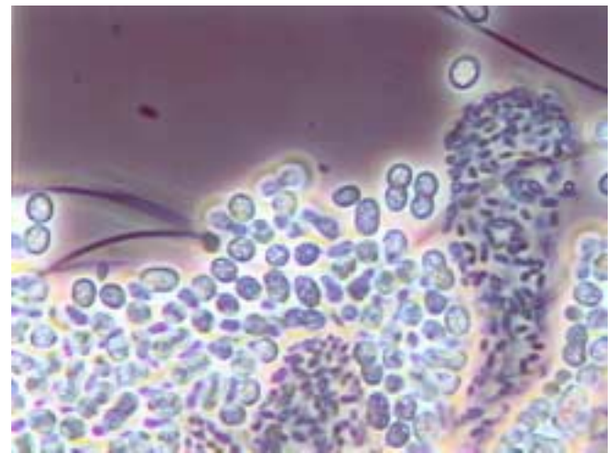

Figure 79 PAO sludge dominated by two different bacterial populations

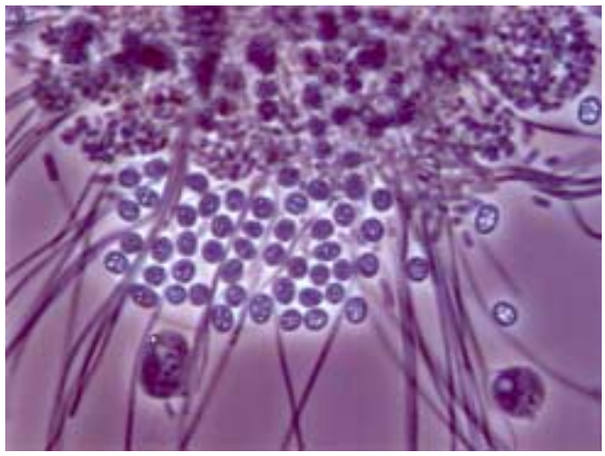

Figure 78 Growth initiation of PAOs

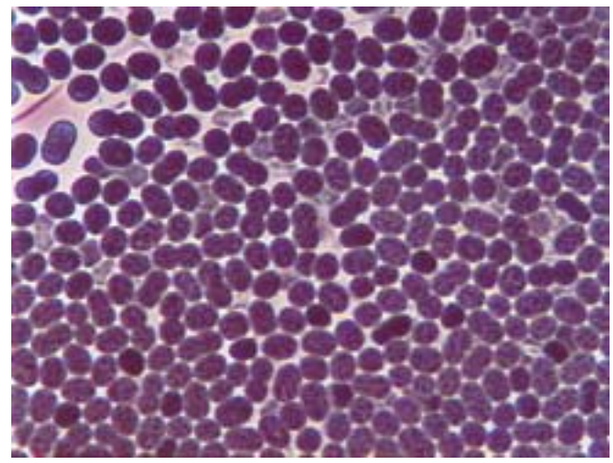

Figure 80 Bacterial population with clear inclusion of storage polymers

4. DISCUSSION

Although operating conditions were set to obtain constant feeding concentrations during the different stages of the research period, experimental results indicate that they were not constant (Table 70).

Experimental evidence was gained that even when peristaltic pumps are used, operating under time controller, the volumes added to the reactor significantly depend on the height of the liquid level in the feeding vessel. Furthermore, a slight acidification in the concentrated feed vessel apparently occurs even though this container was stored in a refrigerator.

Studies concerning EBPR have focussed on the processes occurring in the anaerobic phase, since accumulation of an internal carbon source is the most essential phenomenon to obtain good phosphorus removal capacity. Comparing our experimental results with literature data, it is clear that the ortho-phosphate release to COD uptake ratios mentioned in literature vary as much as our results: 0.22 to 0.40 mg P/mg acetate (Arun *et al.*, 1988), 0.54 to 0.59 mg P/mg acetate (Wentzel *et al.*, 1988), 0.64 up to 0.76 mg P/mg acetate (Arvin and Kristensen, 1985). Smolders *et al.* (1994a) and Liu *et al.* (1996a) found theoretical and experimental evidence that phosphorus release in EBPR processes is linked to the anaerobic pH of the medium during acetate uptake. Since no pH controller was active during this research period, pH could vary slightly. However, this slight pH variation is not in accordance with the pH dependency observed by Smolders (1994a). The statements underlying the theoretical proof formulated by Smolders *et al.* (1994a) are under discussion (Fleit, 1995; Bond *et al.*, 1999). The practical observation that pH influences the anaerobic phosphorus release, however, is not contested (Filipe and Daigger, 1998)

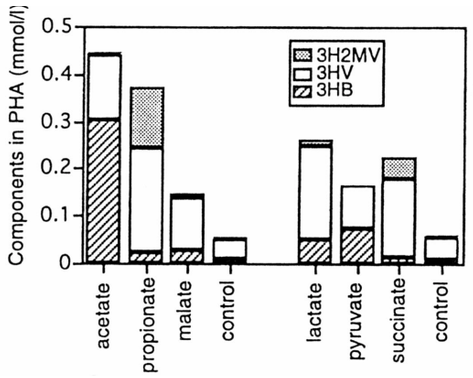

Figure 81 Content of accumulated PHA within 3 hours after injection of different substrates (Satoh et al., 1996)

When lactate is used as carbon source the ortho-phosphate release to COD uptake ratio markedly decreased, in accordance with observations made by Satoh *et al.* (1996). These authors reported lactate to disappear faster from the supernatant than acetate, propionate and malate, although a lower net phosphorus release was observed compared to acetate or propionate. Using acetate as the sole carbon source, mostly PHB was formed, with a maximum of 35% PHB. According to Satoh *et al.* (1996) acetate mainly results in PHB whereas, when lactate is used as carbon source, a clear increase in 3HV units in the PHA copolymer occur (Figure 81), in accordance with our observations.

Smolders *et al.* (1994a) reported a value of at most 10% PHV formed when acetate is used as sole carbon source. Louie *et al.* (2000), however, observed a much higher ratio, namely ranging between 0.6 and 0.75 PHV/PHB formed. Our experimental results indicate an average value of 0.3 mg PHB/mg PHV for acetate feeds and a value of 1 mg PHB/mg PHV when lactate-acetate mixtures are used. These results lay in between the values reported in literature.

According to the Mino-biochemical model for EBPR (Mino *et al.*, 1987; Arun *et al.*, 1988), the uptake of acetate is linked with the formation of PHB at a ratio of 1.49 mg PHB/mg COD. According to the Comeau-Wentzel model (Matsuo, 1985; Comeau *et al.*, 1986; Wentzel *et al.* 1986), however, PHB is formed at a ratio of 1.00 mg PHB/mg COD (Smolders *et al.*, 1994c). In these values, PHV is accounted for as PHB. Our experimental data vary considerably and only for the high feed concentration, our observed result (~1 mg PHA/mg COD) lays in the range of the value calculated by Smolders *et al.* (1994c) for the Comeau-Wentzel model. It is generally accepted now that the Comeau-Wentzel model, with only the TriCarboxylic Acid (TCA) cycle operating, is not valid. Pereira *et al.* (1996), using ^{13}C-NMR, clearly provided evidence for the glycolysis cycle operating anaerobically. A combined action of both the TCA and the glycolysis cycle are put forward. Whereas concurrent operation of both cycles is lately contested as well, no conclusive evidence is yet gained for other pathways, although the model lately proposed by Hesselmann *et al.* (2000) is promising. This discrepancy between own experimental

data and literature references can partly be explained by the poor COD analysis when prefabricated test kids are used and/or partly by inappropriate handling of the samples. Indeed, in a later phase of this research it was clearly indicated that sampling for acetate analysis demands careful handling and immediate addition of formic acid to prevent further reaction, even when samples are filtered immediately using glass microfibre filters (Whatman GF/C, retention of particles of 1.2 µm).

When lactate is added as carbon source, no carbohydrates have to be degraded since reducing power is not necessary. Satoh *et al.* (1992) proposed a model for the direct uptake of lactate and concomitant consumption of glycogen.

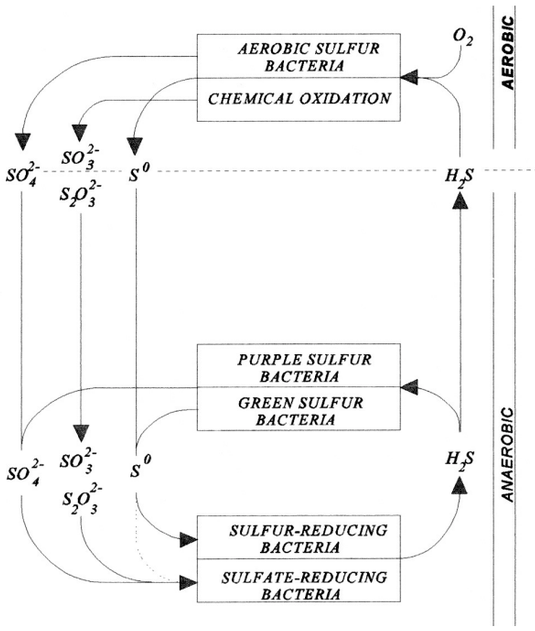

Figure 82 The biological sulphur cycle in activated sludge systems (Widdel, 1988)

Through symbiosis of several bacteria, a complete biological sulphur cycle can exist in anaerobic/aerobic systems (Figure 82). Because H_2S is continuously stripped from the mixed liquor, it is obvious that the complete cycle never occurred in this work. A brief summary of this cycle is given anyway to indicate possible other pathways and growth of other bacterial strains causing deterioration of enhanced biological phosphorus removal. Specialised aerobic, colourless, bacteria are able to oxidise sulphide to sulphate and or elemental sulphur. Anaerobically sulphate is used as electron acceptor for the oxidation of organic substances (Widdel, 1988). Even under anaerobic conditions alone, a complete cycle can exist, where sulphide, formed from reduction of sulphate, is oxidised by photosynthetic bacteria with the production of sulphate or elemental sulphur. When intermediate sulphur forms are present in the anaerobic phase, they will be reduced to sulphide by the sulphate reducing bacteria as well. Possible chemical reactions occurring in the aerobic phase are presented as well.

According to Widdel (1988) sulphate reducing bacteria are, in comparison with denitrifying bacteria, obligate anaerobic bacteria. As a consequence, sulphate reduction can not occur when nitrate is present in the medium. However, Yamamoto-Ikemoto *et al.* (1996) observed simultaneous aerobic sulphate reduction and sulphide oxidation. Possibly, the observed decrease in sulphate concentration has to be attributed to assimilatory processes.

Amongst the sulphate reducing bacteria, two separate metabolic strains are encountered (Widdel, 1988). *Desulfovibrio* is the best studied SRB belonging to the first metabolic group of bacteria that oxidise the carbon source incompletely to acetate. This first group of bacteria does not possess the enzymatic mechanisms that allow them to oxidise acetate. They are nutritionally less versatile than the second group of completely oxidising ones, but they grow faster. Various low molecular weight compounds can serve as hydrogen donor: lactate, acetate, propionate, butyrate, formate, methanol, ethanol, higher fatty acids, aromatic components and molecular hydrogen (Schlegel, 1993). The experimental observations in this study do not allow to make distinction between the possible microbial consortia present in the reactor. Symbioses did occur but acetate analysis do not allow to verify if lactate was fermented to acetate or fully oxidised by the SRBs. Further experimental results could be gained when accurate COD analysis are combined with PHB/PHV analysis. Mass balances, calculating the amounts of PHB and PHV formed per unit of acetate utilised, and taking into account the metabolic reactions for PAOs, should allow to verify whether lactate is fully consumed by the SRBs.

Although the experimental evidence gained provides proof that SRBs and PAOs can co-exist under defined conditions, it was also shown that this co-habitation is vulnerable and dominant growth of filamentous bacteria as *Beggiatoa* spp. can completely suppress the EBPR activity. Although Eikelboom (2000) indicated four filamentous species known to store elemental sulphur granules inside their cells, i.e. *Thiotrix* spp., *Beggiatoa* spp., Type 021N and Type 0914, they stated that *Beggiatoa* spp. is rather uncommon in activated sludge plants. In 1994, Yamamoto-Ikemoto *et al.* proposed a model for the possible ecological interactions among SRBs, PAOs, denitrifying bacteria and filamentous sulphur bacteria based on own experimental evidence and general microbial knowledge (Yamamoto-Ikemoto *et al*., 1991, 1994, 1996) (Figure 83). Denitrifying conditions suppress the growth of sulphate reducing bacteria because of the competition for the available carbon source. In the absence of denitrifiers, the authors observed sulphate reduction in wastewater treatment.

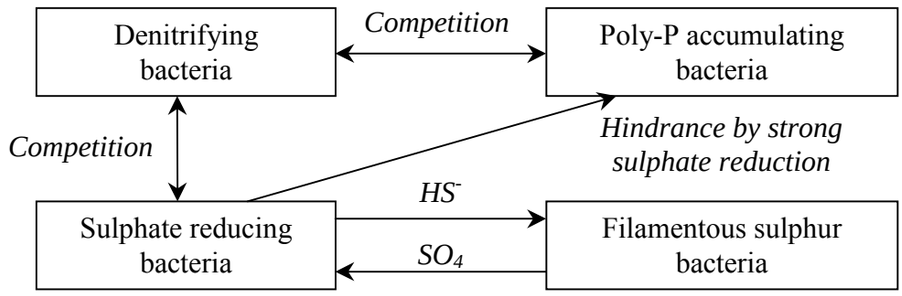

Figure 83 Model of ecological interactions among denitrifying bacteria, PAOs, SRBs and filamentous sulphur bacteria in activated sludge (after Yamamoto-Ikemoto et al., 1994)

According to Yamamoto-Ikemoto *et al*. (1991), deterioration of EBPR activity was linked with high sulphate reduction producing high concentration of sulphide. The high sulphide concentration induced growth of filamentous bacteria, i.e. *Beggiatoa* spp. Consequently, the longer the sulphate reduction activity, the higher the phosphate concentration observed in the effluent. Whereas sulphide was never observed in our system, except for the times when nitrogen bubbling was deliberately switched off, growth of filamentous bacteria was clearly observed. Fast uptake of sulphide by the microbial population is a possible explanation for the sulphide profile to be absent.

5. CONCLUSIONS

Simultaneous occurrence of anaerobic sulphate reduction and phosphorus release was possible, but proved to be a process vulnerable to fast deterioration of EBPR activity due to changed operating conditions. Growth of filamentous bacteria, with *Beggiatoa* spp. as dominant organism, was always observed concurrent with sulphate reduction, even at low levels. It is expected that the higher the sulphate reduction, the more dominant the growth of these filamentous bacteria will become, with fast deterioration of EBPR.

It was observed that PAO activity was suppressed, but could very quickly restore its acetate uptake and overall phosphorus removal capacity. Regrowth of PAOs allowed complete recovery of EBPR activity within 3 sludge ages.

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CHAPTER 6

Temperature effects in Bio-P removal

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Chapter 6

Temperature effects in Bio-P removal

Abstract - This paper discusses the temperature influence in biological phosphorus removal through literature review and own experimental evidence. An SBR (Sequencing Batch Reactor) was operated in an anaerobic-aerobic sequence to cultivate an enriched biological phosphorus removing sludge. The impact of long term temperature changes on the stoichiometry and kinetics of the different processes involved was studied at 20, 15, 10 and 5°C. At 5°C breakthrough of acetate to the aerobic period occurred. It was shown that the stoichiometry of the an-aerobic processes was insensitive to long-term temperature changes, whereas the kinetics of the aerobic and an-aerobic processes where clearly affected. The aerobic phosphorus uptake rate showed a maximum in the interval between 15 and 20°C. All other anaerobic and aerobic conversion rates increased with increasing temperature. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates. It was shown that a prediction of the temperature effect on a full scale biological nutrient removal plant is not a straight forward case because of the different influence of temperature on the sub-processes. All these influences should be accounted for.

1. INTRODUCTION

Biological phosphorus removal from waste water is preferred above chemical phosphate precipitation because of its lower environmental impact. A conventional waste water treatment plant can easily be converted into a biological phosphorus removal plant by providing an anaerobic tank ahead of the aerobic reactor.

Waste water treatment plants can be subjected to wide temperature ranges giving a strong need to determine possible influences of temperature variations on the process. Recently several investigators have been interested in this temperature influence on Enhanced Biological Phosphorus Removal processes (EBPR) leading to a number of papers, unfortunately not always with comparable results. These differences are often due to non comparable experimental set ups. Laboratory as well as full scale results are available, with temperatures ranging from as low as 3°C up to temperatures as high as 45°C.

Most studies can be categorised into those looking at the ***efficiency*** of EBPR processes under varying temperatures (often dealing with full scale waste water treatment plants) and/or publications which focus on the ***kinetics*** of the process (laboratory scale studies primarily). Recently attention is also being paid to the effects of temperature on the ***stoichiometry*** of the EBPR process.

Efficiency

Especially when looking at EBPR efficiency at different temperatures, results from literature are revealing contrasting observations. Where at first it would seem logical to find higher efficiencies at elevated temperatures (20-37°C) (Yeoman et al., 1988a; McClintock et al., 1993; Converti et al., 1995; Jones et al., 1996 and Scheer, 1994), different authors report on improved efficiencies at lower temperatures (5-15°C) (Sell et al., 1981; Kang et al., 1985; Krichten et al., 1985; Barnard et al., 1985; Vinconneau et al., 1985 and Florentz et al., 1987). According to Helmer and Kunst (1997) a drop in temperature from 15°C to 10 and then to 5°C had no significant influence on the efficiency of the EBPR

capacity. From full-scale SBR results (Marklund and Morling, 1994) a sharp decrease in EBPR efficiency was observed when the waste water temperature dropped below 4.5-5°C.

Kinetics

When the kinetics of the EBPR process are being studied, more consistency is observed in the literature. In the temperature range from 5°C to around 30°C, increased P-release and/or P-uptake rates with increased temperatures are being reported by Shapiro et al. (1967), Boughton et al. (1971), Spatzierer et al. (1985), Mamais and Jenkins (1992) and Brdjanovic et al. (1997). Helmer and Kunst (1997), however, reported a higher specific P-uptake at 5°C than at 10°C.

Declining phosphate release and uptake rates were observed at temperatures of 35°C and higher, with a significant inhibition at 42.5°C while no phosphate release or uptake was observed at 45°C, indicating that at this temperature the phosphate removing bacteria were probably dead (Jones and Stephenson, 1996).

Stoichiometry

A recent study showed that the stoichiometry of the anaerobic process is insensitive towards temperature changes whereas some effects on the aerobic stoichiometry was observed (Brdjanovic et al., 1997).

The objective of this paper is to indicate possible causes for the sometimes conflicting observations of temperature influence in biological phosphorus removal waste water treatment plants. Operational parameters that may be influencing the observations are considered and discussed.

In practice biological phosphorus removal is usually integrated with biological nitrogen removal in waste water treatment processes to comply with the actual discharge regulations. The temperature influence in both bio-P and bio-N removal can be different and these differences might influence the overall impact of the temperature influence on the biological phosphorus removal process.

2. METHODS

2.1. *APPARATUS*

The study was carried out in a laboratory fermentor (Biostat) filled with 8 ℓ of mixed liquor. The reactor was operated as a Sequencing Batch (SBR) with a cycle length of 6 hours, notably 15 minutes filling (anaerobic), 1.5 hours anaerobic phase, 3.5 hours aerobic phase, a maximum 10 minutes anoxic in between phase, a minimum 33 minutes settlement phase and a 17 minutes effluent withdrawal period. At the end of the aerobic period 200 ml of mixed liquor was removed to obtain a sludge age of 10 days. 3.8 Litre of supernatant was removed at the end of the cycle and 4 litre of medium was fed at the beginning of the cycle. The hydraulic retention time thus was 12 hours. When performing experiments, filling of the batch reactor was always performed within 3 minutes. In a later stage the reactor configuration was modified to allow a filling period of only 3 minutes in all cases. The anaerobic period was then prolonged with 12 minutes.

The SBR consisted of a Pyrex vessel with an internal diameter of 20 cm and a total height of 40 cm. A constant stirrer speed of 350 rpm was maintained except for the settlement and effluent purge period.

A time controller was used to obtain the settings for the different phases. Nitrogen gas was bubbled through the reactor during the anaerobic phase when the oxygen concentration raised above 0.05 mg O_2/ℓ. When performing experiments, nitrogen was continuously bubbled through the reactor during the anaerobic period. During the aerobic phase the dissolved oxygen (DO) concentration was automatically controlled to obtain a constant value around 2 mg/ℓ. The band width was set at 0.2 mg/ℓ, however, a

practical band width up to 1 mg/ℓ usually occurred due to overshoot in both directions. Gas flows were controlled with massflow controllers (Bronkhorst). The flow rates, however, were fixed at a given value for nitrogen and air and can, during the course of the cycle, only be changed manually. The experiments are performed at controlled temperature and pH (7.5 ± 0.1). The pH was maintained by dosing Na_2CO_3 or HCl (both ± 1N). An external heating/cooling device allows for operating at different, but constant temperatures. The SBR is equipped with an internal coil.

2.2. *MEDIUM*

A non sterilised medium was used containing 0.85g NaAc.$3H_2O$ (400 mg COD/ℓ), 65.81 mg KH_2PO_4 (15 mg P/ℓ), 90 mg $MgSO_4.7H_2O$, 14 mg $CaCl_2.2H_2O$, 36 mg KCl, 107 mg NH_4Cl (28 mg N/ℓ), 1 mg yeast extract and 0.3 ml nutrient solution per litre.

