Sulfur transformations during two-stage anaerobic digestion and intermediate thermal hydrolysis

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In: Science of The Total Environment, 151247, 2021.

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Abstract

The formation of hydrogen sulfide (H_2S) during anaerobic digestion (AD) imposes constraints on the valorisation of biogas. So far, inorganic sulfur compounds -mainly sulfate - have been considered as the main contributors to H_2S formation, while the contribution of organic sulfur compounds is mostly neglected. This study investigates the fate of organic and inorganic sulfur compounds during two-stage anaerobic digestion with intermediate thermal hydrolysis for treatment of primary and secondary sludge in a WWTP treating domestic wastewater. The results of a seven-week monitoring campaign showed an overall decrease of organic sulfur compounds in both stages of anaerobic digestion. Further fractionation of organic sulfur revealed a high conversion of the particulate organic fraction during the first digestion stage and of the soluble organic fraction during the second digestion stage. The decrease of soluble organic sulfur during the second digestion stage was attributed to the solubilisation and hydrolysis of sulfur-containing organic compounds during thermal hydrolysis. In both digestion stages, more organic sulfur was taken up than particulate inorganic sulfur (metal sulfide) was produced, indicating the formation of other reduced sulfur forms (e.g. H_2S). Further batch experiments confirmed the role of organic sulfur uptake in the formation of H_2S during anaerobic digestion as sulfate reduction only partly explained the total sulfide formed (H_2S in biogas and precipitated FeS). Overall, the

conversion of organic sulfur was demonstrated to play a major role in H_2S formation (and thus the biogas quality), especially in case of thermal hydrolysis pretreatment.

Key words: Anaerobic digestion; Intermediate thermal hydrolysis; H_2S formation; organic sulfur; biological sulfate reduction

1 Introduction

Anaerobic digestion has a crucial part in modern wastewater treatment plants (WWTPs). Its primary role is the stabilisation of waste sludge and the reduction of its volume, by transforming organic matter in the absence of oxygen. In addition, biogas is produced, which has a high calorific value and is considered a renewable energy source (Appels et al., 2008). In WWTPs, biogas is used to generate electricity and heat in combined heat and power (CHP) units, or purified for direct injection into the natural gas grid. However, the inevitable presence of H_2S in biogas is problematic causing severe corrosion of electrical equipment, release of sulfur dioxide (SO_2) in cogeneration and boilers, and entailing other operational, health and safety problems, which necessitates its removal from the biogas. Therefore, a good process understanding of how sulfur is transformed to H_2S is important to be able to design appropriate control strategies to decrease H_2S in biogas to low levels.

There are three main chemical forms of sulfur existing in sludge: organic sulfur, soluble and insoluble sulfide and sulfate (Yang et al., 2016). Sulfur is a building block of amino acids and hence presents in proteins, which are the largest fraction of wastewater organic material (Wilson and Novak, 2009). The total sulfur composition of sludge in the anaerobic digesters of WWTPs in 10 cities in the United States of America was composed predominantly of S-containing amino acids (Sommers et al., 1977). Sulfur species undergo biological, chemical and physical reactions during anaerobic digestion process (Fig. 1). Degradable particulate organic sulfur would be converted to soluble organic sulfur in form of soluble protein and amino acids through hydrolysis and further degraded into H_2S and volatile organic sulfur compounds (Du and Parker, 2013). The reduction of sulfate by sulfate reducing bacteria (SRB) is another reaction leading to formation of H_2S. SRBs use sulfate as electron donner and VFAs and H_2 as their substrates to produce H_2S. Dissolved sulfide produced can be transferred to the gas phase as H_2S,

or remain in the liquid and precipitate as metal sulfide. When microaeration (i.e. dosing small amounts of oxygen or air into the anaerobic digester) is applied to the gas phase, H_2S in the biogas is biologically oxidised to elemental sulfur by sulfide oxidising bacteria (Krayzelova et al., 2014).