The nutrient solution contained per litre: 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO_4.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$ and 10 g EDTA. Only reagent grade products were used. The mixture was adopted from Smolders et al. (1995a)

2.3. *ANALYTICAL METHODS*

Orthophosphate analyses, using the ascorbic acid method, were performed with a colorimetric autoanalyser (TrAAcs 800) with a measurement range between 0.5 and 5 mg P/ℓ. Nitrate measurements, using the hydrazine reduction method, and ammonia measurements, using the Berthelot reaction, were also performed on TrAAcs, with a measurement range between 0.2 and 2 mg N/ℓ and 0.5 to 10 mg N/ℓ respectively. Potassium measurements were conducted on a flame photometer. Colorimetric COD analysis were performed according to the Standard Methods using Hach tubes and a Nanocolor colorimeter. Acetate analysis was performed on a Gas Chromatograph (GC) equipped with an FID. For polyhydroxybutyrate (PHB) analysis, lyophilised biomass was subjected to a propylation reaction. The organic phase was analysed by gas chromatography. For MLSS measurements 20 ml mixed liquor was filtered on a Whatman glass microfibre filter (GF/C) in a crucible. Before filtration the crucibles were dried at 550°C and weighed. After filtration the crucibles were first dried at 105°C followed by weighing of the recipient. For VSS determination the crucibles where then heated at 550°C followed by weighing again. The polyphosphate concentration can be approximated by the difference between MLSS and VSS. Polyphosphate can also be obtained performing a total phosphate analysis. Therefore, lyophilised biomass was subjected to a destruction reaction in an H_2SO_4-HNO_3-mixture (1:1). Organic phosphates were thereby converted to orthophosphate and measured as such. Glycogen measurements were not performed. It's initial anaerobic concentration was estimated as 2.5% of the MLSS content. The anaerobic glycogen profile was calculated based on the stoichiometric ratio between acetate-uptake and glycogen derived by Smolders et al. (1995a). Aerobically the glycogen was supposed to be replenished linearly. The active biomass concentration was calculated as the difference between MLSS and the sum of PHB, glycogen and polyphosphate.

2.4. *OPERATION OF THE SEQUENCING BATCH REACTOR*

On an average basis, the unit was sampled twice a week to verify the stability of the phosphorus removal capacity. For this purpose, samples were taken at the end of the previous cycle in the settlement phase, at the end of the feeding phase, at halftime and at the end of the anaerobic phase and at halftime and at the end of the aerobic phase. Samples were then analysed at least for orthophosphate, acetate, nitrate, ammonia and MLSS.

Initially the SBR was operated at 20°C and an SRT of 10 days. Once steady state operation was achieved, experiments were performed followed by switching the temperature to 15°C. The SBR operated at this temperature until steady state again was achieved. This procedure was repeated for 10 and 5°C. The concentration profiles for orthophosphate were used to define steady state. About 2 to 3 sludge ages were necessary to obtain steady state after switching the operational conditions. An SRT of 10 days was only sufficient for complete phosphorus removal at 20, 15 and 10°C. At 5°C incomplete P-removal occurred and acetate broke through to the aerobic phase. No attempts were made to correct for this situation. Short-term temperature shock experiments were performed at the lowest temperature.

When experiments were performed to elucidate the kinetics of the various reactions, in total 30 samples were taken during the anaerobic and aerobic phase of one cycle. Samples were taken every five minutes, gradually enlarging the sampling interval to 20 minutes towards the end of both anaerobic and aerobic phases when limited changes were expected. Samples were analysed for orthophosphate, acetate, nitrate, ammonia and PHB. At five moments samples were taken for MLSS and VSS measurement.

3. RESULTS AND DISCUSSION

3.1. *PERFORMANCE OF THE EBPR ACTIVITY IN THE SBR*

The SBR was initially inoculated with sludge originating from a lab scale Phoredox installation. After some start-up difficulties (Baetens and Hosten, 1996) the SBR has exhibited good phosphorus removal capacity for over a two and a half year period. Anaerobically orthophosphate is released, whereas acetate and glycogen are consumed with concurrent formation of PHB (and PHV not taken into account here). Aerobically, phosphate is stored internally by the bacteria using PHB as carbon source, while glycogen is replenished. Potassium follows the same profile as orthophosphate, according to the formula $K_{1/3}Mg_{1/3}PO_3$ (Comeau et al., 1987). At ambient temperatures the MLSS concentration became fairly stable with an average value of 3.5 g/ℓ. An average VSS/MLSS ratio of 70% was obtained. At 20, 15 and 10°C complete phosphorus removal was achieved over long periods. Maximum anaerobic phosphorus concentrations of 140 mg P/ℓ were recorded. For this temperature range acetate was always fully consumed, whereas at 5°C acetate broke through to the aerobic phase. Nitrification was observed at 20, 15 and 10°C, whereas at 5°C nitrifiers were washed out. Nitrate was never observed anaerobically. It seemed that during the 3 minutes filling period all nitrate present from the preceding aerobic period, was already consumed.

3.2. *QUALITATIVE INTERPRETATION*

In Figure 84 (a-d) the patterns of the relevant parameters are shown, recorded at different steady state temperatures.

From the figures it can be seen that complete anaerobic acetate consumption occurred at temperatures 20, 15 and 10°C, whereas breakthrough to the aerobic phase occurred at 5°C. At this lowest temperature, acetate is aerobically consumed with concomitant PHB production. During aerobic acetate consumption, the phosphate concentration remained constant, a finding in contrast to other authors (Brdjanovic et al., 1998c) who observe a net P-release and PHB-formation when acetate breaks through to the aerobic stage.

From the graphs it can be seen that with decreasing temperature, the PHB concentration slightly increased. After switching the operating temperature to 5°C, however, the PHB concentration in the cells increased drastically and reached a final maximum value of 30% based on MLSS (50% based on VSS). It can be assumed this high value is the maximum storage capacity of the cells. Note that the PHB measurements at 5°C show a much higher scatter than at the other temperatures. No validated explanation

for this observations is found, except maybe that homogenisation of the sludge after freeze-drying is more difficult at high PHB concentration because of the plastic character of the samples.

Whether P-release occurs or phosphorus remains constant during aerobic acetate consumption, fact is that part of the aerobic period is lost for P-uptake. From Figure 1d it can be seen that increasing the aerobic retention time significantly might result in a total P-removal at 5°C as well. The results obtained by Brdjanovic et al. (1998b) showed that complete P-removal could be obtained at 5°C, provided the SRT was increased from 16 to 32 days. PHB concentration then dropped to normal values again and complete anaerobic acetate consumption was achieved.

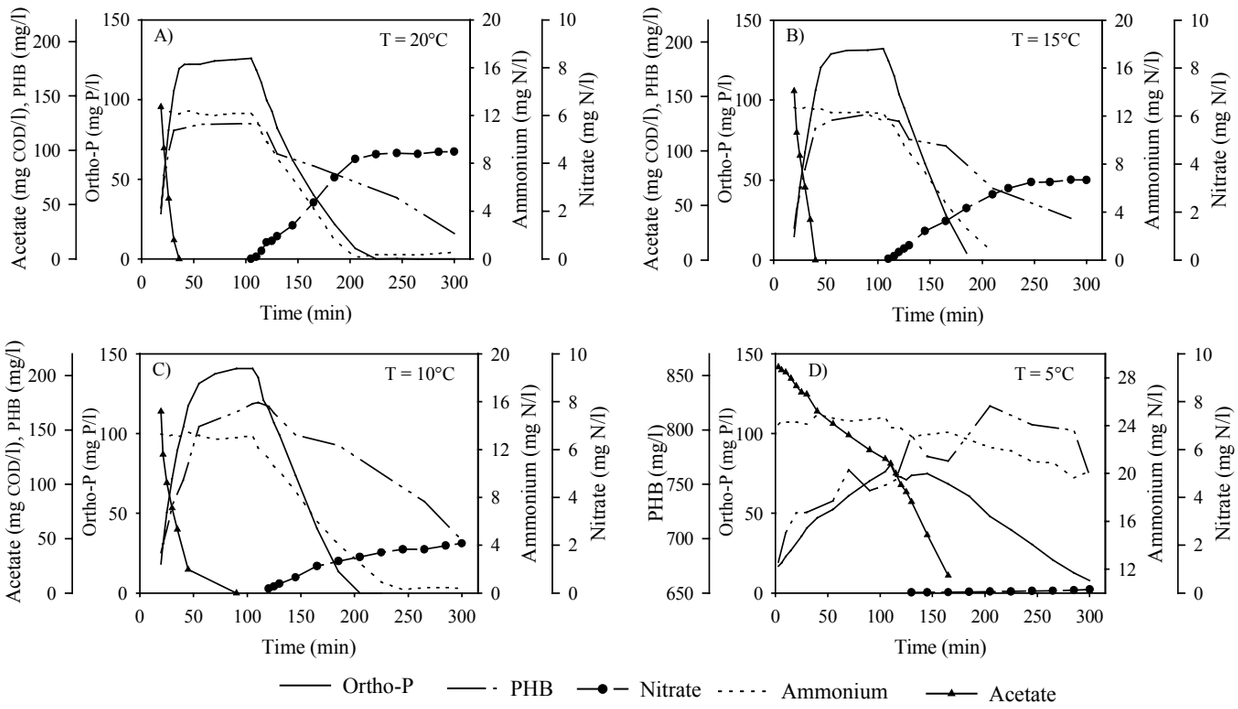

Figure 84 Patterns of relevant parameters recorded at different temperatures (Active biomass concentrations : 20°C: 2.61 g/ℓ; 15°C: 2.06 g/ℓ; 10°C: 2.57 g/ℓ; 5°C: 1.84 g/ℓ) Graph D): use axe graph C) for acetate concentration

3.3. *QUANTITATIVE INTERPRETATION : STOICHIOMETRY AND KINETICS OF THE ANAEROBIC AND AEROBIC PHASE*

The anaerobic and aerobic stoichiometry and kinetics (i.e. maximum specific reaction rates) were calculated for each temperature and represented in Table 71. Calculation of the rates mentioned in Table 71 has been performed using raw data. Initial maximum rates are used. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates by fitting equation 1 to the data. In Table 72 the temperature coefficients θ are represented for the anaerobic and aerobic reaction rates observed.

$$r_T = r_{20} \exp^{(\theta \cdot (T - 20))} \qquad (1)$$

From the values in Table 71, it can be concluded that temperature has little or no impact on anaerobic stoichiometry. On the kinetics, however, in both the aerobic and anaerobic phase, temperature has a strong influence.

Table 71 Stoichiometric and kinetic parameters for the anaerobic and aerobic phases of the EBPR process

		20°C	15°C	10°C (a)	10°C (b)	5°C
Anaerobic period						
Max. spec. Acetate uptake rate	g COD/g active BM/h	0.243	0.193	0.171	0.113	0.058
Max. spec. P-release rate	g P/g active BM/h	0.137	0.122	0.105	0.069	0.033
Stoichiometry	g P/g COD	*0.58±0.04*	*0.63±0.01*	*0.61±0.06*	*0.61*	*0.56*
Max. spec. PHB production	g PHB/g active BM/h	0.149	0.108	0.094	0.067	0.036
Stoichiometry	g PHB/g COD	*0.64*	*0.56*	*0.54±0.08*	*0.60*	*0.59*
Aerobic period						
Max spec. P-uptake rate	g P/g active BM/h	0.0373	0.0532	0.0411	0.0397	0.027
Max spec. PHB consump. rate	g PHB/g active BM/h	0.0256	0.0222	0.0138	0.0089	n.a.
Max spec. total NH_4-uptake rate	g N/g active BM/h	0.0035	0.0034	0.0028	0.0025	0.0002
Max spec. tot. NO_3-prod rate	mg N/g active BM/h	1.056	0.889	0.548	0.306	0

(a)-(b) Time gap of 4 months in between measurements.

Whereas nearly all rates show a declining trend with decreasing temperature over the complete temperature range, the aerobic specific P-uptake rate seems to attain a maximum value between 20 and 15°C. Brdjanovic et al. (1997) observed also only a maximum for the phosphate uptake rate.

From the results obtained from experiments performed at 10°C with a considerable acclimation time in between, it can be seen that the observed rates differ, but the stoichiometry remains constant. This observation indicates a potential difficulty when one tries to compare rates recorded by different authors. However, stoichiometry appears not to be affected. For the calculation of the temperature coefficients θ the results from 10b have been used because of the longer acclimatisation time applied for the experiment.

The anaerobic P-release/HAc uptake ratio reported by Brdjanovic et al. (1997) is lower than the value reported here. This is due to the higher pH applied in our experiments and may be also caused by not accounting for endogenous P-release here. The stoichiometric PHB/HAc ratio is lower than reported by Brdjanovic et al. (1997) since here PHV production is not yet taken into account.

For the calculation of the NH_4-uptake NO_3-production rates the total active biomass was considered. Due to the small fraction of nitrifiers and denitrifiers present the relative influence of a decreasing amount of these organisms will have an important influence on the rate calculation. For the calculation of the other rates, this problem is less important because of the much higher fraction of Poly-P organisms. The values mentioned in Table 71 for NH_4-uptake NO_3-production rates should thus be regarded with some scepticism.

Table 72 Temperature coefficients θ ($°C^{-1}$) and standard error for the anaerobic and aerobic reaction rates.

Anaerobic phase	θ ± stand. error	Aerobic phase	θ ± stand. error
Max spec. Acetate uptake rate	0.0764 ±0.011	Max spec. P-uptake rate (5-15°C)	0.0648 ± 0.003
Max spec. P-release rate	0.0683 ± 0.015	Max spec. PHB cons. rate	0.0742 ± 0.028
Max spec. PHB production rate	0.0816 ± 0.007	Max specific NO_3-prod rate	0.0842 ± 0.033

According to the classification of the temperature coefficients in ASM2 and the values obtained in this study, BPR has a low to medium degree of temperature dependency. The temperature coefficient

observed for nitrification, however, is much lower than typical values mentioned in literature (θ = 0.113°C^{-1})

Generally speaking the experimental results obtained using the enriched culture are consistent with the results obtained by Brdjanovic et al. (1997, 1998b) who used a similar experimental set-up. However from the introduction in this paper it shows that in practice many conflicting results are obtained. In the following section of this paper possible causes which can explain the results are indicated.

3.4. *BIOMASS ACCLIMATION TO TEMPERATURE*

Whereas short term temperature changes are supposed only to influence the kinetics and eventually the stoichiometry, long term temperature changes might also influence the biomass population. Hence, a distinction should be made between experimental results on pure cultures of bio-P organisms, or results obtained with enriched and mixed cultures. In the literature no results where found for temperature effects on pure cultures.

3.4.1. Enriched cultures

In two recent papers Brdjanovic et al. (1997, 1998a) studied short term as well as long term temperature effects on stoichiometry and kinetics of the anaerobic and aerobic phases of the biological phosphorus removal process. The process was operated in such a way to enrich the biomass with P-removing organisms. From their results it can be concluded that the anaerobic temperature coefficient (θ) obtained from long and short term tests were similar and consistent with results observed by Jones and Stephenson (1996), with θ being approximately 0.077 C^{-1}. In contrast to this observation under anaerobic conditions, different temperature coefficients for the aerobic phase were obtained depending on either long or short term tests. This observation was attributed by the authors to a probable population shift. Their conclusion is supported by molecular ecological techniques showing a distinct population shift in this enrichment culture with temperature.

Jones and Stephenson (1996) studied the temperature dependency of the EBPR process at different temperatures performing batch tests using sludge that first had been acclimatised to different temperatures. For anaerobic P-release and even more apparent for aerobic P-uptake, θ values generally decreased with increasing temperatures. According to these results the authors describe the EBPR process to be more sensitive to temperature changes at lower temperatures. A population shift is not discussed, but could be an alternative explanation for their observations.

Sell et al. (1981) and Krichten et al. (1985) explained the enhanced EBPR efficiency at lower temperatures by stating that the EBPR bacteria were psychrophilic. Above 10°C the non-EBPR mesophilic bacteria would then compete for substrates with the psychrophiles resulting in less phosphate removal at higher temperatures. Helmer and Kunst (1997) also attribute their results to an accumulation of cold tolerant P-removing bacteria.

3.4.2. Mixed Cultures

When using mixed cultures, as in full-scale studies, population shifts are of even greater importance. When operating at constant MCRTs, nitrification can easily be suppressed at lower temperatures, resulting in washout of nitrifiers and, henceforth, denitrifiers. Furthermore, lower temperatures might lead to incomplete anaerobic substrate uptake, resulting in a breakthrough of substrate to the aerobic stage. This will result in an increase of heterotrophic bacteria.

3.5. *TEMPERATURE INFLUENCE ON ACTIVE BIOMASS CONCENTRATION*

Mathematical models that include the presence of EBPR in activated sludge processes, such as the Activated Sludge Model No2 (ASM2) (Henze et al., 1995), University of Cape Town Activated Sludge Model (UCTPHO) (Dold et al., 1994) or the metabolic model of the EBPR (Smolders et al., 1995a) take into account the active biomass instead of the MLSS measurement. The active biomass can be calculated from the MLSS measurement obtained through standard procedures by subtracting the PHB, poly-P and glycogen content. While the active biomass remains constant under anaerobiosis (no growth and negligible decay), the MLSS value will decrease due to loss of poly-P and its comparatively higher molecular weight than stored acetate. Aerobically, the active biomass will increase due to growth and the poly-P content will increase whereas the PHB concentration will decrease. An overall increase in active biomass as well as MLSS should be observed. At decreasing temperatures less orthophosphate will be taken up and thus less poly-P will be formed. The ratio active biomass/ MLSS will thus be a function of temperature. This phenomena will result in different specific P-release and P-uptake rates when MLSS instead of active biomass is used as biomass count. The observed higher specific P-uptake rate at 5°C (Helmer and Kunst, 1997), might be explained by this MLSS based calculation.

3.6. *TEMPERATURE INFLUENCE ON OBSERVED YIELD AND SRT*

With increasing temperature the ATP requirement for maintenance increases (Brdjanovic et al., 1997) causing a decrease of the substrate available for net biomass growth. Decay processes increase with increasing temperature as well. Both phenomena result in less biomass production for the same amount of substrate used, causing a decrease in the net observed yield and thus a decrease in phosphorus removal capacity.

Working with full-scale continuous flow waste water treatment plants, the biomass concentration in the reaction basins is maintained constant through adjustment of the sludge wasted. When temperature decreases sludge production increases and more sludge needs to be purged to maintain this constant biomass concentration. With decreasing temperature a decrease in solids retention time (SRT) and thus an increase in the observed yield occurs.

Within the temperature range 13.5 to 20°C, it was demonstrated that EBPR functions efficiently and independently of SRT for aerobic SRTs above 2.1 days. At lower SRT values EBPR capability maybe lost at an aerobic SRT that depends on temperature. Higher temperatures allow efficient EBPR to be maintained at lower SRT values (Mamais and Jenkins, 1992). The authors observed washout of P-removing organisms at an aerobic SRT of 2.1 days at a temperature of 13.5 °C while washout only occurred at aerobic SRT of 1.5 days for a temperature of 20°C. Their results are in good agreement with observations made by McClintock et al. (1993) and Shao et at. (1991) who observed washout for an aerobic SRT of 2.5 days at 10°C and 1.45 days at 23°C respectively.

3.7. *TEMPERATURE INFLUENCE ON NITRIFICATION AND DENITRIFICATION*

A prerequisite for biological phosphorus removal to occur, is the existence of a true anaerobic zone, preceding an anoxic or aerobic stage. A carbon source should be available for the micro-organisms in this true anaerobic zone. Poly-P organisms will store this carbon source as polyhydroxyalkanoates for later use in the anoxic or aerobic stage. Nitrate entering the anaerobic zone will cause denitrification, depleting part of the carbon source present. Several process configurations have thus been modified to avoid breakthrough of nitrate in the anaerobic stage as much as possible (e.g. Modified UCT).

Whereas the influence of temperature on the bio-P process still remains partially unresolved, the influence of temperature on nitrification is well described and reveals a high correlation between temperature and

nitrification (θ=0.113 C^{-1}). So, when temperature decreases, less ammonium might be converted to nitrate, causing less nitrate to enter the anaerobic stage. With decreasing temperature, more carbon will thus be available for poly-P bacteria. An increase in the fraction of poly-P bacteria will occur due to wash out of the nitrifiers and denitrifiers.

For denitrification with acetic acid, 2.16 g C-HAc/g $N\text{-}NO_3$ are consumed (Henze et al., 1996). When denitrification occurs together with phosphorus removal, a higher acetic acid uptake rate will be observed. With decreasing temperature denitrification will disappear because of wash out of nitrifiers and thus denitrifiers. The resulting decrease in acetic acid uptake rate will then not only be caused by the influence on the bio-P process only, but also because of the lack of nitrate production, and thus the lack of denitrification.

3.8. *TEMPERATURE INFLUENCE ON SIMULTANEOUS PRECIPITATION REACTIONS*

Ever since the discovery of biological phosphorus removal, researchers also focussed on the possible contribution of simultaneous phosphorus precipitation reactions. Orthophosphates are known to form stable complexes with many cations. In most waste waters primarily calcium and magnesium are of importance but also sodium is often taken into account. When cation concentrations are high enough precipitation should be considered. Moreover the temperature influence on the precipitation will influence the observed overall temperature effects on the biological phosphorus removal process. Unfortunately the temperature effect on CaP-precipitation seems to be a complicated process with no straight forward answers considering the overall effect. The difficulty is that the probability of CaP-precipitation decreases with decreasing temperature but if the solubility product once is exceeded the formation of hydroxyapatit is quicker at lower temperatures (Maurer and Boller, 1998).

3.9. *TEMPERATURE INFLUENCE ON ANAEROBIC VOLATILE FATTY ACID (VFA) AVAILABILITY*

When the anaerobic uptake of acetate is incomplete at lower temperatures, leading to a breakthrough of acetate to the aerobic phase, an increase of heterotrophic bacteria will occur. However, it should also be taken into account that for real waste water treatment plants the available carbon source first has to be fermented to short-chain-fatty acids. This process can take place in a prefermentor, but also occurs naturally in slow-flowing outfall sewers or in pumping stations and force mains. Generally, it was found that without this incidental fermentation or prefermentation, it is very difficult to produce sufficient VFAs in the anaerobic zone of an EBPR plant when winter mixed liquor temperatures are below 17°C. Below this point the additional secondary release of phosphates in the anaerobic zone is more than the uptake possible through the production of VFA by increasing the anaerobic retention time (Randall et al., 1992). Low temperature will thus cause less hydrolysis and fermentation, causing less PHB to be formed anaerobically, resulting in less energy availability for later aerobic phosphate uptake.

3.10. *TEMPERATURE INFLUENCE IN BIO-P REMOVAL : AN OVERVIEW*

In Table 73 an overview is given of the expected temperature effects on the different processes occurring in a biological nutrient removal plant. In this table an important terminology, namely the storage capacity, is introduced. This storage capacity is not the capacity of one single organism, but the overall storage capacity given by the product of the individual storage capacity and the amount of organisms.

Table 73 The effect of a decreased temperature in Bio-P removal

Level	Action	Resulting effect	Effect
Organism	• Lower rates	• Kinetic limitation	-
	• Lower decay resulting in higher observed yield	• Less limitation due to increased storage capacity	+
Population	• Acclimation leading to population shift within PAOs	• Changed Arrhenius coefficients, changed kinetics	+/-
	• Less nitrification, less nitrate	• More substrate for PAOs, more storage capacity	+
	• Less fermentation	• Less substrate for PAOs, less storage capacity	-
Physical-chemical	• Precipitation	• Probability of precipitation decreases	-
		• If solubility product is exceeded, precipitate formation quicker	+

4. CONCLUSIONS

This paper discusses the temperature influence on biological phosphorus removal through literature review and own experimental evidence. From this review it becomes obvious that temperature has an important influence on bio-P processes, especially due to the storage processes involved. As such, MLSS measurements are not a good representation of the active biomass. Temperature changes can also cause a shift in composition. When comparing results between different researchers, attention should be focussed on differences between several operational conditions and all rates should be expressed on the basis of active phosphorus removal biomass.

An SBR (Sequencing Batch Reactor) was operated in an anaerobic-aerobic sequence to cultivate an enriched biological phosphorus removing sludge. The impact of long term temperature changes on the stoichiometry and kinetics of the different processes involved was studied at 20, 15, 10 and 5°C. At 5°C breakthrough of acetate to the aerobic period occurred.

It was shown that the stoichiometry of the anaerobic processes was insensitive to long term temperature changes, whereas the kinetics of the aerobic and anaerobic processes where clearly affected. The aerobic phosphorus uptake rate showed a maximum in the interval between 15 and 20°C. All other anaerobic and aerobic conversion rates increased with increasing temperature. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates.

It was shown that a prediction of the temperature effect on a full scale biological nutrient removal plant is not a straight forward case because of the different influence of temperature on the sub-processes. All these influences should be accounted for.

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CHAPTER 7

Optimal Experimental Design for the Calibration of Models of Phosphorus Removing Activated Sludge Systems

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Chapter 7

Optimal Experimental Design for the Calibration of Models of Phosphorus Removing Activated Sludge Systems

Abstract - In the field of waste water treatment, the Activated Sludge Models have been introduced as a tool for upgrading, optimising and controlling the process. The identification of these models is in general characterised by two important features, i.e. the models are often highly complex and there is a lack of reliable sensors for on-line measurement. The structural and practical identifiability of the parameters in these models is addressed in this paper. It focuses on optimal experimental design of experiments (OED), i.e. procedures to design a limited number of experiments that give the experimenter the maximum information content. The experimenter will select the experimental region and perform one experiment. From then onwards following experimental conditions will be chosen from the experimental region solely based on their predicted information content. The method is applied to calibrate models of phosphorus removing activated sludge systems. The D-criterion was found to give the best performance for the selection of the experimental conditions.

1. INTRODUCTION

In the field of waste water treatment, the *Activated Sludge Models* No 1, 2 and 3 (AS Models) (Henze *et al.* 1987, 1995 and Gujer *et al.* 1999) have been introduced by the IAWQ Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment Processes. These models are used as a tool for upgrading of existing plants or as an aid in process control. In these models many years of laboratory and field experience from individual researchers have been summarised. These researchers were able to define experimental conditions, e.g. by working with pure culture experiments, that allowed them to describe very specific sub-processes. The AS Models therefore take into account an important number of components and processes. Taking these models to a full scale waste water treatment plant to describe, optimise and control the process asks for a thorough understanding of the model and the possibility to measure its individual components. One of the constraints will immediately be that many of *the model components cannot easily be measured*. This should be taken into account when the model parameters have to be estimated from the plant operating conditions. Moreover, although today mathematical models related to ASM1 and ASM2 are implemented in various computer codes, there are *no general procedures for estimating the parameters*. Theoretically, at first the researcher should check whether it is mathematically possible to determine the parameters in the models, i.e. *verify the structural identifiability* of the model. Secondly the experimenter has to *verify the practical identifiability* of the model parameters, i.e. to check whether or not the available data are informative enough to identify the parameters accurately. Several papers have been published presenting procedures for the selection of structurally identifiable parameters (for an overview see below), with the aim of selecting only those parameters which can be estimated uniquely from the data set used. This data set then has be optimised, i.e. the experimental conditions should be designed to maximise its information content, i.e. to increase the practical identifiability of the parameters. This paper addresses in particular the latter by focussing on the theory of optimal design of experiments by describing procedures to design a limited number of experiments that give the experimenter the best information content. In this paper we will not use data from a full scale WWTP nor will we take into account all processes occurring in these treatment plants.

We only want to demonstrate the power of experimental design to increase the information content obtained during experimental runs without having to perform an excessive amount of experiments.

The paper is set up as follows. First, the theoretical framework of building a model, including a discussion on structural and practical identifiability, is introduced. Next, the theory for optimal experimental design to maximise the practical identifiability is explained. The practical framework starts with a description of the processes occurring in the biological phosphorus removal. This section comprises information on prior experiments performed to gain information on certain model parameters. Next, the definition of the experimental grid for further experiments is discussed. To conclude this section the mathematical calculation of the Fisher Information matrix (FIM) is addressed. The equivalence between the covariance and the sensitivity function method for the calculation of the FIM is evaluated. Different criteria for the selection of the following experimental conditions are analysed for best performance.

2. THEORETICAL FRAMEWORK

The identification of the dynamical models describing activated sludge processes is in general characterised by two important features (Dochain *et al.*, 1995):

- The models are often highly complex and are usually high-order non-linear systems incorporating a large number of state variables and parameters.
- There is a lack of cheap and reliable sensors for on-line measurement of the key state variables.

A priori knowledge on the identifiability of the parameters will be essential and statistical methods will be necessary to design the experiments and to evaluate the parameters obtained. In the following sections these features will be briefly explained to set the ideas.

2.1. *BUILDING A MODEL*

The final aim of model building is to obtain a useful model for the system under consideration. As such, the model structure and the parameters have to be identified, in this case a dynamic model describing the activated sludge process. The ideal model (M_I) is the model that contains all process characteristics This ideal model describes the process using the ideal model structure (S_I) and the ideal parameters (P_I). However, only these process characteristics that are identifiable, i.e. structurally and practically identifiable, can be determined. The model that contains only the identifiable process characteristics is called the user model (M_U). With this user model some process characteristics will be identified erroneously. Therefore, the user model structure (S_U) and the user parameters (P_U) will be different from the ideal model structure and model parameters. Due to noise on the experimental data, the estimated user model parameters will differ from the exact model parameters P*. As such, the error, i.e. the difference between the ideal model and the user model, can be divided in two parts:

$$M_I(S_I, P_I) - M_U(S_U, P_U) = \underbrace{M_I(S_I, P_I) - M_U(S_U, P^*)}_{BE} + \underbrace{M_U(S_U, P^*) - M_U(S_U, P_U)}_{VE}$$

The bias error (BE), is due to the difference between the ideal model structure and the user model structure selected from the candidate user models. This error becomes smaller the better the user model represents reality. BE is independent of the number of experimental data. The variance error (VE) in the above equation is caused by the noise on the experimental data and can be minimised by minimising the noise level and/or increasing the number of experimental data.

Once a user model selected and the user model structure defined, the user parameters have to be determined. But first another question has to be addressed, are all of these parameter identifiable? This

question even results in two questions which have to be asked, are the parameters theoretically, i.e. structurally, identifiable and if yes, are they practically identifiable. In the following subsections this is explained.

2.1.1. Structural identifiability

To explain the idea of structural identifiability a known model structure with perfect noise free data should be considered. Is it in this case evident that all parameters in the model are identifiable? Consider for instance the following simple equation: $y = ax_1 + bx_2 + c(x_1 + x_2)$, where x_1 and x_2 are two variables, y is the measured variable and a, b and c are the model parameters. From this simple example it is obvious that only the parameter combinations (a+c) and (b+c) can be identified unless the value of one of them is a priori known. For linear equations it seems an easy task to decide whether or not the parameters can be identified from the structural point of view. Despite this a number of different tests for parameter identifiability were developed for linear models. An overview of these different methods is given in Godfrey and DiStefano (1985)).

For models that are non-linear in the parameters the problem is a lot more complex. In this case several structural identifiability tests exist, but they are usually very complex. The following list summarises the available methods, including both theoretical and application oriented references that have focussed on the Monod kinetics, generally used for the simulation of WasteWater Treatment Plants (WWTPs).

1. Transformation of the non-linear model into a linear model (Godfrey and DiStefano, 1985; Walter, 1982; Ljung and Glad, 1994; Dochain *et al.*, 1995; Bourrel *et al*., 1998, Sperandio, 1998).
2. Similarity transformation approach or local state isomorphism (Vajda *et al.*, 1989; Chappell *et al.*, 1990; Chappell and Godfrey, 1992; Walter and Pronzato, 1995 ; Julien, 1997; Julien *et al.*, 1998).
3. Study of the observability properties of non-linear systems (Casti, 1985; Bourrel *et al.*, 1998).
4. Series expansions :

   4.1. Taylor series expansion (Pohjanpalo, 1978, Walter, 1982; Godfrey and DiStefano, 1985; Chappell *et al.*, 1990; Walter and Pronzato, 1995; Holmberg, 1982; Dochain *et al.* 1995; Jeppsson, 1996; Bourrel *et al.*, 1998; Sperandio, 1998; Petersen, 2000).

   4.2. Generating series (Walter, 1982; Walter and Lecourtier, 1982; Walter and Pronzato, 1995; Petersen ,2000).

For the series expansions, the user has to suggest the model parameters or parameter combinations that are thought to be identifiable. As such, the procedure becomes iterative, since so far there are no general rules for selecting the "right" combinations (Dochain *et al.*, 1995). Recently, Petersen (2000) has created some general rules that allow to get good parameter combinations for AS Models. For a model with zero input the generating series approach is equivalent to the Taylor series approach (Walter, 1982). However, for models that include inputs, the generating series approach usually results in simpler equation structures than the Taylor series approach (Raksanyi *et al.*, 1985; Walter and Prozato, 1995), although this was questioned by Godfrey and DiStefano (1985). In general, it can be difficult to judge in advance which approach of structural identifiability analysis is the most suitable one for a particular model under study (Chappell *et al.*, 1990).

The general biological processes occurring in waste water treatment plants, i.e. carbon, nitrogen and phosphorus removal, are generally divided in three main groups according to the bacteria involved in the process: heterotrophic organisms will consume available carbon sources, phosphorus accumulating organisms have the extra ability to store internal polyphosphate chains, thus removing phosphorus compounds from the wastewater, and nitrifying organism transform ammonia to nitrate. Nitrate is transformed to nitrogen gas by denitrifying organisms belonging to the group of heterotrophic and/or the

phosphorus accumulating organisms. In the characterisation of these biological degradation processes, Monod-type growth kinetics are most often used to describe the observations (Activated Sludge Model No.1 (ASM1, Henze *et al.*, 1987)). For these models it will be shown that the structural identifiability will depend not only on the model structure but also on the variables being measured in the course of the experiment. Indeed, due to the complexity of the Activated Sludge Models and the scarce availability of (on-line) measurement methods, it remains impossible to measure all of the state variables used in these models. Researchers are therefore continuously seeking for possibilities to minimise the necessary experimental effort, verifying however that the structural identifiability remains guaranteed. In the study of heterotrophic substrate degradation via the Monod model where measurements of both substrate and biomass were assumed to be available, it was proven that all parameters were structurally identifiable (Holmberg, 1982). In a similar study assuming only biomass measurements were available, it was not possible to identify all parameters structurally (Chappell and Godfrey, 1992). In both studies (Holmberg, 1982; Chappell and Godfrey, 1992) it was assumed that growth took place. In the work of Dochain *et al.* (1995) growth was neglected in the heterotrophic substrate degradation model, and oxygen uptake rate data were considered as measurements. It appeared that in this situation only certain combinations of model parameters were structurally identifiable. If, however, growth is explicitly accounted for, the structural parameter identification improves because the identifiable parameter combinations obtained assuming no growth (Dochain *et al.*, 1995) can be split up further (Sperandio, 1998, Petersen, 2000). Bourrel *et al.* (1998) studied the structural identifiability of the Monod kinetics for the denitrification process in a biofilm model assuming steady state with respect to growth. It was shown that depending on the measured state variables (nitrate, nitrite, carbon substrate) different parameter combinations were structurally identifiable. In another study the identifiability of a reduced order model used to control nitrification and denitrification by applying measurements of oxygen and nitrate was investigated (Julien, 1997; Julien *et al.*, 1998). Also in this study it appeared that some parameters were uniquely identifiable whereas others were only identifiable through combinations. In the study of Petersen (2000), the added value of combination of respirometric and titrimetric data was shown and a generalisation of results was obtained that allows to quickly deduce structurally identifiable parameter combinations from an ASM-like model written in its matrix formation.

2.1.2. Practical identifiability

While the structural identifiability is studied under the assumption of perfect, i.e. noiseless data, the problem of highly correlated parameters arises when a limited set of experimental, noise-corrupted data is used for parameter estimation. Under such conditions the uniqueness of parameter estimates predicted by the theoretical analyses, may no longer be guaranteed, because a change in one parameter can be compensated almost completely by a proportional shift in another one, still producing a satisfying fit between experimental data and model predictions. In addition, the numerical algorithms that perform the non-linear estimation show poor convergence when faced with this type of ill-conditioned optimisation problems, the estimates being very sensitive to the initial parameter values given to the algorithm.

It has been shown that the practical identifiability not only depends on the noise level of the data, but also depends on the experimental conditions themselves. Holmberg (1982) shows that the practical identifiability of Monod parameters from batch experiments depends on the chosen initial substrate concentration. The author states that the optimal initial substrate concentration depends not only on the noise level of the data but also on the sampling instants. At the moment only a limited number of studies in the domain of biotechnology (Baltes *et al.* 1994; Versyck *et al.*, 1998; Vanrolleghem *et al.*, 1995; Munack, 1991; Petersen, 2000) have addressed the problem of designing experiments by which more informative data can be collected.

Practical identifiability is thus related to the quality of the data and is related to their information content, i.e. whether or not the available data are informative enough to identify the model parameters accurately.

2.1.3. Optimal experimental design to maximise the practical identifiability

In the following subsection, the maximisation of the practical identifiability is tackled by defining optimal experimental design to increase the information content of the data.

2.1.3.1. ***Theory***

Above, practical identifiability has always been related to the information content of the data. In this section the information content of the data will be explained mathematically. Parameter estimation typically comprises the minimisation of the following *objective functional* (J) by finding the optimal choice of the parameters θ:

$$J(\theta) = \left[\underline{y} - \underline{\hat{y}}(\theta)\right]^T . Q . \left[\underline{y} - \underline{\hat{y}}(\theta)\right] \qquad [1]$$

with: $\underline{y}$ is the vector of the N measured values at times t_i (i= 1 …N)
$\underline{\hat{y}}(\theta)$ is the vector of N model predictions at times t_i (i= 1 …N)
θ are the model parameters
Q is a square matrix with user-supplied weighing coefficients

For non-linear models, the *expected value* of the *objective functional* for a parameter set slightly different from the optimal one can be approximated by (Munack, 1989)

$$E\left[J(\theta + \delta\theta)\right] \cong \delta\theta^T . \underbrace{\left[\left(\frac{\partial \underline{y}}{\partial \theta}\right)^T . Q . \left(\frac{\partial \underline{y}}{\partial \theta}\right)\right]}_{\text{Fisher Information Matrix}} . \delta\theta + \text{constant} \qquad [2]$$

For a parameter to be practically identifiable, a small change in the parameter value should result in a significant change of the (expected value of the) objective functional. From equation 2 it can be seen that for a small change in the parameter value, this difference will be more significant when the value for the so-called Fisher Information Matrix (FIM) is higher. From this equation, it is thus obvious that the FIM plays a central role to increase the practical identifiability of the parameters. Depending on the experimental conditions, different experiments will reveal more or less information, e.g. complete flat profiles, i.e. revealing no dynamics, will provide less information than profiles with more curvature. Since the FIM summarises the information content of the experiment, the choice of the experiments to be performed will be based on the value of the FIM.

From the above statements it is obvious that optimal experimental design can be based on the FIM matrix. How this FIM is used and needs or can be calculated will be explained in the following sections. It has to be stressed that for models non-linear in the parameters, the Fisher information matrix is parameter dependent through the local evaluation (i.e. at each t_i) of $\partial y/\partial \theta$. Non-accurate parameter estimates may therefore lead to on inefficient experimental layout.

2.1.3.2. ***Planning experiments based on the objective functional***

In the previous section it has been indicated that the objective functional and thus the FIM plays a central role in defining the experiment with the highest information content. From the beginning of an experimental set-up, i.e. the definition of the experimental conditions such as feed concentration, experimental temperature, length of the experiment, etc, this method can be used. The general methodology is explained by means of Figure 85. Before the procedure is really started the experimenter needs to have an initial guess of the parameter values. These values can be obtained from literature or when little trust can be based on these values or when no literature values are available, initial parameter values can be based on at least one experiment, i.e. an initial experiment has to be performed. With a parameter estimation algorithm an initial set of parameter values is obtained.

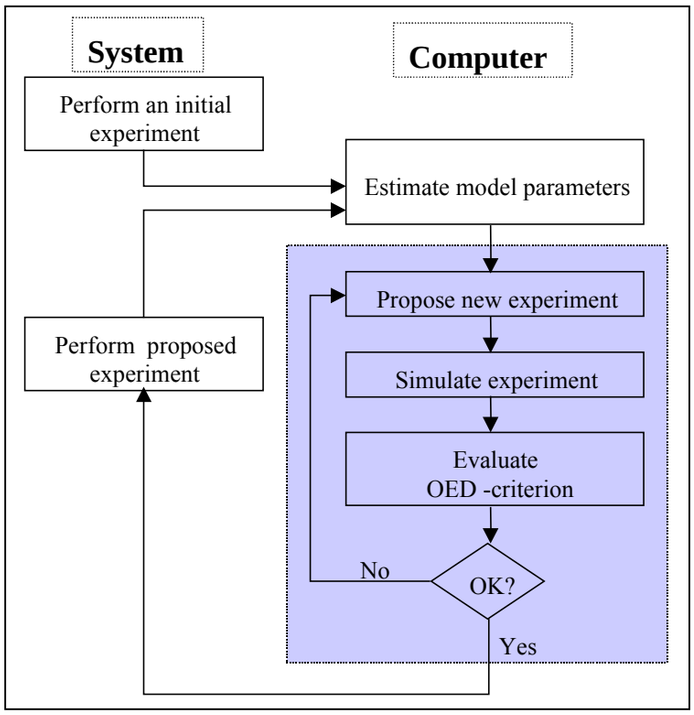

Figure 85 General methodology to plan experiments

From this point onwards OED is imple mented to determine the next experiment that will be performed. Therefore a new (virtual) experiment is now proposed within the experimental boundaries, e.g. maximum tem perature, feed concentration, time measure ment constraints. The experiment is simu lated (computer) using the set of parameter values obtained from the initial experiment. Mathematically the OED-criterion can now be evaluated by calculating the sensitivity functions (see equation 2). This procedure has to be repeated for different (virtual) experiments within the experimental boundaries. The experimental settings that reveal the highest value for the FIM will be used to perform the next experiment (system). The set of parameter values obtained from this and previous experiments (system) will now be used as new input (computer) to propose the next experiment. This methodology will provide the experimenter with the minimal set of experiments to be performed in order to obtain the maximal information content, i.e. an optimal experimental design is proposed.

2.1.3.3. ***Calculation of the FIM***

For the practical implementation of the OED, it is necessary to calculate the value of the FIM for each proposed experiment. To calculate the elements of the FIM the sensitivity functions have to be calculated. These sensitivity functions can be calculated analytically, which is a laborious task, or the calculations can be based on a numerical approximation. In the latter case model predictions are calculated where for each simulation run a single parameter is perturbed slightly from its optimal value. As such, at each measurement time instant a number of model predictions is available corresponding to the number of parameters plus one. To obtain the approximated value for the sensitivities, the difference is made between the slightly perturbed predictions and the "true" model predictions. Dividing this difference by the perturbation of the parameter gives the sensitivity. This calculation has to be performed for each response (y_k), for all parameters (θ_p) at each time instant (t_n). The accuracy of the numerical approximation largely depends on the magnitude of the parameter perturbation. It is suggested to verify the results by calculating the matrix using a fixed parameter perturbation and subsequently recalculating the matrix using a parameter perturbation half the previous one. If no difference is noticed in the sensitivities, the fixed value is used for all subsequent calculation. Unfortunately, both methods are still (computer) time consuming. Indeed, as indicated above, for every measurement point (i.e. time) the change in the value of the response (y) has to be calculated for a small change in the parameters, and this for all parameters.

Fortunately calculation of the FIM can be performed using a third method. Indeed, the elements of the FIM are the elements of the inverse of the covariance matrix of the parameter estimates (Godfrey and DiStefano, 1985). This covariance matrix can be obtained from mathematical parameter estimation algorithms. In the "covariance method" for the optimal experimental design of experiments, the steps

between simulating the experiment and evaluating the objective functional (Figure 86) are different. Indeed, for the covariance method experimental data, now including noise, have to be generated. Therefore noise is added to the simulated data. These "created" concentration profiles are then used as virtual experimental data and used as input data for parameter estimation. From the parameter estimation algorithm also the covariance matrix is obtained. This matrix can then be inverted to obtain the FIM. This procedure has to be repeated for each possible next experiment.

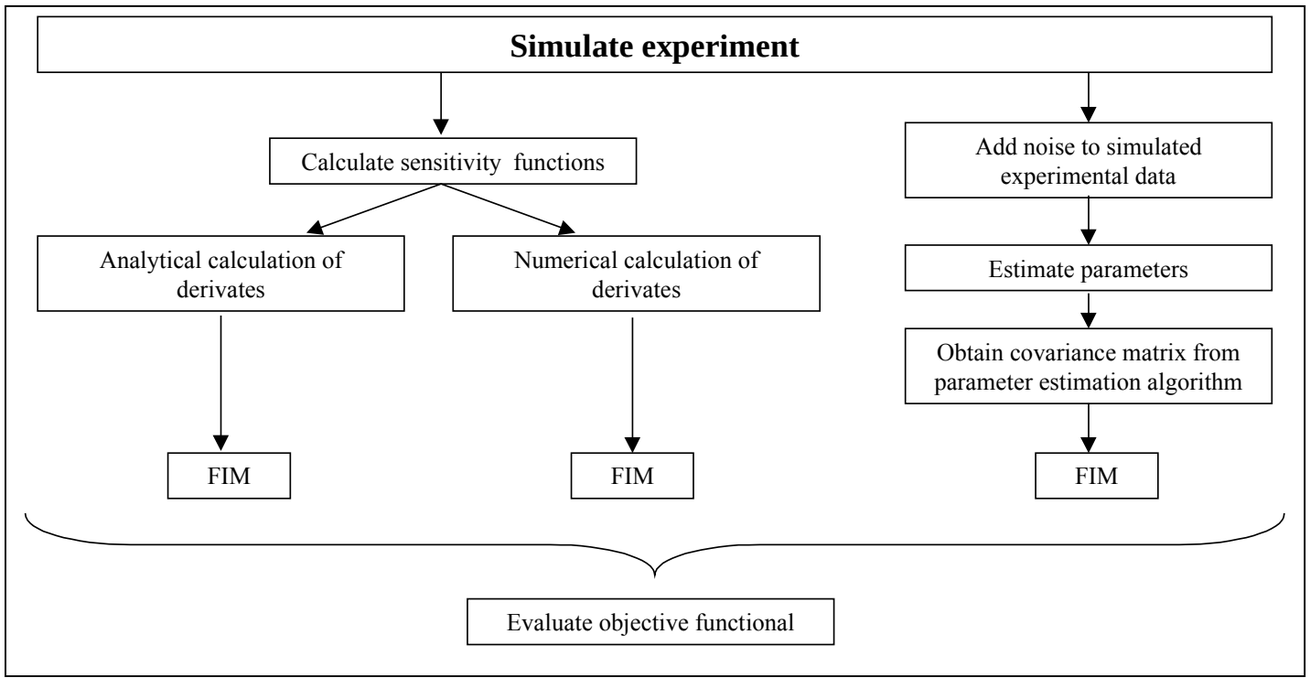

Figure 86 Possible ways to calculate the FIM and obtain the objective functional

2.1.3.4. ***The information content of the data: criteria used for maximisation of the FIM***

In the preceding paragraphs it has been stressed that the value for the FIM has to be maximised. Since the FIM is a matrix, the terminology maximising its value needs to be addressed. Several decision criteria exist that will be explained further. All of these criteria are based on the shape of the confidence region of the parameters. Actually, each parameter obtained from a parameter estimation procedure is situated within a confidence region. For linear models and for one parameter this will be a symmetric interval, for two parameters the confidence region will be an ellipse, whereas for n parameters the confidence region will be an n-dimensional ellipsoid. The volume of this ellipsoid is proportional to the inverse of the determinant of the FIM (Hosten, 1990):

$$V \approx \frac{\text{constant}}{\sqrt{\lambda_1 \lambda_2 ... \lambda_p}} = \frac{\text{constant}}{\sqrt{\det(FIM)}} \quad \text{with} \quad \begin{array}{l} p = \text{number of parameters} \\ \lambda \text{ the eigenvalues of the parameter covariance matrix} \end{array}$$

For non-linear models linearisation procedures are used for the calculation of the FIM. Thus, the ellipse or n-dimensional ellipsoid obtained are an approximation of the true confidence region.

The criteria used in practice to minimise the volume are (Hosten, 1990)

1 *D-optimal criterion (i.e. the volume criterion)*

This criterion will select for the experimental conditions that will *minimise the volume of the ellipsoid*. This volume is inversely proportional to the determinant of the FIM. Maximising the determinant of the FIM physically means minimising the geometric average of the errors on the parameter values.

2 *E-optimal criterion (i.e. the shape criterion)*

This criterion will select for experimental conditions that will *reshape the ellipsoid towards a sphere*. Practically this means that the longest axis has to be shortened, which is equivalent to maximising the smallest eigenvalue of the FIM. Physically this means minimising the largest of all parameter errors.

3 *Modified E-optimal criterion*

This criterion is a slight modification of the above criterion, i.e. instead of only taking into account the smallest eigenvalue, the ratio of the largest to the smallest eigenvalue will be minimised. This ratio expresses the stiffness of the FIM. The more important the stiffness becomes, the more difficult it becomes to invert the matrix until finally a singular matrix is obtained and the information content becomes zero. The modified E-optimal criterion will thus give rise to a FIM that is as far away as possible from singularity.

4 *Modified A-optimal criterion*

This criterion will maximise the trace of the FIM, i.e. it allows to maximise the sum of the eigenvalues of the FIM. Physically this criterion will minimise the arithmetic mean of the error on the parameter estimates.

5 *A-optimal criterion*

This criterion will minimise the trace of the inverse of the FIM. Physically this criterion will, as with the modified A-optimal criterion, minimise the arithmetic mean of the error on the parameter estimates.

3. PRACTICAL FRAMEWORK

3.1. *THE BIOLOGICAL PHOSPHORUS REMOVAL PROCESS*

In this study the technique of Optimal Experimental Design is applied to model the biological phosphorus removal processes occurring in a Sequencing Batch Reactor (SBR) fed with a synthetic wastewater (Baetens *et al.*, 1996). During one batch cycle two separate phases can be identified. During the first phase, called the anaerobic phase since no oxygen is present, micro-organism store acetate as an internal carbon source (PolyHydroxyButyrate, PHB). The energy required to produce PHB is obtained from the breakdown of an internal high energy PolyPhosphate chain. When the oxygen supply is switched on, the second phase starts, called the aerobic phase. During this phase the stored PHB is consumed, generating energy for growth and (excess) uptake of ortho-phosphate from the liquid phase. In Figure 87 a typical profile recorded during an experimental run performed on the SBR, is presented. The release and uptake of potassium follows the same profile as orthophosphate, according to the formula $Mg_{1/3}K_{1/3}P$ (Comeau *et al.*, 1987).

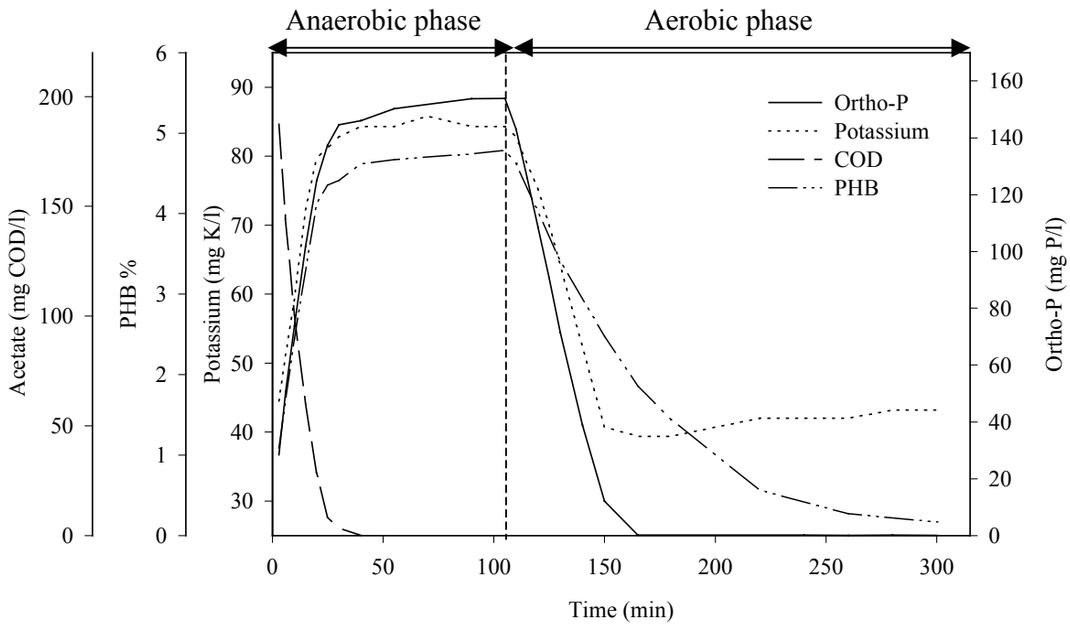

Figure 87 Typical profile recorded during an experiment

The rate equations considered for the anaerobic and aerobic phases are similar to the ASM2d model (Henze *et al.*, 1995) but comprise less components. Alkalinity and oxygen are not considered, since they were kept at a constant level throughout the different phases.

$$r_{an(aerobic)} = k_{PHB} \cdot \frac{C_{Ac}}{K_{Ac} + C_{Ac}} \cdot \frac{X_{PP}}{K_{pp}^{an} + X_{PP}} \cdot X_{PAO}$$

$$r_{ae(robic)} = k_{pp} \cdot \frac{X_{PHB}}{K_{PHB} + X_{PHB}} \cdot \frac{C_{PO_4^{3-}}}{K_{PO_4^{3-}} + C_{PO_4^{3-}}} \cdot \frac{K_{pp}^{ae} + X_{PP}}{X_{PP}} \cdot X_{PAO}$$

The mass balances for the anaerobic reactions read (no flow terms appear since the processes are studied in a batch reactor):

$$\frac{dC_{PO_4^{3-}}}{dt} = f_{pp} \cdot r_{an} \quad \& \quad \frac{dC_{Ac}}{dt} = \beta \cdot r_{an} \quad \& \quad \frac{dC_{K^+}}{dt} = f_{KP} \cdot f_{PP} \cdot r_{an} \quad \&$$

$$\frac{dX_{PHB}}{dt} = r_{an} \quad \& \quad \frac{dX_{PP}}{dt} = -f_{PP} \cdot r_{an}$$

The mass balances for the aerobic reactions read:

$$\frac{dC_{PO_4^{3-}}}{dt} = \alpha \cdot r_{ae} \quad \& \quad \frac{dC_{K^+}}{dt} = \alpha \cdot f_{KP} \cdot r_{ae} \quad \&$$

$$\frac{dX_{PHB}}{dt} = r_{ae} - k_x \cdot X_{PHB} \quad \& \quad \frac{dX_{PP}}{dt} = -\alpha \cdot r_{ae}$$

With:

C_{Ac} = acetate concentration (mg COD/ℓ)

$C_{PO_4^{3-}}$ = orthophosphate concentration (mg P/ℓ)

X_{PP} = internal polyphosphate concentration (mg P/ℓ)

X_{PHB} = internal polyhydroxybutyrate concentration (mg PHB/ℓ)

X_{PAO} = concentration of P - removing organisms (mg sludge/ℓ)

K_j = Monod coefficients related to the limiting component j (mg $comp_j$/ℓ)

k_j = rate coefficients related to the component j

α = ratio phosphate uptake/PHB use (mg P/mg PHB)

β = ratio acetate uptake/PHB formation (mg COD/mg PHB)

f_{PP} = ratio phoshate release / PHB formation (mg P/mg PHB)

f_{KP} = ratio phosphate release / potassium release (mg K/mg P)

Some of the parameters were fixed at literature values or own values.

$\alpha = 5.34$ mg P / mg PHB	(Smolders, 1995)
$K_{Ac} = 4$ mg COD / ℓ	(Henze et al., 1995)
$K_{PP}^{an} = 10$ mg P / g sludge	own results
$K_{PHB} = 10$ mg PHB / g sludge	(Henze et al., 1995)
$K_{PO_4^{3-}} = 0.2$ mg P / ℓ	(Henze et al., 1995)

Therefore, only 6 parameters remain to be estimated: $k_{PHB}, k_{PP}, k_X, \beta, f_{PP}, f_{KP}$

These parameters were estimated on the basis of the generalised least squares criterion for multi-response systems, using a Levenberg-Marquardt algorithm (Hosten, 1990).

3.2. *MODEL PARAMETERS FROM INITIAL EXPERIMENTS*

Prior to the experiments the sludge used was subjected to a constant feeding regime for several batch cycles, the number of batch cycles depending on the sludge age. The sludge is then under "steady state conditions". Five experiments were performed and used for preliminary parameter estimation. These five experiments could be subdivided in two groups: experiments 1&2 were performed at a temperature of 20.3°C whereas experiments 3,4&5 were performed at 15.4°C. Simultaneous parameter estimation, by combining all five experiments, was not performed at this stage because the temperature dependency of the parameters was not accounted for in the above kinetic equations (Baetens *et al.*, 1999). In Table 74 the results of the preliminary parameter estimation are presented. All parameters were significantly different from zero at the 95% confidence level. At a temperature of 20.3°C the parameters β and f_{pp} were relatively strongly correlated. At a temperature of 15.4°C, no significant correlation between the parameters was observed. These parameter values were subsequently used as input for determination of the FIM according to the schemes in Figure 85 and Figure 86.

Table 74 Preliminary parameter estimates

Parameter	Parameter set 1 obtained from experiments 1&2 at 20.3°C	Parameter set 2 obtained from Experiments 3,4&5 at 15.4°C	Unit
f_{PP}	1.106	1.051	mg P/mg PHB
k_{PHB}	130.4	113.5	mg COD/g sludge/h
β	1.708	1.580	mg COD/mg PHB
f_{KP}	0.2766	0.2178	mg K/mg P
k_{PP}	5.081	7.660	mg P/ g sludge/h
k_x	0.7695	0.5850	h^{-1}

3.3. *PROPOSE NEW EXPERIMENT : DEFINITION OF THE EXPERIMENTAL GRID*

In the OED, the best experiment was chosen from a three dimensional experimental grid determined by the following degrees of freedom

- The initial phosphate concentration $[PO_4]_0$
- The initial acetate concentration $[Ac^-]_0$
- Time in between two measurements

Practically the definition of an experimental grid is not necessary, using the principals of OED an experiment can be defined solely taking into account the experimental boundaries. For practical reason it is more convenient to define in advance an experimental grid when working with biological systems.

The choice of *the boundaries of the experimental grid* for the first two variables were based on literature values and own experience. For phosphorus these boundaries were set at 2 and 20 mg P/ℓ, for acetate the boundary values were 200 and 600 mg COD/ℓ. Too high an initial acetate concentration might lead to an acetate break through to the aerobic period in the SBR cycle. Since aerobic acetate uptake is not taken into consideration in the model, this phenomenon had to be avoided.

For the selection of the time in between two measurements it is quite obvious that the higher the frequency the greater the information content of the experiment will be. The procedure was tested for an interval of 10 and 5 minutes. An important difference in information content existed between both. Further decreasing the time interval was physically impossible with the set-up under investigation, i.e. samples cannot be taken any faster manually. Consequently, the results discussed in the following are only based on the five minutes interval.

Table 75 Discrete experimental grid

Grid point	$[PO_4]_0$ mg P/ℓ	$[Ac^-]_0$ mg COD/ℓ	Grid point	$[PO_4]_0$ mg P/ℓ	$[Ac^-]_0$ mg COD/ℓ	Grid point	$[PO_4]_0$ mg P/ℓ	$[Ac^-]_0$ mg COD/ℓ
A	5	200	M	10	200	Y	15	200
B	5	225	N	10	225	Z	15	225
C	5	250	O	10	250	AA	15	250
D	5	275	P	10	275	BB	15	275
E	5	300	Q	10	300	CC	15	300
F	5	325	R	10	325	DD	15	325
G	5	350	S	10	350	EE	15	350
H	5	375	T	10	375	FF	15	375
I	5	400	U	10	400	GG	15	400
J	5	425	V	10	425	HH	15	425
K	5	450	W	10	450	II	15	450
L	5	475	X	10	475	JJ	15	475

In Table 75 the *discrete experimental grid* used is shown. Possible initial phosphate concentrations are 5, 10 or 15 mg P/ℓ while initial acetate concentrations range between 200, and 475 mg COD/ℓ, with discrete steps of 25 mg COD/ℓ. The experimental grid thus consists of 36 grid points. Each grid point corresponds to a virtual experiment that is evaluated in terms of the expected information content.

The FIM was calculated in each of the grid points. From the FIM four out of five of the OED criteria described in the theoretical framework were calculated, i.e. the D- and E-optimal criterion and the modified A- and E-optimal criterion. The A-optimal criterion was not considered since it doesn't rely on the properties of the FIM but on the properties of its inverse, the error covariance matrix. The discussion of the results is further concentrated on the D- and E-criteria.

3.4. *CALCULATION OF FIM*

For the calculation of the FIM both the covariance and the sensitivity technique were used. The noise applied for the covariance method had a normal distribution with a standard deviation of $\sigma = 0.1$.

For the calculation of the FIM based on the sensitivity technique the numerical approximation was chosen instead of the laborious analytical calculations. In this study a value of 10^{-6} for the parameter perturbation gave satisfactory results.

4. RESULTS AND DISCUSSION

4.1. *EQUIVALENCE BETWEEN THE COVARIANCE AND THE SENSITIVITY METHOD*

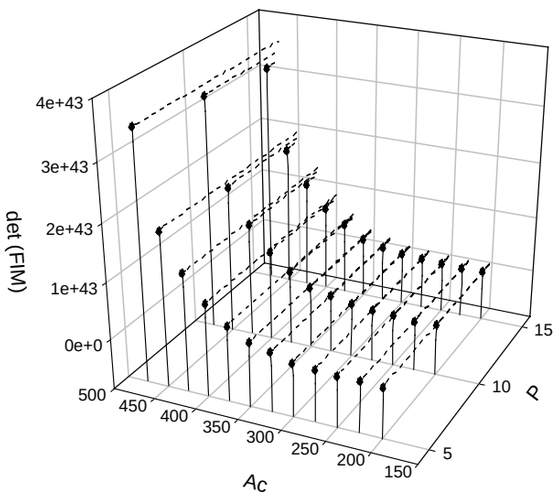

Figure 88 D-criterion using the covariance method

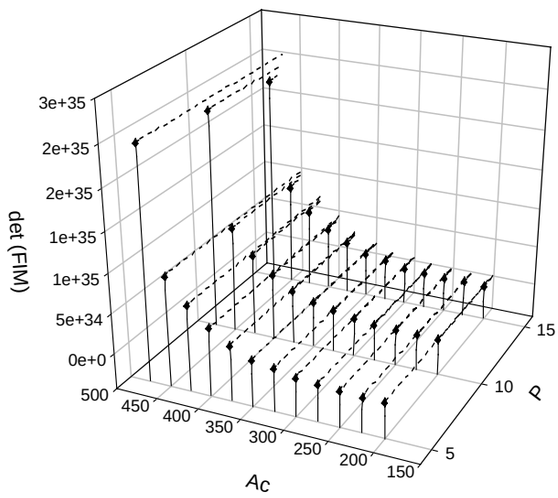

Figure 89 D-criterion using the sensitivity method

In Figure 88and Figure 89 the D-criterion, i.e. the maximisation of the determinant of the FIM, is shown both for the covariance and the sensitivity method. From both graphs it can easily be verified that both criteria yield similar three dimensional shapes with the highest values for det(FIM) being found at the highest acetate concentration. The values for det(FIM) decrease nearly exponentially with decreasing acetate concentration but remain constant with changing orthophosphate concentration at a fixed acetate level. These graphs provide extra evidence that the covariance method and the sensitivity method yield the same results in terms of optimal experimental conditions. However, the numerical values obtained with the covariance method are higher. The same tendency was observed for the other experimental

points. Theory predicts that both methods should also yield the same numerical values. The observed differences result from the fact that noise has been added to the experimental data during the parameter estimation procedure in the covariance method. Upon calculation of the FIM from the sensitivity method noise is not considered since only the covariance matrix of the parameters is taken into account.

The computer time necessary for determining the FIM using the covariance method was found to be significantly larger than by using the sensitivity method (8 times longer), because the time consuming parameter estimation steps don't have to be performed when using the sensitivity method. It should, however, be stressed that for the sensitivity method, data handling can become a difficulty for some software packages. Indeed, the calculation of all FIM elements asks for large amounts of stored data.

4.2. *THE MOST CONSISTENT OED CRITERION*

The model parameters obtained from the initial experiments are now used to select the best experiment from the experimental grid, choosing those experimental settings that reveal the highest value for the FIM. The calculations were performed using the parameter sets obtained from each experiment separately (not shown in Table 74) and using the parameter sets 1 and 2 for the combination of experiments 1&2 at 20,3°C and for the combination of experiments 3,4&5 at 15,4°C respectively (Table 74).

From Table 76 it is clear that the D-criterion is very consistent in its choice of OED. The modified E criterion is the least consistent.

OED based solely on the parameter set obtained from experiment 3 yielded different results for all criteria. Indeed, the parameter set obtained from the preliminary experiment 3 differs from the parameter sets obtained from experiment 4 or 5 separately or 3,4&5 combined. This different OED is explained by the fact that the Fisher information matrix is parameter dependent through the local evaluation (i.e. at each t_i) of $\partial y/\partial \theta$ as was already stated before.

The difference between L and JJ is only the amount of phosphate. In the next section it is shown that the criteria are the least sensitive to this degree of freedom.

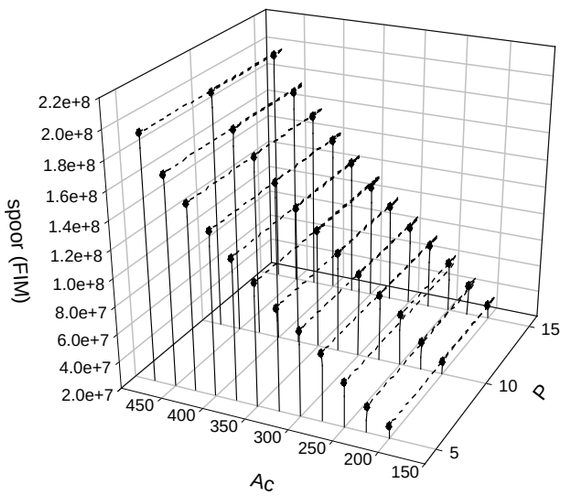

Figure 90 Modified A-criterion

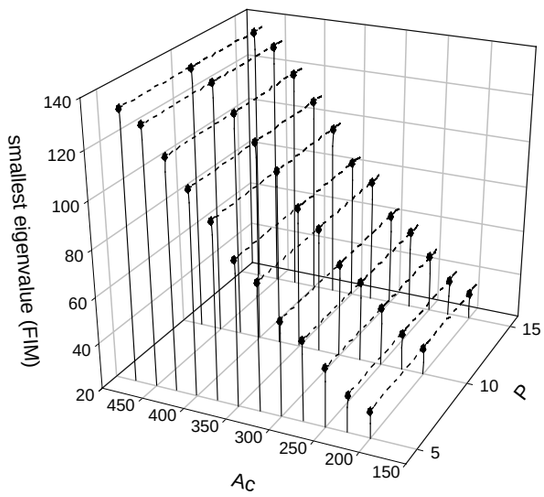

Figure 91 E-criterion

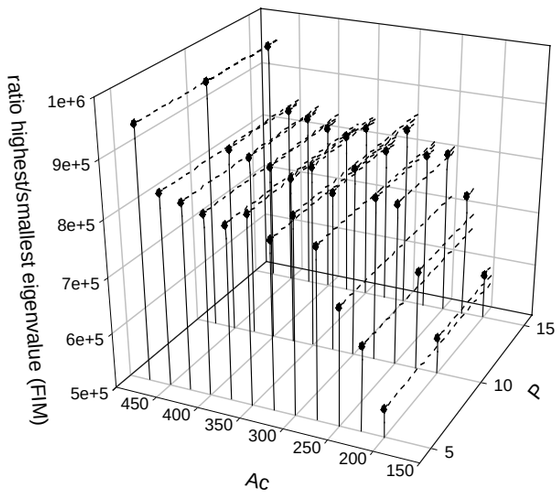

Figure 92 Modified E-criterion

Figure 90, Figure 91 and Figure 92, show the results for the different criteria for the parameter set obtained from experiment 1. It is obvious that, except for the modified E criterion, the higher the initial acetate concentration the more information is gained. Only for the D-criterion a slight increase in information content with a lowered phosphate concentration is indicated. Since the modified D-criterion appears able to consistently reveal the best experiment, this criterion is to be preferred.

Table 76 OED for parameter sets obtained from each experiment or each experimental group

Parameter set obtained from:	D-criterion	E-criterion	Modified A criterion	Modified E criterion
Exp 1 (20,3°C)	L	L	L	A
Exp 2 (20,3°C)	L	J until L	K until L	GG
Exp 1&2 (20,3°C)	L	L	L	J
Exp 3 (15,4°C)	J until L	F until L	C	CC until JJ
Exp 4 (15,4°C)	HH until JJ	BB until JJ	HH until JJ	D
Exp 5 (15,4°C)	FF until JJ	CC until JJ	DD until JJ	J until L, V until Z, HH until JJ
Exp 3,4&5 (15,4°C)	JJ	L	JJ	F

4.3. *OPTIMAL EXPERIMENTAL DESIGN RESULTS : DISCUSSION*

From Table 76 it is clear that the two temperature levels yielded different optimal experiments. However, the difference between both has only to do with the initial phosphate concentration, i.e. for the highest temperature the experiment should be performed at 475 mg COD/ℓ and 5 mg P/ℓ, whereas for the lowest temperature level 15 mg P/ℓ is preferred. The difference between both is subtle and is explained below.

For the acetate concentration it is clear that the higher the initial concentration, the longer it takes before its concentration drops to zero in the anaerobic phase and the more informative data points are gained from the experiment, i.e. the more information is gained on the dynamics of the process. Moreover, at the moment acetate is fully consumed, the other concentrations, whose dynamic variations also depend on the acetate content, give a plateau as well. So, the higher the initial acetate concentration the higher will be the information content for the other responses as well. However, it should be stressed that the acetate concentration should not be too high either. Indeed, breakthrough of acetate to the aerobic phase has to be avoided since no aerobic processes for acetate consumption are taken into consideration. Even more importantly, breakthrough of acetate to the aerobic phase will cause that the parameter K_{Ac} in the model for anaerobic acetate consumption can no longer be identified, i.e. det FIM will become singular. A practical identifiability problem will occur.

An explanation for the insensitivity of the criteria towards the initial phosphate concentration can be found in the fact that as long the organisms can fully consume the available phosphate, no additional information is gained by increasing its value. Indeed, when the phosphate concentration becomes nearly zero most information is gained, i.e. to accurately determine the relevant Monod coefficients.

For the experiments performed at 15.4°C it is found that more information can be obtained by working at the highest allowed initial phosphate concentration. The explanation is to be found in the parameter values themselves. Indeed, it is shown that the rate coefficient for aerobic phosphate uptake has its maximum at this temperature (Baetens *et al.*, 1999). Hence, the higher the initial phosphate concentration, the longer it takes before complete consumption of phosphate occurs and thus the higher the information content will be of the experiment.

As a general conclusion it needs to be stressed that OED is a method to define experiments to gain information on the parameters of the process at its current state. For example, the criterion indicates that an experiment has to be performed at an initial concentration of 475 mg/ℓ, clearly higher than the feed used in the period leading towards a steady state regime before experiments 1 and 2 were performed. The calculated optimal experiment should be performed by taking sludge from the reactor under the steady state conditions, and subjecting this sludge to one, and only one, cycle where the initial acetate concentration is increased to 475 mg/ℓ. During the next cycle in the SBR, the sludge should then again be fed with its normal steady state feed. Indeed, if the system would be subjected continuously to this new, high acetate concentration, the system will adapt to reach a new "steady state". As natural consequence the biomass concentration will clearly increase. The model parameters might change as well.

5. CONCLUSIONS

It is practically shown that the covariance and the sensitivity method lead to the same patterns of OED criteria values and that both methods can be used to select the experimental settings for most accurate parameter estimation. Moreover, using the numerical method as an approximation method for calculating the sensitivity matrix gives the same results, except for an important difference, namely that the time consuming analytical calculation can be avoided. Since using the numerical sensitivity method also reduces the necessary computer time compared to the covariance method, the numerical sensitivity method is clearly the preferred method for OED calculation.

Comparison between the different criteria on which experimental design can be based, has led to the conclusion that the D-criterion is the most powerful of all. Moreover, especially for the calibration of phosphorus removing activated sludge systems, the D-criterion was capable of sensing the subtle difference between initial phosphate concentrations.

This research was performed before the temperature dependency of the parameters was fully examined. With information gained later (Baetens *et al*., 1999) the OED procedure should be repeated with a model that accounts for the temperature dependency of the process and for which a single set of parameters is estimated taking into account all experiments together.

Acknowledgement

This work is dedicated to Professor Hosten who died too early to see this work being published. He dedicated his professional career to mathematical modelling in a never lasting effort to teach his students the importance of a thorough study of parameter estimation.

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CHAPTER 8

Model based upgrading for nutrient removal: Modelling study – Efficiently calibrating ASM2d

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Chapter 8

Model based upgrading for nutrient removal: Modelling study – Efficiently calibrating ASM2d

Abstract - Models can play an important role in the evaluation of upgrading strategies for biological nutrient removal. In this paper the calibration of ASM2d to a pilot plant with an intermittent aerobic/anoxic reactor is discussed. The performed modelling study was part of a retrofit study. An existing plant, removing only COD and where phos-phorus was chemical precipitated, had to be upgraded towards full biological nutrient removal. Emphasis was put on the practical aspects of calibrating ASM no 2d efficiently. The calibration procedure was based on an 'expert approach' rather than on a system engineering approach. With only changing three parameters (reduction factors, lysis rates for PAOs and the decay rate for autotrophs), the model proved well capable of describing the performance of the pilot plant. A second set of parameter adjustments was tested. Good results were obtained as well, but more parameters had to be changed.

New findings are that oxygen entering the treatment plant via the influent has an important influence on the simulated phosphate effluent concentrations. Further, reactions occurring in the final clarifier effect the effluent concentrations. This is indicated by the necessity to introduce and assign a virtual volume to the settler where reaction can occur. Both factors are extremely important to focus attention to because they are different between a pilot plant and a full scale system.

1. INTRODUCTION

Wastewater treatment plants built in the past were mainly designed for COD removal. Stricter environmental regulations necessitate the removal of nitrogen and phosphorus in the treatment plants. Including biological nutrient removal in existing wastewater treatment facilities means reassigning existing tanks for new purposes, redesigning the flow scheme of the plant and when necessary the installation of new tanks.

The high number of reactions, the interaction between the different processes, the very strict regulations and the limited space available for upgrading or building new treatment plants necessitates nowadays the application of models for designing these facilities. An IAWQ task group recently presented ASM2d for modelling nutrient removal (Henze et al., 1999). This is the first paper where this model is used to describe a pilot plant and predict the behaviour of an upgraded treatment plant.

The parameters in the ASM models are average values often based on laboratory experiments performed under extreme conditions favouring particular processes. These parameters can be very case specific and new values should be assigned based on pilot and or full scale data. Indeed, the quality of the predictions depends on the quality of the parameters of the model and of the quality of the wastewater and plant characterisation. In the case of upgrading an installation, investing money in prior pilot scale experiments with different scenarios will result in better modelling results and maximised design knowledge. A good strategic set-up benefits the success of the calibration procedure.

Although it is often said that for the application of dynamic processes, default values may be retained for stoichiometry and most kinetic constants, it will be shown in this paper that ASM2d has some

shortcomings which can be overcome by adjusting certain parameters. Other parameters will be shown to be process and influent dependent.

Wastewater characterisation was performed using the Dutch standards proposed by STOWA (Roeleveld et al., 1998). Laboratory experiments were used to obtain a first indication of the fraction of PAOs, to differentiate between denitrifying and non-denitrifying PAOs and to have an idea of the denitrifying capacity of the heterotrophs. In this paper the standard notation of the IAWQ task group has been used.

2. MATERIALS AND METHODS

2.1. *INSTALLATIONS*

The projects involves upgrading an existing plant for COD removal to a full biological nutrient removal plant, including biological nitrogen and phosphorus removal (Boonen et al., submitted). A pilot plant was built and long-term pilot investigations with different AS processes were carried out for approximately 10 months (Bixio et al., 1999). An intensive measurement campaign was set up with the pilot plant consisting of an anaerobic tank of 1 m^3, an aerobic/anoxic tank of 6 m^3 and a settler of 3.5 m^3. Influent was taken from the WWTP after the grit chamber and fed at a constant flow rate of 12 m^3/d to the pilot plant without primary settling (Bixio et al., 1999). The aerobic/anoxic tank was operated intermittently with a complete cycle of 5000 seconds of which 2000 seconds with aeration switched on. The oxygen concentration was regulated at 1 mg O_2/ℓ. Excess sludge was taken from this tank at intermitted intervals to set a sludge retention time of 14 days. The return flow was set to 81.6 m^3/d.

2.2. *MEASUREMENT CAMPAIGN*

Three automatic samplers (AS900, Sigma) were located respectively at the entrance of the biological unit, at the end of the anaerobic compartment and at the effluent 24h composite samples were taken during a period of 48 days. Within that period, 2h composite samples were collected for 48 hours. VFA determination was performed only for these 2h samples. All samples were cooled at 4°C and transported to the laboratory every day for analysis.

Influent samples were analysed for COD, COD_f, BOD, BOD_f, SS, VSS, KjN, NH_4-N, NO_3-N, NO_2-N, TP and PO_4-P. Samples of the effluent of the anaerobic tank were analysed for COD, COD_f, SS, VSS, KjN, KjN_f, NH_4-N, NO_3-N, NO_2-N and TP. Within the biological reactors ammonia, nitrate and dissolved oxygen were monitored on-line. The effluent samples were analysed for COD, COD_f, BOD, SS, KjN, NH_4-N, NO_3-N, NO_2-N, TP and PO_4-P.

2.3. *SLUDGE CHARACTERISATION*

Two jacketed laboratory fermenters of 2 litres were used to perform batch tests to determine the fraction of phosphate removing organisms and the reduction factors for anoxic acetate uptake and anoxic phosphate uptake. The activated sludge used was taken from the aerobic basin of the pilot installation preferably one day prior to the batch experiments.

The values resulting from the experiments are used as indication for increasing or decreasing the relevant parameters. The tests were performed at a controlled temperature of 20°C and pH of 7.0 ± 0.1.

Prior to the experiments phosphate is added to the sludge to allow for eventual aerobic phosphate uptake. Then the sludge is subjected to an anaerobic stage with acetate feed, followed by an aerobic or anoxic period. To this end the mixed liquor is divided in two equal amounts after the anaerobic period. Phosphate, ammonium, nitrate, VSS and MLSS are measured following a pre-set sampling program.

2.4. *INFLUENT CHARACTERISATION*

Since the IAWQ models (ASM1, ASM2, ASM2d) were published, a variety of methods emerged to characterise the different components of the wastewater. For the purpose of this study the wastewater characterisation was performed using the Dutch guidelines formulated and published by STOWA in 1996 (STOWA, 1996). This method is based on a physico-chemical method to characterise the soluble and particulate fractions, combined with a BOD-measurement for characterising the biodegradable fraction of the influent COD.

In Table 77 the average measurement data obtained during the measurement campaign are presented along with their standard deviations (34 measurement points). In the last column the set of equations needed for the influent characterisation according to the STOWA guidelines are presented. Since for ASM2d no soluble inert production is included in the model, i.e. f_{SI} being the stoichiometric parameter for production of S_I in the hydrolysis process is set to zero, the proposed equation, $S_I = 0.9\ COD_{effl}$, was replaced by $S_I = COD_{effl}$.

Table 77 Average measurement data and conversion formulas (Roeleveld et al., 1998)

Measured value			Conversion equations for model values
Influent measurement data			
	Average	Stdv	
COD	524.7	188.4	$COD = S_A + S_F + S_I + X_I + X_S$ (gives $\mathbf{X_I}$)
BOD_5	203.4	76.1	$BCOD = \frac{1}{1-f_{BOD}} * \frac{1}{1-e^{-k_{BOD}t}} * BOD_5 = S_A + S_F + X_S$ (gives $\mathbf{X_S}$)
COD_f	221.6	109.8	$COD_f = S_A + S_F + S_I$ (gives $\mathbf{S_F}$)
VFA	16.5	6.7	S_A (gives $\mathbf{S_A}$)
K_j-N	46.1	5.9	$S_{NH4} = K_j\text{-}N - (i_{NSI} * S_I + i_{NSF} * S_F + i_{NXI} * X_I + i_{NXS} * X_S + i_{NXBIO} * X_{BIO})$
TP	7.6	1.5	$S_{PO4} = TP - (i_{PSI} * S_I + i_{PSF} * S_F + i_{PXI} * X_I + i_{PXS} * X_S + i_{PXBIO} * X_{BIO})$
S_{NH4}	31.1	5.5	
S_{NO3}	0		
S_{PO4}	4	0.8	
Effluent measurement data			
COD_f	38	4	$S_I = COD_f$ (gives $\mathbf{S_I}$)

2.5. *SIMULATION ENVIRONMENT*

For simulation purposes the software package Simba 3.2+ (c) ifak 1997, based on Matlab and Simulink, was used. The plant lay-out consists of one anaerobic tank, one intermittent aerobic/anoxic tank and one settler. A proportional controller was used to set the oxygen concentration at 1 mg O_2/ℓ.

To account for the processes taking place in the settler, a virtual anoxic reactor is implemented in the return sludge line of the model of the plant. Indeed, during the passage through the settler, biological processes continue. These processes are not accounted for in the model as it is when a standard point settler model is used. The virtual reactor has a volume corresponding to the residence time of the sludge in the settler.

To calculate the residence time of the sludge in the settler the settling velocity and the return flow rate have been used. With a return flow rate of 81.6 m³/d and a superficial area of 5 m², the underflow velocity

equals 0.68 m/h. Considering an average settling velocity of 2 m/h, an overall velocity of 2.68 m/h is obtained. This value was rounded to 2.5 m/h. As the sludge enters the settler at approximately half its height, a residence time of the sludge in the settler of 15 minutes is obtained. A virtual reactor with a volume resulting in such a residence time was implemented. The reactor volume was set at 0.95 m^3.

3. RESULTS

3.1. *SLUDGE CHARACTERISATION*

From the experiments an uptake rate of 5.4 mg P/g VSS/h was determined. For enhanced cultures of PAOs a value of 55 mg P/g VSS/h is measured (Smolders, 1995), indicating that about 10% of the sludge population can be considered to be PAOs. The ratio between acetate uptake under aerobic conditions and acetate uptake under anoxic conditions revealed a value of 0.40 for the reduction factor. In ASM2d the default value for η_{HNO3} is 0.8. The experiment thus indicates that the number of denitrifying heterotrophic bacteria is probably lower for this plant than the average assumed value. For the reduction factor for denitrification by PAOs a similar value was found.

3.2. *INFLUENT CHARACTERISATION*

The rate constant of the BOD test (k_{BOD} , measured by an external laboratory) resulted in a value of 0.38 d^{-1} for the 24 hour grab samples and a value of 0.28 d^{-1} for the period in which 2 hour grab samples were collected. Using these values, the influent concentrations of the different fractions were calculated. However, the calculated values revealed high concentrations for the particulate inert fraction in the influent X_I. The actual value of this component in the reaction basins depends on the influent concentration via $X_{I,\ reaction\ basin} = X_{I,\ influent}$ *SRT/HRT, supplemented with the formation of inerts in the lysis process. Only taking the $X_{Iinfluent}$ gave a higher value than the observed total sludge concentration of 4.63 g COD/ℓ. The estimation of k_{BOD} has in practise proven to be difficult. At the same time this value has a large influence on the fraction of inerts in the sludge, and thereby on the total sludge production (Brdjanovic et al., submitted). It was decided to start with a value of 0.23 d^{-1} or k_{BOD}, this value is used in the Netherlands as an average value when reliable measurements are lacking. In Table 78 the obtained influent composition is given.

Table 78 Calculated influent concentration

S_{O2}	**4**	g O_2/m^3	X_I	**136.5**	g COD/m^3
S_F	**167.1**	g COD/m^3	X_S	**166.6**	g COD/m^3
S_A	**16.5**	g COD/m^3	X_H	**0**	g COD/m^3
S_{NH4}	**31.3**	g N/m^3	X_{PAO}	**0**	g COD/m^3
S_{NO3}	**0.0**	g N/m^3	X_{PP}	**0**	g P/m^3
S_{PO4}	**4.3**	g P/m^3	X_{PHA}	**0**	g COD/m^3
S_I (from $COD_{effluent}$)	**38**	g COD/m^3	X_{AUT}	**0**	g COD/m^3
S_{ALK}	**5.0**	mol HCO_3/m^3	X_{TSS}	**227.3**	g TSS/m^3
S_{N2}	**0**	g N/m^3	SS	**333.1**	g/m^3

$X_{TSS} = i_{TSSXI} * X_I + i_{TSSXS} * X_S + i_{TSSXBIO} * X_{BIO}$ as suggested as default in ASM2d.
$SS = X_I + X_S + X_H + X_{PAO} + X_{AUT}$, based on a COD/VSS ratio of 1.33 and a VSS/SS ratio of 0.75 as observed for the plant.

3.3. *CALIBRATION PROCEDURE*

Step 1: Using the default parameters of ASM2d

The calibration procedure was started using the default values of ASM2d and using the influent concentrations as given in Table 78. With the standard k_{BOD} value of 0.23 d^{-1} reasonably good match between simulated and measured total solids concentration was obtained. The concentrations for the soluble components, however, did not correspond satisfactorily. In Table 79 the effluent concentrations as measured during an extensive measurement campaign are summarised. In Table 80 the simulated effluent concentrations are given. It can be seen from these tables that ammonium is far too high and nitrate and phosphate are too low if ASM2d default values are used.

Table 79 Effluent measurement data during extensive measurement

Component	Average	Stdv	Unit
BOD	7	1	g BOD/m^3
COD	51	9	g COD/m^3
COD_f	38	4	g COD/m^3
SS	18	9	g /m^3
TKN	3.97	2	g N/m^3
NH_4	1.79	2	g N/m^3
NO_3	4.13	3	g N/m^3
PO_4	2.23	1	g P/m^3

Table 80 Summary of calibration results using ASM2d default values

SNH_4 (mg N/ℓ)			SNO_3 (mg N/ℓ)			SPO_4 (mg N/ℓ)		
low	ok	high	low	ok	high	low	ok	high
		12.99	0.63			1.14		

Step 2: Determining a set of parameters subject to calibration

A range of parameters which are unknown or plant specific can be proposed a priori. These parameters are the most logical to change in the calibration procedure.

The reduction factors for anoxic acetate and phosphate uptake (η_{HNO3} and η_{PNO3}) are considered to be plant and influent dependent. The experimentally obtained values were used as an indication, not as exact values since the experimental conditions in the lab might have been somewhat different than in the real plant.

In the activated sludge models cell lysis is incorporated. This lysis is modelled such that it leads to generation of particulate substrate, which by a hydrolysis process is converted into soluble substrate. The substrate is then converted to biomass again by growth processes. Aside whether this proposed mechanism is correct or not, a good description of the activated sludge process is obtained (van Loosdrecht and Henze, 1999). Lysis of heterotrophic bacteria leads to new heterotrophic bacteria using this "death-regeneration concept". In ASM2d, however, lysis of autotrophic and phosphorus removing bacteria also leads to the creation of new heterotrophic biomass, instead of new respectively autotrophic and phosphorus removing bacteria. Pure and enriched culture experiments used to determine the lysis rates of PAOs and nitrifiers (b_{PAO}, b_{PP}, b_{PHA} and b_{AUT}), will give different values as those which will have to be used in the model of the pilot plant. The value proposed by Wentzel (Wentzel et al., 1989b) or by Smolders (1995) calculated from a maintenance coefficient is indeed lower than suggested for ASM2d.

As such the rates for *lysis of autotrophic and phosphorus removing bacteria* are considered to be subject to changes during calibration procedures.

In the model, the *hydrolysis process* is assumed to be carried out by heterotrophic bacteria. However, the exact mechanisms are totally unknown. Since only heterotrophic bacteria are considered in ASM2d, the hydrolysis rate can be adjusted by changing the not known parameters for η_{LNO3} and $\eta_{fe.}$

Oxygen concentration gradients in the reactors due to non homogeneous mixing will affect the overall observed conversions in the anaerobic and anoxic reactors. Floc internal oxygen gradients will also influence the process in the aerobic and anoxic compartments. These effects are compensated for by the 'affinity' and 'inhibition' constants for oxygen. This means that the parameters K_{HNO3} and K_{NO2} are process dependent and influenced by the type of flocs formed and the mixing intensity in the system. When a pilot plant is compared to a full scale possible changes in these affinity constants have therefore to be envisaged.

The calibration was thus started taking the following parameters into account: η_{LNO3}, η_{fe}, η_{HNO3}, η_{PNO3}, b_{PAO}, b_{PP}, b_{PHA} and b_{AUT} (reduced set). A second calibration sequel was done incorporating K_{HNO3} and K_{NO2} (full set).

Step 3: Parameter adjustments

The calibration procedure is started by adjusting the nitrification kinetics to decrease the ammonia concentration. This can be achieved by decreasing the decay rate for nitrifiers (b_{AUT}). Lowering the saturation coefficient for oxygen (K_{NO2}) will also decrease the ammonia concentration. It was found that adjusting the nitrification kinetics had little impact on the concentrations for nitrate and phosphate.

The next step in the calibration procedure consisted of adjusting the denitrification kinetic parameters. Decreasing the saturation/inhibition coefficient for nitrate (K_{HNO3}) is one way of decreasing the nitrate concentration. In ASM2d denitrification can be performed by heterotrophic as well as phosphorus removing bacteria. So, reducing denitrification, as was necessary in this case, can also be obtained by lowering the available COD amount. This was achieved by decreasing the hydrolysis rate. However, decreasing the available COD also has an impact on the phosphate removal capacity of the plant. As such, starting with adjusting denitrification, necessitated the simultaneous adjustment of the parameters for phosphorus removal. Where initially the effluent phosphate concentration was too low, this shifted while adjusting for the effluent nitrate concentration.

To increase the effluent nitrate concentration the reduction factor for denitrification (η_{HNO3}) was decreased and hydrolysis was lowered by decreasing the reduction factors for hydrolysis (η_{LNO3}, η_{fe}).

Finally, to increase phosphorus removal, the rates for lysis of X_{PAO}, X_{PP} and X_{PHA} (b_{PAO}, b_{PP} and b_{PHA}) were decreased. From own experimental evidence it was found indeed that the reduction factor for anoxic activity of the PAOs should be lowered. The value was changed in approximately the same way as the other reduction parameters in the model.

In Table 81 and Table 82 the initial and final parameters are given along with the resulting effluent concentrations for both calibration exercises.

Table 81 Effluent prediction by the calibrated model

	SNH_4 (mg N/ℓ)			SNO_3 (mg N/ℓ)			SPO_4 (mg N/ℓ)		
	low	ok	high	low	ok	high	low	ok	high
full set		**1.82**			**4.18**			**2.09**	
reduced set		**1.67**			**4.20**			**2.16**	

Table 82 Summary of calibration parameters

	η_{LNO3}	η_{fe}	η_{HNO3}	η_{PNO3}	b_{PAO}[1] (d^{-1})	b_{PP}[1] (d^{-1})	b_{PHA}[1] (d^{-1})	b_{AUT}[1] (d^{-1})	K_{HNO3} ($gN\ m^{-3}$)	K_{NO2} ($gO_2\ m^{-3}$)
ASM2d	0.60	0.40	0.80	0.60	0.20	0.20	0.20	0.15	0.5	0.5
full set	**0.25**	**0.15**	**0.20**	**0.20**	**0.03**	**0.03**	**0.03**	**0.1175**	**0.2**	**0.2**
reduced set	**0.25**	**0.15**	**0.26**	**0.20**	**0.069**	**0.069**	**0.069**	**0.065**	ASM2d	ASM2d

[1] the temperature dependence of these parameters as suggested in ASM2d is not changed, only the constant.

Calibration efforts should preferable result in as few parameters to be changed as possible. Indeed, changing many parameters will probably always lead to a set of values giving satisfactory results. For interpretation reasons and to facilitate the comparison between results of different authors, a selected set of parameter adjustments is to be preferred. As such the reduced set of parameters was chosen for the model to be used in testing upgrading scenarios. In Figure 93 the dynamic concentration profiles in the intermittent reactor and for the effluent are shown for this set of parameters.

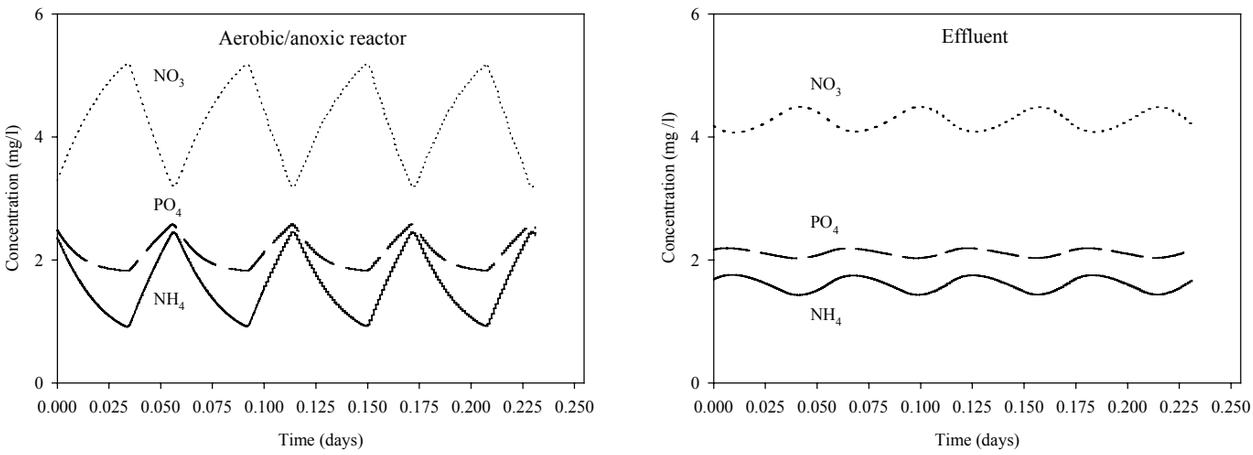

Figure 93 Dynamic concentration profiles in the anoxic/aerobic reactor and in the influent

4. DISCUSSION

The number of parameters involved in the ASM2d along with its non-linear character cause identification problems if a straightforward systems engineering approach is used with a reasonable and cost-effective measuring campaign. For ASM1 with its lower number of reactions taken into account, many authors have investigated through sensitivity analysis which parameters are the most sensitive. Indeed, when a parameter is known to be very difficult to assess through experimental procedure, investing time and money to obtain more reliable values for it is rather senseless when its value has little impact on the simulation results.

From theoretical sensitivity analyses Y_H, b_H, Y_A, and b_A often are reported along with μ_A and η_g (phosphorus removal is not taken into account in ASM1, so parameters connected to these reactions are not encountered) (Weijers en Vanrolleghem, 1997). Calibration efforts using data from real wastewater treatment plants revealed somewhat different results, meaning that the yield coefficient does not appear to be as important, but μ_A, μ_H and K_S remain to be important (Vanrolleghem and Coen, 1995b). It should, however, be remembered that the degree of sensitivity is system specific.

The heuristic calibration method used in this study reveals differences compared to the above mentioned results. It was decided from the beginning to use a 'knowledge based' approach rather than a 'black box' approach as e.g. in most parameter sensitivity studies. It was decided e.g. not to change Y and μ values, considering these values as properly known. A limited set of parameters to be adjusted to obtain satisfactory simulations results was found. It is unclear why in this treatment plant the reduction factors are relatively low. It could be that this was due to some operational conditions, like a relatively high oxygen input in the anaerobic reactor, or a direct feeding of anaerobic effluent in the aerobic reactor. All other parameter changes are in accordance with prior experimental evidence or understanding of the model.

4.1. *IMPORTANCE OF REACTIONS IN SETTLER*

Simulations were performed to evaluate the influence of an increased residence time in the settler. To this end the size of the virtual anoxic basin was increased from 0 to 1.75 m³. From Table 83 it can be seen that the size of this reactor indeed has an important impact on the overall results. Due to the increased size of the reactor less nitrate is returned to the inlet of the anaerobic reactor, leaving more COD available for the phosphorus removing bacteria. Hence, an increased phosphorus removal capacity is obtained. The effluent nitrate concentration decreased as well. For further calibration purposes, measurement of at least nitrate in the return flows could be considered. This is an important factor when pilot plant data are used for evaluating the full scale operation.

Table 83 Dynamic concentration profiles in the anoxic/aerobic reactor and in the influent

	SNH_4 (mg N/ℓ)			SNO_3 (mg N/ℓ)			SPO_4 (mg N/ℓ)		
	low	ok	high	low	ok	high	low	ok	high
Reference (Vol = 0.95 m³)		1.67			4.20			2.16	
Volume = 0 m³	**1.48**					**5.29**			**4.68**
Volume = 0.52 m³		1.57				**4.58**			**3.19**
Volume = 1.75 m³			**1.96**	**3.58**			**0.81**		

4.2. *OXYGEN ENTERING THE PLANT VIA THE INFLUENT*

During calibration the importance of oxygen entering the plant was noticed. Initially it was suggested that no oxygen entered the plant. This working hypothesis was checked in practice and turned out to be invalid: influent concentrations were on average 4 mg O_2/ℓ. This higher oxygen level is due to the aeration action of the influent screw pumps and an aerated grit chamber. In Table 84 the simulation results are given when different inlet oxygen concentrations are considered. From the results it is shown that phosphorus removal increases with decreasing influent oxygen. It is clear that the effect is of such importance that a good measurement of this value is required in a model calibration campaign. Therefore it needs to be evaluated when predictions for the full scale plant are made.

Table 84 Influence of influent oxygen

	SNH_4 (mg N/ℓ)			SNO_3 (mg N/ℓ)			SPO_4 (mg N/ℓ)		
	low	ok	high	low	ok	high	low	ok	high
Reference (O_2 = 4 mg/ℓ)		1.67			4.20			2.16	
Inlet oxygen = 3 mg/ℓ		1.68			4.14		**1.96**		
Inlet oxygen = 2 mg/ℓ		1.70			4.08		**1.75**		
Inlet oxygen = 1 mg/ℓ		1.71			4.02		**1.55**		
Inlet oxygen = 0 mg/ℓ		1.73		**3.96**			**1.37**		

In Figure 94 the influence of influent oxygen and of the virtual anoxic reactor size on the effluent concentrations are shown. Whereas the influence of the size of the virtual reactor is important on NH_4, NO_3 as well as PO_4, the influence of oxygen penetrating the system via the influent is only noticeable on the effluent PO_4 concentration.

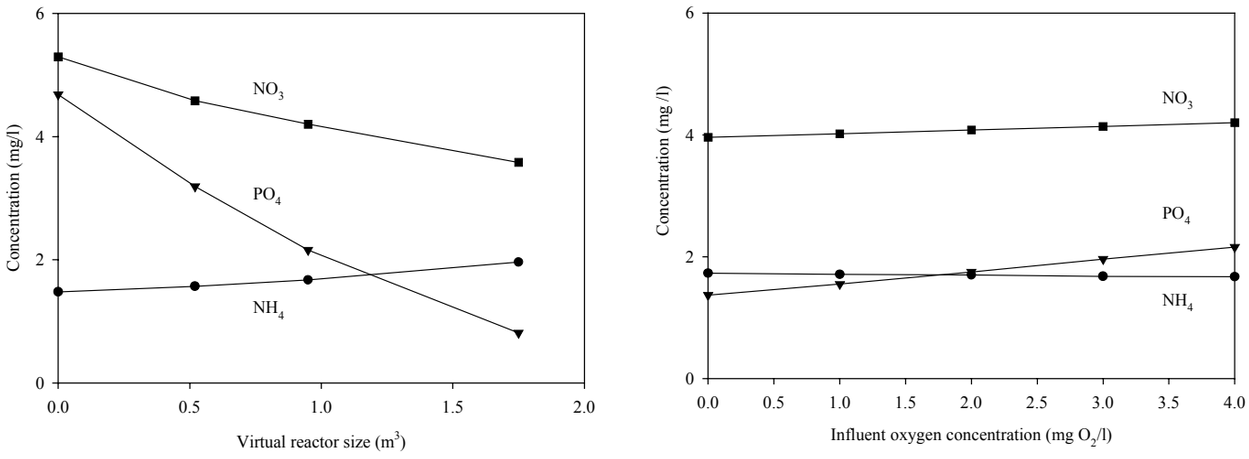

Figure 94 Influence of the virtual reactor size and the inlet oxygen concentration on the effluent concentrations

5. CONCLUSIONS

It was shown that based on understanding of the model, parameter changed could be proposed leading to a limited set of adjustments necessary in the calibration procedure, allowing a relative quick calibration.

There are several aspects of importance when the model of a pilot plant is used for predicting the full scale behaviour. These were in this study: (i) oxygen entering the treatment plant via the influent (ii) reactions occurring in the final clarifier, which may be accounted for by e.g. a virtual settler compartment (iii) mixing intensities which might affect the values of the affinity/inhibition constants for oxygen.

Finally it could be that the relatively low reduction factor for anoxic reactions is due to anomalies introduced by the pilot plant set-up (e.g. relative large oxygen diffusion through the air/water interface in the anoxic phase). In general, higher reduction factors are found for full scale applications.

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CONCLUSIONS and PERSPECTIVES

Conclusions and Perspectives

This research was inspired by increased public awareness to protect and preserve our environment. Pollution prevention is a broad research field, going from air, soil and water pollution as the areas gaining most attention, to light and noise pollution considered as general items affecting every day life of people in general. Because of the disturbing news on increased eutrophication of the European aquatic environment, this study focussed on prevention of water pollution with a special emphasis on phosphorus removal. In practice, both nitrogen and phosphorus are often the limiting factors for biological growth in natural waters. Since cyanobacteria are capable of fixing molecular nitrogen eutrophication prevention only needs a lowering of phosphate availability. Indeed, experiments indicated that no eutrophication occurred when the phosphorus concentration was reduced to 8-10 µg P/ℓ, even when the nitrogen concentration amounted to 4-5 mg N /ℓ. Promising news from South African researchers concerning a possibility to remove phosphorus from wastewaters using an environmentally friendly, biological process in wastewater treatment plants (WWTPs), i.e. by means of Enhanced Biological Phosphorus Removal (EBPR), led to an increased number of international studies on the different aspects of EBPR. Enforcement of more stringent legislation further stimulated the interest to remove phosphorus from wastewater by means of a biological process rather than a chemical precipitation. The investment costs of chemical treatment plants are lower than for biological treatment plants, but the annual operating costs are significantly higher. Moreover, the EBPR process produces a qualitatively rich sludge, effectively improving the sustainability of the biological treatment process. Additionally, only minor process modifications, i.e. adding an anaerobic phase ahead of the aerobic phase, are necessary to upgrade existing treatment plants to achieve EBPR.

The EBPR process can, very basically, be described as a twofold process

(a) In an anaerobic phase cell internal polyphosphate chains (poly-P) are hydrolysed to orthophosphates, a process which delivers the necessary energy for the PolyPhosphate Accumulating Organisms (PAOs) to produce storage polymers as poly-β-hydroxybutyrate (PHB)

(b) In the following anoxic or aerobic phase the internal carbon polymers are utilised creating energy for growth and excess phosphate uptake from the wastewater.

Since the discovery of the EBPR process (first indications in 1959 with a real breakthrough in 1976) an important number of articles has been published by researchers with different fields of expertise. These studies focussed on the underlying biochemical mechanisms, the micro-organisms involved in the process, the external factors influencing the process, the engineering aspects of EBPR, simulation of the process as well as experiences with full-scale applications.

Internationally, with the publication in 1987 of the first Activated Sludge Model (ASM1) by the Task group on Mathematical Modelling for Design and Operation of Activated Sludge Processes for domestic wastewater treatment plants and with the publication of a kinetic model for Biological Phosphorus Removal in 1989 by the South African researchers, an increased number of research topics concerning the different aspects of EBPR have been initiated. Despite all these efforts, research results appeared to be incoherent and engineering firms, constructing wastewater treatment plants, and plant operators remained sceptic. In this study attention was therefore focussed on a thorough modelling of the EBPR process.

Very soon it became apparent from an extensive literature review that modelling the EBPR process is a huge and difficult task. Often, conflicting literature data added to the general confusion. From an engineering point of view it is thought the EBPR process can be modelled taking into account the observable variables, e.g. orthophosphate, COD, ammonia, .., and by understanding of the overall processes occurring, i.e. storage of orthophosphate as polyphosphate, storage of substrate and subsequent

use for growth and energy requirements, … Although this pragmatic engineers' approach is commonly used for chemical processes, it soon became apparent that biological processes occurring in wastewater treatment plants are much more complicated and this pragmatic approach is insufficient to model the biological processes adequately. For modelling purposes it became apparent that a close co-operation between different departments was necessary. Whereas this was certainly not practically feasible from the beginning, this study was finally only possible by bringing together information gained from biochemists, micro-biologists, chemists, bio-engineers (sanitation engineers), mathematicians and process engineers. This study, initiated at the Department of Technical Chemistry and Environmental Technology of the Ghent University (Faculty of Civil Engineering), is the result of a direct collaboration between BIOMATH of the Ghent University (Faculty of Agricultural and Applied Biological Sciences), the Department of Biochemical Engineering of the Delft University of Technology (The Netherlands) and the Department for Microbiology of the Ghent University (Faculty of Sciences). Close contacts with the Laboratory of Environmental Engineering of the Helsinki University of Technology (Finland), with the Department of Chemical Engineering and the Department of Environmental Science and Engineering of the Technical University of Denmark and with the Department of Chemistry, University "La Sapienza" (Italy) were greatly appreciated.

From an engineering point of view it might be disputed whether all this information is necessary. Moreover, it may by stated that it should even be feasible to model the process without this input. However, from the following paragraphs it will become apparent that the above co-operation was essential to obtain the results listed in the following paragraphs. Moreover, bringing the EBPR knowledge in Belgium at a higher level will request an even more intense collaboration between the different experts.

Unravelling the EBPR process, it became apparent that measuring soluble wastewater components was insufficient to model the process. Indeed, the basic outline of the process described above, reveals that internal storage components are involved. Initially, attention was focussed on the analysis of PolyHydroxyAlkanoates (PHAs). As a key storage polymer in PAOs, the analysis of PHA is essential for kinetic modelling of EBPR processes. Therefore, in the early stage of this research an analysis procedure for the determination of PHAs in activated sludge samples was tested and validated by means of a Round Robin test. Aiming at an overall approach, biomass originating from different EBPR treatment units has been used. The results from this research are presented in *Chapter 2*. Each lab used slightly modified methods. It was observed that the use of an internal standard improved the reproducibility of the method, that using dichloro(m)ethane as solvent instead of chloroform improves the GC analysis and that shaking the reaction tubes during heating improves the esterification reaction. It was shown that the standard deviation of measurements in each lab and the reproducibility between the labs was very good. Experimental results obtained by different laboratories using this analysis method can be interpreted in a uniform way. The gas chromatographic method allows for PHV, PH2MB and PH2MV analysis as well.

Later, also glycogen appeared to be a key storage polymer. Indeed, using ^{13}C-NMR analysis techniques, it was proven that glycogen participates in the EBPR process, but modelling efforts need accurate and quantitative analysis of this component. With the publication of the Activated Sludge Model No3 (ASM3), internal storage components and their analysis gained even more importance. However, due to the difficulties for quantitative glycogen measurement, internal storage components are still presented as a lumped term. Extending ASM3 with EBPR processes asks for inclusion of glycogen as a separate component to account properly for the processes occurring and to account for competition between different organisms for the available substrate. Efforts were undertaken to analyse these components together with other research laboratories. In the framework of the COST 682 action a one month research study at the University of Denmark, in co-operation with researchers from the Helsinki University of Technology and Delft University of Technology, indicated that glycogen determination did not deliver promising results, i.e. standard deviations were too high to use the methods tested for modelling purposes. Analysis of glycogen in activated sludge samples was hampered by the cell structure of bacteria, i.e.

membrane linked lipids could not be separated from the cell internal glycogen polymer. Own research efforts to determine glycogen had to be ceased despite the importance of this component. Therefore, conclusive results were not achieved in the scope of this study. In the framework of the COST 624 action, other research groups continued their efforts to improve the glycogen analysis procedure. Recent research results are evaluated as encouraging (Aurola and van Loosdrecht, personal communication).

In *Chapter 3* three of the most cited EBPR configurations were compared in order to obtain information on the best process configuration. It was shown that the SBR configuration was obviously the better one for modelling the EBPR process. Indeed, it allowed the selective growth of PAOs. Moreover it was proven to be extremely robust. Among the continuous processes the UCT process should be preferred for these wastewaters containing high ammonia concentrations. In practice the increased number of recycles causes increased pumping costs and thus increased operating cost of the process. The microbial diversity developed in the continuous installations fed with a complex substrate such as meat extract did not allow for stable process operation. Complete phosphorus removal could not be achieved. Using acetate as sole carbon source, caused production of extracellular polymers with subsequent blockage of tubes. The more recycles are involved the higher the chance for possible blockage, a phenomenon often observed in the lab-scale installations. During stable operating conditions and using acetate as sole carbon source resulted in complete phosphorus removal. From the experimental evidence gained on the SBR installation it was observed that using acetic acid instead of sodium acetate did not result in any phosphorus removing profile. It was observed that special care is necessary to avoid even very low oxygen concentrations in the non-aerated phases. During a short period deterioration of the EBPR activity was observed. No conclusive evidence was gained to explain this phenomenon. A possible explanation could be linked to the growth of Glycogen Accumulating Organisms (GAOs) competing with PAOs for the available substrate with deterioration of EBPR activity as a consequence. Because of the practical relevance of these observations, i.e. full-scale WWTP still exhibit unexpected increases in effluent phosphorus concentrations, more research is necessary concerning growth of GAOs. Analysis of glycogen could have delivered the necessary information to decide if GAOs had outcompeted PAOs during periods of bad EBPR performance. Analysis of the microbial population's composition using different techniques could have revealed an increased dominance of other bacterial strains. This information could have provided extra evidence concerning the dominant PAO strains and the dominant GAO strains. It is therefore recommended to invest in additional microbial structure analysis. Unravelling the biochemical pathways and microbial knowledge on the different communities involved could prevent growth of GAOs and concurrent EBPR deterioration. Thus, although microbiology is often ignored by engineers modelling EBPR processes, the possible competition between different microbial populations already indicates the importance of microbial community structure in activated sludges. Moreover, thorough knowledge of the microbial populations involved will allow to develop WWTP concepts that enhance growth of PAOs and to achieve higher phosphorus removal efficiencies and more stable process operation.

Modelling the anaerobic and aerobic processes in *Chapter 4*, it was observed that using simple models, the experimentally observed profiles could nearly perfectly be simulated. The results were validated using experimental data obtained in batch experiments conducted with sludge from the continuous installations. Comparing results with other papers it was observed that in general researchers are using very similar kinetic expressions. However, kinetic and stoichiometric parameter values often differ. With the publication of ASM2 and later ASM2d, average values are suggested, applicable for domestic sewage treatment plants. As for the results presented in chapter 4, the general conclusion should be that using simple equations as used by others and presented in ASM2(d), it is possible to obtain a parameter set which predicts the observed concentration profiles in a lab-scale SBR installation nearly perfectly. From a research point of view, it was then decided to invest no more time in fine-tuning the determination of these parameters. Indeed, in terms of added value for our modelling purposes it became far more important to investigate external parameters influencing the EBPR process. Gathering this information,

standard models as ASM2 can then be extended taking into account these external influences. Further research was then oriented towards other carbon sources (Chapter 5) and temperature influence (Chapter 6).

As it was suggested from the results of Chapter 3, microbial population structure is important. Isolates were characterised by gaschromatographic analysis of their methylated fatty acids (FAME). These patterns were then compared with the Microbial Identification Software (MIS) databank. Results were presented in *Chapter 4* as well. More than 60% of the isolates could be identified. It was clearly observed that the dominant microbial population did not belong to the genus *Acinetobacter*. Four different groups of organisms were present, each representing about 10% of the total bacterial population. Two groups could not be identified, one group belonged to the genus *Pseudomonas* and the fourth group contained representatives of the lactic acid bacteria. Although the latter group was doubted, it might be an indication that cell lysis and hydrolysis are also important in enhanced culture. Further research could reveal this statement. Modelling efforts could then take these processes into account.

Research results evaluating the influence of external parameters on the EBPR performance indicated that sulphates, after reduction to sulphide, can negatively influence the process. The possibility of this phenomenon to occur in full-scale plants is realistic. In *Chapter 5* experimental evidence is gathered that using a mixture of acetic acid and lactic acid as carbon sources allows for simultaneous removal of phosphorus and sulphate. Sulphate reduction induces growth of filamentous bacteria (*Beggiatoa*) oxidising the produced sulphide. After several operating cycles the filamentous population became dominant and phosphorus removal did not occur anymore. Optimising the process, i.e. when care is taken that the active biomass is retained in the reactor, these results provide a solid basis for concurrent phosphorus and sulphate removal. The research results indicated that growth of filamentous bacteria occurred rapidly but did not become dominant initially. These results were obtained using a SBR configuration probably with a limited number of different microbial organisms. Extrapolating these results to full-scale continuous installations asks for careful interpretation and additional experimental evidence is necessary. The filamentous bacteria, when present in limited amounts, can be entrapped in bigger flocs, thus not causing operating problems. However, operating conditions could already favour partial growth of (other) filamentous bacteria. Additional sulphate reduction could then increase the amount of filamentous bacteria to a level detrimental for the overall performance of the WWTP. The results from Chapter 5 prove that for WWTP treating sewage with increased sulphate concentrations, additional reactions have to be included in standard activated sludge models. Doing this, simulation may help to define operating conditions optimising the symbiosis between the two concerned microbial populations.

Chapter 6 focussed on the effects of temperature on the EBPR performance. It was observed that literature references showed no consensus at all regarding the influence of temperature on the EBPR process. Most studies can be categorised into investigations of the efficiency of EBPR processes under varying temperatures (often dealing with full-scale waste water treatment plants) and into publications focussing on the kinetics of the process (laboratory scale studies primarily). Recently attention is also being paid to the effects of temperature on the stoichiometry of the EBPR process. Especially when looking at EBPR efficiency at different temperatures, results from literature are revealing contrasting observations. Where at first it would seem logical to find higher efficiencies at elevated temperatures (20-37°C), different authors report on improved efficiencies at lower temperatures (5-15°C). Looking at the kinetics of the EBPR process more consistency was observed. In the temperature range from 5°C to around 30°C, increased P-release and/or P-uptake rates with increased temperatures are reported. However, a specific P-uptake higher at 5°C than at 10°C was also observed.

Table 1: *The effect of a decreased temperature on Bio-P removal*

Level	Action	Resulting effect	Effect
Organism	• Lower rates • Lower decay resulting in higher observed yield	• Kinetic limitation • Less limitation due to increased storage capacity	- +
Population	• Acclimation leading to population shift within PAOs • Less nitrification, less nitrate • Less fermentation	• Changed Arrhenius coefficients, changed kinetics • More substrate for PAOs, more storage capacity • Less substrate for PAOs, less storage capacity	+/- + -
Physical-chemical	• Precipitation	• Probability of precipitation decreases • If solubility product is exceeded, precipitate formation quicker	- +

Own experimental results indicated that the stoichiometry of the anaerobic processes was insensitive to long-term temperature changes, whereas the kinetics of the aerobic and anaerobic processes where clearly affected. The aerobic phosphorus uptake rate showed a maximum in the interval between 15 and 20°C. All other anaerobic and aerobic conversion rates increased with increasing temperature. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates. It was shown that a prediction of the temperature effect on a full-scale biological nutrient removal plant is not straight-forward because of the different temperature influences on the sub-processes. A matrix structure was proposed to account for the different influences. From Table 1 it is obvious that taking the temperature in activated sludge models into account is necessary to predict the overall influence of temperature on a full-scale wastewater treatment plant. The simplified Arrhenius equation can be used for this purpose.

The best practices presented in *Chapter 7* propose the design of a limited number of experiments giving rise to a maximal information content. The structural and practical identifiability of the parameters are addressed. The D-criterion was found to give the best performance for the selection of the experimental conditions. Selecting a limited number of experiments limits the intrinsic difficulties the identification of these models is in general faced with, i.e. the model complexity and the time consuming analysis of all components involved. Especially for the calibration of phosphorus removing activated sludge systems, the D-criterion was capable of sensing the subtle difference between initial phosphate concentrations. This research was performed before the temperature dependency of the parameters was fully examined. The OED procedure should be repeated with a model that accounts for the temperature dependency of the process and for which a single set of parameters is estimated taking into account all experiments together. As a general conclusion it needs to be stressed that OED is a method to define experiments to gain information on the parameters of the process at its current state. Indeed, if the system would be subjected continuously to this new, high acetate concentration, the system will adapt to reach a new "steady state".

One of the final aims of all modelling efforts should be to obtain a model applicable for the design of wastewater treatment plants or for upgrading existing plants. Here emphasis should be put on the difference between modelling chemical processes and modelling biological processes. Whereas for the first, extrapolation of lab-scale models to full-scale plants is rather straightforward, biological processes are much more complicated. Many of the parameters if activated sludge process models depend not only on the biomass presented in WWTP but also on the operating conditions of the WWTP. Whereas these problems were already pointed out for WWTP only removing COD and nitrogen, adding EBPR to the overall process makes it even more difficult. In Chapter 4 it was mentioned that a set of parameter values

could be obtained describing the observed profiles excellently. Can these parameters be used for full-scale processes? Which parameters should be altered when calibrating existing activated sludge models? In *Chapter 8* emphasis was therefore put on the practical aspects of efficiently calibrating ASM2d. The study was part of a retrofit exercise. An existing plant, removing only COD and where phosphorus was chemically precipitated, had to be upgraded towards full biological nutrient removal. A pilot plant, built on-site, was operated treating sewage from the full-scale treatment plant. Concentration profiles were recorded and used for calibration of ASM2d. Whereas calibration procedures for carbon and nitrogen removal are encountered in literature, calibration procedures for models including phosphorus removal do not yet exist. With the large number of parameters present in the ASM2d, theoretically it is always possible to find a set of parameters describing the observed data. However, for practical calibration purposes, a procedure, changing only a limited number of parameters, is necessary. The calibration procedure suggested in this chapter was based on an 'expert approach' rather than on a system engineering approach. By only changing a limited number of parameters the model proved well capable of describing the performance of the pilot plant. A parameter set was defined that should be considered for calibration purposes of activated sludge systems including EBPR. Whereas for the maximum specific growth rates, it is suggested in literature that lab-scale experiments, using sludge from the full-scale treatment plant, provide the true values, it was decided not to alter these values in ASM2d. Indeed, values suggested in ASM2d are suggested for WWTP treating domestic sewage. The treatment plant considered in this study, although also partly treating industrial waste, could be considered as an average treatment plant. The heterotrophic community present in the activated sludge is thought to be an average consortium. Therefore, since the maximum growth rate depends on the actual bacteria present, ASM2d standard values were used. For the fractions of bacteria present, i.e. the fraction of denitrifiers (PAOs and non-PAOs), lab-scale experiments were performed to gain additional information. The reduction factors are considered to be plant and influent dependent. Therefore, the experimentally obtained values were used as an indication, not as exact values since the experimental conditions in the lab might have been somewhat different than in the real plant. The lab-scale values were thus considered as an indication whether the ASM2d value should be increased or decreased. Full-scale calibration indeed proved to be necessary to obtain the actual value. The decay rates used in ASM2d should not be considered only to depend on the microbial population. Indeed, in ASM2d, lysis of autotrophic and phosphorus removing bacteria leads to the creation of new heterotrophic biomass, instead of new autotrophic and phosphorus removing bacteria respectively. Decay rates were thus included in the calibration procedure. Last but not least, the half saturation coefficients for nitrate and oxygen were considered during the calibration procedure. Indeed, whereas half-saturation coefficients for pure culture are regarded as pure biological parameters that correlate for the affinity of the biomass for substrates, in full-scale plants, micro-organisms grow in flocs. The size and structure of the flocs, determined by the operating conditions of the WWTP, determine substrate diffusion. Whereas ASM2d contains many more half-saturation coefficients, initially only these two parameters were considered important for the calibration procedure. Calibrating ASM2d, based on the "expert approach" delivered excellent results. Mathematical calibration, i.e. taking into account the most sensitive parameters, might obtain a set of parameters equally describing the experimental results. However, the expert approach allows to determine a set of parameters generally applicable for calibration exercises. With the publication of ASM3 and the soon to be published ASM3 + EBPR models, new calibration procedures will be necessary. Whereas for the reduction factors and the half-saturation coefficients the same ideas as stated above can be applied, the decay rates probably should not be considered as crucial parameters anymore. Indeed, within ASM3 the principle of endogenous respiration decay has been introduced. As for the maximum growth rate, the decay coefficients can then be taken from the default values proposed for the models (still to be published). Additional calibration exercises have to support this statement. If the statement is correct, calibrating ASM3 + EBPR should be a fairly straightforward case.

During the calibration exercise in Chapter 8, it was observed that oxygen entering the treatment plant via the influent has an important influence on the simulated phosphate effluent concentrations. Although the anaerobic phase of the pilot plants appeared to be a true anaerobic phase, i.e. no oxygen was detected, simulations indicated the importance of oxygen in the influent on the overall EBPR performance. In fact, part of the COD present in the influent is immediately consumed by the heterotrophic biomass using the oxygen as electron acceptor. The process is that fast that the available oxygen in the influent is immediately depleted. Moreover, reactions occurring in the final clarifier affected the effluent concentrations and should be accounted for when simulating the overall process for instance by introducing a virtual anoxic reactor in the sludge return line.

Epilogue

During the scope of this study an important number of research projects concerning EBPR were also initiated internationally. Despite these efforts, a general consensus, neither regarding the overall process, nor regarding the specific aspects, could not yet be achieved. Especially the underlying biochemical models are continuously revised based on new information. With the publication of ASM3, a new era has been introduced. Hopefully research efforts will now be concentrated on this model, with the final aim to find a consensus in accepting or rejecting this model for future modelling efforts. The sooner this decision can be made, the sooner researchers can extend the model to make it applicable for a broader range of applications. Calibration efforts can then be focussed on ASM3 rather than older models.

The results obtained in the scope of this study, although obtained in a period where not even ASM2 was the standard, contain many topics directly applicable for future research. With the introduction of ASM3, where the internal storage components for all micro-organisms involved in the activated sludge process play a crucial role, precision and accuracy of measurements of these storage components are even more important than for previous models. In *Chapter 2* a generally acceptable and reliable analysis technique for PHA is established. This analysis technique is directly applicable for analysis of PHA in the diverse microbial populations of ASM3. Additional information gained from glycogen measurement will allow further fine tuning of the model. *Chapters 3 and 4* advocate the use of SBRs for cultivation of an enhanced culture of PAOs. Experimental results obtained with these units are especially suited for modelling purposes. Indeed, the number of influences on the process can be limited, and, moreover, can be controlled. From the literature review and experimental evidence, it was shown that not one bacterial strain dominates the process, but probably different micro-organisms are involved. Microbial identification of these strains is advised. *Chapter 5* identifies the boundary conditions for future simultaneous biological removal of sulphate and phosphorus in activated sludge systems. Where to date the temperature influence in existing models is only taken into account in a very basic way, the results obtained in *Chapter 6* form the basis for proper implementation of the temperature influence in activated sludge processes. *Chapter 7* provides the onset for the identification of an optimal design of experiments to unravel the influence of external parameters on the EBPR process. It was clearly demonstrated that, despite the complex character of the process, that, with a limited set of experiments, sufficient information can be gained to model the different aspects of the EBPR process. The results obtained in *Chapter 8* indicated that standard models as ASM2d are directly applicable for simulation and upgrading of full-scale plants, provided calibration of a limited set of parameters is performed. This chapter emphasises on the importance of common sense before relying fully on mathematical parameter sensitivity methods. Moreover, implementing the simplified reactions proposed in ASM3 it was suggested that even less parameter adaptation might be necessary.

The future aim in modelling WWTPs, particularly encompassing for EBPR processes, should be to develop a unified activated sludge model, accepted and "shared" by the scientific community, preferentially based on metabolic modelling of the processes. This model should be updated in a transparent way and thus be accessible to a broad technical public. However, in reality, because of

confidentiality issues prior to publication, it will remain difficult to stimulate researchers to communicate on their ongoing research. At least, researchers should be urged to publish their results according to a generally adopted methodology.

With all the efforts nowadays invested in research related to wastewater treatment - wastewater characterisation, on-line monitoring, design of wastewater treatment systems, modelling and calibration studies - a centralised non-commercial body, funded on a European or even world-wide scale, steering research and its practical application, could well prove to be a successful investment. Both researchers and end-users should be active participants. The centralised body should solve the paradigm lock by urging end-users, e.g. WWTP designers and policy makers, to properly formulate their needs to the researchers and to sensitise the researchers to produce concrete results on a short term basis.

As such future research, performed at universities and/or within companies, can be divided into two distinct levels, that should interact at the appropriate time. Fundamental research will certainly lead to new insights and new models. However, not all results should necessarily end up in a general unified model. Results relevant for full scale applications can well be introduced in the model as appendix, clearly indicating the boundary limits for their application.

Future applied level of research, focussed on direct applicability, with research results transparent continuously

- Existing research, published and non-published data related to the modelling and calibration of activated sludge models for pilot and full scale installations should be gathered.
- A uniform parameter estimation procedure has to be adopted for this level of research. Preferentially the procedure should be based on a human expert basis (cf. chapter 8) rather than a mathematical one.
- Additionally to the above statement a methodology should be provided to the end-users, indicating clearly the boundary limits of the parameters and alerting the end-users whenever these limits are reached indicating that the model used is not applicable in this particular situation and optimally providing the end-users with similar cases studies and the suggested approach.
- Since one of the major remarks formulated against activated sludge models is the high detail of characterisation necessary to apply these models for full scale WWTPs, attention should be focussed on minimising this level for practical implementation and incorporating flexibility in the treatment designs to account for changing conditions not accounted for using the reduced characterisation detail.
- Research on upgrading existing treatment plants to include for EBPR such as in Belgium (6 plants foreseen for upgrading in the near future, with only 6 plants already in operation or under construction, Aquafin (2000)), allows to gather a maximum input of valuable information.
- For the design of novel treatment configurations, sustainability in the broad sense and in particular related to creating a sustainable phosphorus cycle should be favoured. Re-use of phosphorus in the fertiliser and non-fertiliser phosphate industry provides good opportunities. Direct applications of waste sludge in the agriculture should be further explored.

Future fundamental research, focussed on increasing the basic knowledge to improve the predictive capabilities of unified activated sludge models

- To allow for proper description of EBPR processes, glycogen measurements need to be brought to the same level as the PHA analysis in this study (cf. Chapter 2). Currently the analysis methods are hampered due to interference with cell membrane lipids. Research efforts are important, but a general methodology is lacking. Moreover, methods proposed nowadays might well prove to be

too labour intensive to be generally applicable. Therefore a general, non-labour intensive analysis method is still needed.

- To allow fast introduction of ASM3 like models in practice, basic research needs to be focussed on the internal storage products, i.e. the different pathways possible. Indeed, whereas ASM3 already uses the concept of internal storage prior to carbon utilisation, and whereas the importance of storage polymers in EBPR processes is well proven, too much confusion still exists concerning the different possible pathways.
- Research has mainly been focussed on acetate as carbon source for EBPR processes. With the discovery of possible deterioration of EBPR activity with glucose feeds, the latter has gained importance again in recent studies. However, too little is still known on the possible effects of mixed substrates. Combining lab-scale experiments using mixed substrates and modelling efforts, based on increased knowledge on internal storage products and the different pathways, should reveal the fate of mixed substrates in WWTP.
- The effects of pH and temperature on EBPR have been clearly indicated in literature. This influence should be incorporated in a unified model. More research is still necessary to assess the exact influence on the many parameters of the model.
- With enforced legislation, local wastewater treatment is gaining importance. Increased future application of EBPR configurations for industrial wastewater treatment is expected. The unified model should be assessed for its applicability in these particular cases. Basic research should provide special models, under the form of appendices, i.e. case specific parameter sets and/or additional rate equations, to be applied in these specific cases.
- Future fundamental research efforts could be limited by using the Optimal Experimental Design methodology as presented in Chapter 7.

SAMENVATTING

Samenvatting

De strengere milieuwetgeving wakkert de interesse voor het verwijderen van fosfor uit afvalwater op een biologische eerder dan via klassieke chemische werkwijzen sterk aan. De verwerkingskosten van het kwalitatief rijkere slib van het biologische fosforverwijderingsproces blijken thans immers de hogere investeringskosten t.o.v. een chemische verwijdering te compenseren. Bovendien volstaat het voorschakelen van een anaërobe reactor om een conventionele afvalwaterbehandeling uit te breiden tot het "Enhanced Biological Phosphorus Removal" (EBPR) proces.

Het EBPR proces kan kort omschreven worden als een tweeledig proces :

(a) In een anaërobe fase worden cel-interne fosfaatketens afgebroken tot orthofosfaten en leveren hierbij de noodzakelijke energie voor het opslaan van interne koolstof polymeren zoals poly-β-hydroxybutyraat (PHB) in de PolyFosfaatAccumulerende bacteriën (PAO).

(b) Deze interne polymeren worden in de daaropvolgende aërobe of anoxische fase verbruikt en leveren hierbij de energie die nodig is om het fosfaat uit het afvalwater op te nemen.

Sinds de ontdekking van het EBPR proces (eerste indicatie in 1959 met doorbraak in 1976) zijn tot op vandaag vanuit diverse expertisedomeinen bijzonder veel wetenschappelijke artikels over dit onderwerp verschenen. Deze studies betreffen in hoofdzaak de onderliggende biochemische mechanismen van het EBPR proces, de betrokken polyfosfaataccumulerende organismen, externe factoren die het proces beïnvloeden, de procestechnologische simulaties ervan, evenals de eerste modelleringen en "real scale" ervaringen ermee.

De vaak tegenstrijdige literatuurgegevens werden kritisch geëvalueerd met bijzondere aandacht voor de al dan niet complementariteit van de resultaten die uitgaan van verschillende technisch-wetenschappelijke disciplines. Ondanks het gebruik van het industrie-standaard Activated Sludge Model (ASM, sinds 1987 met upgrades in de jaren '90) voor de modellering van het EBPR proces werd geen consensus bereikt over de kinetiek, de stoichiometrie, de relevante externe parameters, zelfs niet over de nomenclatuur m.b.t. de complexe onderliggende biochemische processen en vooral, er was geen echt "globale aanpak" beschikbaar. Hierdoor werden teveel parameters geselecteerd op mathematische eerder dan op biochemische basis.

Deze doctoraatsstudie draagt ertoe bij om het "overall proces" van biologische fosforverwijdering op een gedegen wetenschappelijke basis te modelleren, zodat op korte termijn de "real scale" toepassingen ervan door de industriële operator zullen kunnen worden "beheerd" uitgaande van een beperkte set aan "bewezen" sturingsparameters.

De verschillende actiepunten van de studie kunnen onder vier hoofdtaken worden gecatalogeerd :

(a) De studie bevat een exhaustief overzicht van de resultaten uit de voorvermelde literatuurstudies, van impact van nitraatintrede in de anaërobe fase van het EBPR proces tot de ervaringen met "real scale" installaties.

(b) De studie evalueert de vroegere studies m.b.t. EBPR in functie van de huidige stand van de techniek.

(c) De studie identificeert de pijnpunten van de vooropgestelde biochemische mechanismen en procesconfiguraties, evalueert ze zelf via enkele gerichte onderzoekingen en geeft de "best practices" op een bevattelijke manier mee.

(d) De studie identificeert de oorzaken van de niet-geslaagde toepassing van de Aktief Slib Modellen (ASM's) voor het EBPR proces en voorziet de noodzakelijke "best practices" voor een geslaagde toepassin, dus o.a. voor de identificatie van de optimale sturingsparameters voor de toekomstige "plant operator".

Voor de delen (a) en (b) wordt expliciet gerefereerd naar de paragrafen 2 en 3 van Hoofdstuk 1, de literatuurstudie. Hierna volgen aandachtspunten en voorbeelden die ressorteren onder de delen (c) en (d), waarvoor tevens wordt verwezen naar paragraaf 4 van Hoofdstuk 1.

In dit onderzoek werd een procedure voor gaschromatografische analyse van PHA's (het voornaamste interne polymeer opgeslagen in de PAO's) in biomassa op punt gesteld en gevalideerd via een ringtest (Round Robin test). Met het oog op een globale aanpak werd biomassa aangewend afkomstig van verschillende afvalwaterbehandelingsinstallaties, zowel met volschalige als met laboratorium EBPR activiteit. De resultaten van dit vergelijkend onderzoek worden besproken in ***Hoofdstuk 2***. Uit het onderzoek is gebleken dat, mits de nodige aandacht voor een aantal parameters, de betreffende geoptimaliseerde analysetechniek betrouwbare resultaten oplevert en dat het aanwenden van dezelfde analysetechnieken en -procedures de enige echte garantie is om resultaten van verschillende laboratoria op een gedegen wetenschappelijke basis met elkaar te "kunnen" vergelijken. De analysetechniek die oorspronkelijk was ontwikkeld in het kader van het EBPR proces, zal ook in de toekomst een belangrijke toegevoegde waarde hebben bij het evalueren van en verder optimaliseren van het derde actief slib model (ASM3) waarbij interne opslag polymeren een sleutelrol spelen. Uit de ringtest zijn een aantal aandachtspunten naar voor gekomen die nauwkeurig dienen te worden opgevolgd tijdens het uitvoeren van de analyse. Zo blijkt dat de biomassa tijdens de veresteringreacties bij 100°C regelmatig dient vermengd te worden, dit om het eventueel samenklitten ervan te verhinderen. Uit de gezamenlijke test bleek nogmaals dat ook voor deze analyse het toevoegen van een inwendige standaard de reproduceerbaarheid ervan beduidend verhoogt. Reeds door vele onderzoekers wordt dit erkend, maar nog steeds wordt het niet universeel toegepast. Als praktische aanbeveling wordt meegegeven om bij elke analysereeks een monster met gekende samenstelling als standaard op te nemen. Het gebruik van gevriesdroogde biomassa laat toe om, enerzijds, de monsters voor langere tijd te bewaren en om, anderzijds, gekende hoeveelheden van deze biomassa af te wegen. Hierdoor worden onnauwkeurigheden bij het nemen van gemengde slibmonsters wordt vermeden. Indien verwacht wordt dat de biomassa een lage concentratie aan PHB bevat, wordt aangeraden om hogere hoeveelheden af te wegen.

In ***Hoofdstuk 3*** worden drie van de in de literatuur meest aangehaalde EBPR procesconfiguraties vergeleken met als oogmerk uitsluitsel te geven over de meest geschikte opstelling voor het modelleren van het EBPR proces. Er wordt aangetoond dat de SBR wel degelijk de meeste garanties op een correcte kinetische modellering van het proces biedt (eenvoudiger opvolging en sturing), terwijl bij de continue installaties de opstelling in de "University of Cape Town" het haalt van de vaak in de literatuur vermelde Phoredox-installatie. In dit laatste type EBPR-systeem bestaat immers een verhoogd risico op nitraat-intrede in de anaërobe fase. Door de bijkomende retourstromen noodzakelijk voor de procesvoering door UCT, zijn de eraan verbonden werkingskosten (pompenergie) evenwel hoger dan voor de Phoredoxinstallatie. Initieel werden de continue installaties gevoed met een gemengde koolstofbron bestaande uit vleesextract. De microbiële diversiteit die daarbij ontstond liet echter niet toe om een stabiele procesvoering te garanderen : volledige fosfaatverwijdering kon niet worden bereikt. In navolging van de SBR installatie, gevoed met een zuivere acetaatvoeding, werden de continue installaties later eveneens gevoed met deze koolstofbron. Volledige fosfaatverwijdering kon reeds op korte termijn worden bereikt, maar de opbouw van extracellulaire koolstofpolymeren veroorzaakte regelmatig verstoppingen van de leidingen. De vorming van deze extracellulaire componenten wordt ook in bepaalde volschalige toepassingen waargenomen, maar heeft daar een geringere invloed op de globale procesvoering vanwege de grotere dimensies van leidingen en pompinstallaties. Bij het opstarten van de SBR installaties is gebleken dat fosfor volledig verwijderd werd tijdens het aërobe deel van het proces. De verwijderingsefficiëntie van fosfor bleek hoger bij het gebruik van natriumacetaat dan bij azijnzuur als koolstofbron. Tijdens het uitvoeren van de verschillende experimenten werd duidelijk dat speciale voorzorgen onontbeerlijk zijn bij de monstername om zuurstofintrede tijdens het anaërobe deel van het proces te vermijden. Een op het eerste zicht onverklaarbare afname in de fosfaatverwijderingscapaciteit

werd tijdelijk waargenomen. Daarbij bleek dat hoge PHB-concentraties in de PAO's geen sluitende garantie vormen voor een netto fosforreductie. De SBR installatie bleek evenwel zeer robust en tijdelijke storingen hadden slecht een kortstondige invloed op de EBPR efficiëntie.

Bij het modelleren van de anaërobe en aërobe reacties in *Hoofdstuk 4* is gebleken dat de verschillende procesparameters nauwkeurig konden worden bepaald met een eenvoudig model. De experimenteel waargenomen profielen konden met het model bijna perfect worden gesimuleerd. De resultaten werden gevalideerd aan de hand van experimentele waarnemingen op slib afkomstig van de continue installaties. De verschillende installaties werden microbiologisch opgevolgd waarbij duidelijk werd aangetoond dat *Acinetobacter*, nog al te dikwijls het meest geciteerde organisme in relatie tot EBPR, zeker niet het dominante organisme was noch in de SBR configuratie, noch in de beide continue installaties. In de SBR configuratie bleken vier verschillende microbiële groepen aanwezig te zijn, die elk 10% uitmaakten van de totale microbiële populatie. Twee groepen konden niet worden geïdentifieerd, één groep behoorde tot het genus *Pseudomonas* en een andere bevatte vertegenwoordigers van de melkzuurbacteriën.

Recente onderzoekingen tonen aan dat sulfaten, na reductie tot sulfide, het EBPR proces beduidend kunnen beïnvloeden. De kans dat een dergelijk fenomeen zich bij "real scale" toepassingen voordoet, is vrij reëel. De eerste aanwijzingen in die zin worden thans ingezameld. In *Hoofdstuk 5* wordt proefondervindelijk aangetoond dat het gebruik van een mengsel van azijnzuur en melkzuur als koolstofbron kan leiden tot de simultane verwijdering van fosfor en sulfaat uit afvalwater. Sulfaatreductie geeft daarbij aanleiding tot groei van filamenteuse bacteriën (*Beggiatoa)* die het geproduceerde sulfide oxideren. Echter, na enkele maanden van werking met de SBR werd de filamenteuse sulfide-oxiderende populatie dermate dominant dat het EBPR proces stilviel. Wordt dit onderzoek verder geoptimaliseerd, m.a.w., wordt ervoor gewaakt dat de EBPR actieve biomassa niet wordt uitgewassen, dan legt deze studie meteen de basis voor de simultane verwijdering van fosfor en sulfaten uit afvalwater.

In *Hoofdstuk 6* wordt aan de parameter "temperatuur" bijzondere aandacht besteed aangezien deze parameter slechts in beperkte mate gestuurd kan worden. De literatuurgegevens m.b.t. de invloed van de temperatuur op het EBPR proces leiden niet tot éénduidige conclusies. Uit de experimenten is gebleken dat de stoichiometrie van de anaërobe processen ongevoelig is aan temperatuursverschillen, dit in tegenstelling met de kinetiek van zowel de aërobe als de anaërobe processen. De opnamesnelheid van fosfaat bleek maximaal tussen 15 en 20 °C, terwijl alle andere reacties versnelden bij verhoging van de temperatuur. Een Arrhenius vergelijking werd opgesteld. In dit hoofdstuk wordt gewezen op het belang van een globale aanpak (stoichiometrie, kinetiek en efficiëntie van het EBPR proces), precieze kennis van de werkingsomstandigheden en het gebruik van een éénduidige definitie voor de gemeten parameter. De tegenstrijdige literatuurreferenties en de bijhorende discussies kunnen dikwijls herleid worden tot een gebrek aan gedetailleerde informatie vermeld in de desbetreffende artikels. De invloed van de temperatuur op het EBPR proces kan niet worden teruggebracht tot een eenvoudige relatie, maar er moet worden rekening gehouden met de invloed op en van de overige processen zoals mogelijke precipitatie, nitrificatie/denitrificatie, etc.

De betreffende "best practices" uit *Hoofdstuk 7* stellen het ontwerp van een beperkt aantal experimenten voor die een maximale informatie-output voor de parameterschatting genereren. De structurele en praktische identificeerbaarheid van de betreffende parameters werd de vereiste aandacht gegeven. Er werd aangetoond dat in het geval van EBPR, de optimale experimentele condities geselecteerd worden aan de hand van het zogenaamde D-criterium. Er bleek duidelijk dat, ondanks de model complexiteit, een beperkte set aan experimentele waarnemingen voldoende informatie oplevert om de invloed van verschillende procesparameters op het globale EBPR proces te identificeren. Specifiek met betrekking tot het EBPR proces bleek het D-criterium in staat om de kleine verschillen in initiële fosfaatconcentraties te detecteren. Dit onderzoek werd uitgevoerd op een ogenblik dat de temperatuurinvloed op het EBPR proces nog niet volledig in kaart was gebracht. De voorgestelde procedure voor het vastleggen van het

optimaal experimenteel ontwerp dient preferentieel opnieuw te worden uitgevoerd, rekening houdend met de in dit werk bepaalde temperatuurinvloed. De aandacht wordt gevestigd, dat de methodologie voor het bepalen van het optimaal experimenteel ontwerp, experimenten vooropstelt om de parameters te bepalen van het proces in zijn huidige stabiele toestand. Dit houdt in dat de vooropgestelde experimentele condities enkele tijdens één cyclus van het batchproces mogen worden vastgelegd. Wordt het systeem gedurende meerdere cyclussen aan de nieuwe experimentele condities onderworpen, dat zal het systeem naar een nieuwe stabiele state toestand evolueren.

In *Hoofdstuk 8* werd via een praktisch voorbeeld een pragmatische, expert gebaseerde aanpak voor een correcte voorspelling van het het volschalig gedrag voorgesteld, waarbij voorvermelde pijnpunten van ASM's minder optreden. Via het testen van verscheidene scenario's op een pilootinstallatie eerder dan op basis van laboratoriumproeven, werden de voor de modellering meest significante parameters afgeleid evenals een maximale ontwerpkennis voor de volschalige installatie. Op basis van logische deductie, empirische gegevens én sensitiviteitsanalyse werd het aantal voor de simulatie echt significante parameters nog verder gereduceerd (expert gebaseerde aanpak). Tot slot bleek dat bij het transponeren van de resultaten van de simulaties op pilootschaal naar de "real scale" installatie een aantal (evidente) verschillen in werkingsomstandigheden vrij eenvoudig in de voorspellingen te kunnen ingepast worden. Door het kunnen selecteren van een beperkte set te bepalen parameters verdwijnen de twee voornaamste pijnpunten voor een correct gebruik van de aktief slib modellen : complexiteit en gebrek aan betrouwbare metingen.

Tijdens het uitvoeren van deze studie werd ook op internationale schaal heel wat bijkomend onderzoek verricht met betrekking tot het EBPR proces. Tot op heden is er geen wetenschappelijke consensus, noch over het globale proces, noch over de specifieke aspecten ervan. Vooral de onderliggende biochemische modellen worden permanent aangepast in functie van de meest recente onderzoeksresultaten. Met de invoering van het ASM3 model waarin interne opslagcomponenten voor alle micro-organismen in het actief slib proces een cruciale rol gaan spelen, worden de precisie en de accuraatheid van de analyseresultaten van deze inwendige opslagcomponenten nog bepalender dan bij vorige modellen. In *Hoofdstuk 2* wordt een algemeen aanvaardbare en betrouwbare analysetechniek voor PHA vastgelegd. Deze analysetechniek is met de invoering van ASM3 direct toepasbaar voor diverse micro-organismen. *Hoofdstukken 3 en 4* pleiten voor het optimaal bedrijven van een SBR met als doel de kweek van een aangerijkt PAO slib voor de kinetische modellering. Hierdoor wordt immers het aantal externe procesparameters beperkt, waarbij de resterende parameters bovendien "stuurbaar" worden. *Hoofdstuk 5* identificeert de randvoorwaarden voor een toekomstige simultane biologische verwijdering van fosfaten en sulfaten in actief slib systemen. Daar waar tot op heden de temperatuurinvloed op het EBPR proces slechts heel rudimentair tussenkomt in de bestaande modellen, legt *Hoofdstuk 6* de basis voor het effectief implementeren van de temperatuurinvloed in de actief slib modellen. *Hoofdstuk 7* vormt de aanzet tot de identificatie van het optimaal experimenteel ontwerp voor de bepaling van de invloed van een beperkte set van externe parameters. Er werd aangetoond dat ondanks het complexe karakter van het proces, met een eenvoudige set aan experimentele waarnemingen, voldoende informatie kan worden bekomen om de verschillende deelaspecten te modelleren. De resultaten uit *Hoofdstuk 8* geven aan dat standaard modellen zoals ASM2d direct toepasbaar zijn voor simulatie en upgrading van volle schaal installaties, mits calibratie van een beperkte set logisch afgeleide parameters. Dit hoofdstuk pleit voor een maximale aflijning van de probleemstelling en een optimale selectie van de oplossingsroutes op basis van gezond verstand het geheel te bekijken alvorens over te gaan naar meer complexe wiskundige programma's.

SUMMARY

Summary

Enforcement of more stringent legislation to protect our environment stimulates the interest to remove phosphorus from wastewater by means of a biological process rather than a chemical precipitation. The investment costs of chemical treatment plants are lower than for biological treatment plants, but the annual operating costs are significantly higher. Moreover, the Enhanced Biological Phosphorus Removal (EBPR) plant produces a qualitatively rich sludge, effectively improving the sustainability of the biological treatment process. Additionally, only minor process modifications, i.e. adding an anaerobic phase ahead of the aerobic phase, are necessary to upgrade existing treatment plants to achieve EBPR.

The EBPR process can be described as a twofold process

(a) In an anaerobic phase cell internal polyphosphate chains are hydrolysed to orthophosphates, a process which delivers the necessary energy for the PolyPhosphate Accumulating Organisms (PAOs) to store internal storage polymers as poly-β-hydroxybutyrate (PHB)

(b) In the following anoxic or aerobic phase the internal carbon polymers are utilised creating energy for growth and excess phosphate uptake from the wastewater.

Since the discovery of the EBPR process (first indications in 1959 with a real breakthrough in 1976) an important number of articles has been published by researchers with different fields of expertise. These studies comprise the underlying biochemical mechanisms, the micro-organisms involved in the process, the external factors focussed on the process, the engineering aspects of EBPR, simulation of the process as well as experiences with full scale applications.

The often conflicting literature data were evaluated critically with special interest for the complementarity of the results emerging from the different disciplines. Despite the development of a standard Activated Sludge Model (ASM1 in 1987 with new releases in the 1990's) no real consensus was reached for modelling the EBPR process. The kinetic and stoichiometric parameters are discussed and even for the nomenclature no global approach could be reached. Moreover, too many parameters have been determined rather on a mathematical basis than a biochemical one.

This Ph.D. study contributes to the modelling of the overall process of biological phosphorus removal on a well founded scientific basis, such that on a short term real scale applications can be managed properly by the industrial operator using a limited number of proven parameters.

A summary of the different aspects dealt with in this study are formulated as:

(a) The study encompasses an exhaustive overview of the different results published in literature, from the impact of possible nitrate introduction in the anaerobic phase to experiences with full scale applications

(b) The study evaluates the early EBPR studies with the current state-of–the–art.

(c) The studies identify the bottlenecks for biochemical mechanisms and process configurations, practically evaluates them and makes suggestions for best practices.

(d) The study identifies the causes for failed EBPR activity.

For parts (a) and (b) the reader is referred to paragraphs 2 and 3 in chapter 1, the literature review. In the following sections the results from published articles dealing with parts (c) and (d) are elaborated on. Paragraph 4 from chapter 1 also deals with these aspects.

In the early stage of this research an analysis procedure for the determination of PHAs (the most important carbon storage polymer in PAOs) in activated sludge samples was tested and validated by means of a Round Robin test. Aiming at an overall approach, the biomass were sampled from different

EBPR treatment units. The results from this research are presented in *Chapter 2*. Each lab used slightly modified methods. It was observed that the use of an internal standard improved the reproducibility of the method, that using dichloro(m)ethane as solvent instead of chloroform improves the GC analysis and that shaking the reaction tubes during heating improves the esterification reaction. It was shown that the standard deviation of measurements in each lab and the reproducibility between the labs was very good. Experimental results obtained by different laboratories using this analysis method can be compared. The gas chromatographic method allows for PHV, PH2MB and PH2MV analysis as well. With the publication of the Activated Sludge Model No3 and the general acceptance that storage polymers are of prime importance for non-PAOs, analysis of internal storage components becomes a prerequisite for modelling the activated sludge processes.

In *Chapter 3* three of the most cited EBPR configurations were compared with the aim to obtain information on the best process configuration. It was shown that the SBR configuration was the better configuration for modelling the EBPR process. Among the continuous processes the UCT process will be better for these wastewaters containing high ammonia concentrations. In practice the increased number of recycles causes increased pumping costs and thus increased operating cost of the process. The microbial diversity developed in the continuous installations fed with a complex substrate as meat extract did not allow for stable process operation. Complete phosphorus removal could not be achieved. Using acetate as sole carbon sources, caused production of extracellular polymers with subsequent blockage of tubes. The more recycles are involved the higher the chance for possible blockage, a phenomenon often observed in lab-scale installations. During stable operating conditions and using acetate as sole carbon source revealed complete phosphorus removal. From the experimental evidence gained on the SBR installation it was observed that using acetic acid instead of sodium acetate did not result in any phosphorus removal profile. It was observed that special care is necessary when performing experiments to avoid even extremely low amounts of oxygen entering the reactor during anaerobic conditions. During a short period deterioration of the EBPR activity was observed. No conclusive evidence was gained to explain this phenomenon. It was observed that during that period very high internal PHB concentrations were observed. Overall, It was proven that the SBR configuration was extremely robust and fast recovery was always observed.

Modelling the anaerobic and aerobic processes in *Chapter 4* it was observed that, the experimentally observed profiles could perfectly be simulated using simple models. The results were validated using experimental data collected in batch experiments with sludge from the continuous installations. Identification of the microbial community present in the SBR system revealed that four different groups of organisms were present, each representing about 10% of the total bacterial population. Two groups could not be identified, one group belonged to the genus *Pseudomonas* and the fourth group contained representatives of the lactic acid bacteria. It was clearly observed that the dominant microbial population did not belong to the genus *Acinetobacter*.

Recent research indicates that sulphates, after reduction to sulphide, can negatively influence the process. The possibility for the phenomena to occur in full scale plants is realistic. In *Chapter 5* experimental evidence is gathered that a mixture of acetic acid and lactic acid as carbon sources allows for simultaneous removal of phosphorus and sulphate. Sulphate reduction induces growth of filamentous bacteria (*Beggiatoa*) that oxidise the produced sulphide. After several operating cycles the filamentous population became dominant and phosphorus removal did not occur anymore. Optimising the process, i.e. by taking care that the active biomass is retained in the reactor, these results provide a solid basis for concurrent phosphorus and sulphate removal.

In *Chapter 6* attention is focussed on the effects of temperature on the EBPR performance. It was observed no consensus existed at all regarding the influence of temperature on the EBPR process. It was shown that the stoichiometry of the anaerobic processes was insensitive to long-term temperature

changes, whereas the kinetics of the aerobic and anaerobic processes where clearly affected. The aerobic phosphorus uptake rate showed a maximum in the interval between 15 and 20°C. All other anaerobic and aerobic conversion rates increased with increasing temperature. A simplified Arrhenius equation was used to describe the effect of temperature on the reaction rates. It was shown that a prediction of the temperature effect on a full scale biological nutrient removal plant is not straightforward because of the different influence of temperature on the sub-processes. All these influences should be accounted for.

The best practices presented in *Chapter 7* focuses on optimal experimental design of experiments (OED), i.e. procedures to design a limited number of experiments giving rise to a maximal information content. The experimenter will select the experimental region and perform one experiment. From then onwards following experimental conditions will be chosen from the experimental region solely based on their predicted information content. The method is applied to calibrate models of phosphorus removing activated sludge systems. The structural and practical identifiability of the parameters are addressed. The D-criterion was found to give the best performance for the selection of the experimental conditions. The selection of a limited number of experiments reduces the intrinsic difficulties the identification of these models is in general faced with, i.e. the model complexity and the lack of reliable measurements.

In *Chapter 8* emphasis was put on the practical aspects of efficiently calibrating ASM2d. The calibration procedure was based on an 'expert approach' rather than on a system engineering approach. With only changing a limited number of parameters the model proved well capable of describing the performance of the pilot plant. New findings are that oxygen entering the treatment plant via the influent has an important influence on the simulated phosphate effluent concentrations. Further, reactions occurring in the final clarifier affect the effluent concentrations and should be accounted for when simulating the overall process by introducing for instance, a virtual anoxic reactor in the sludge return line.

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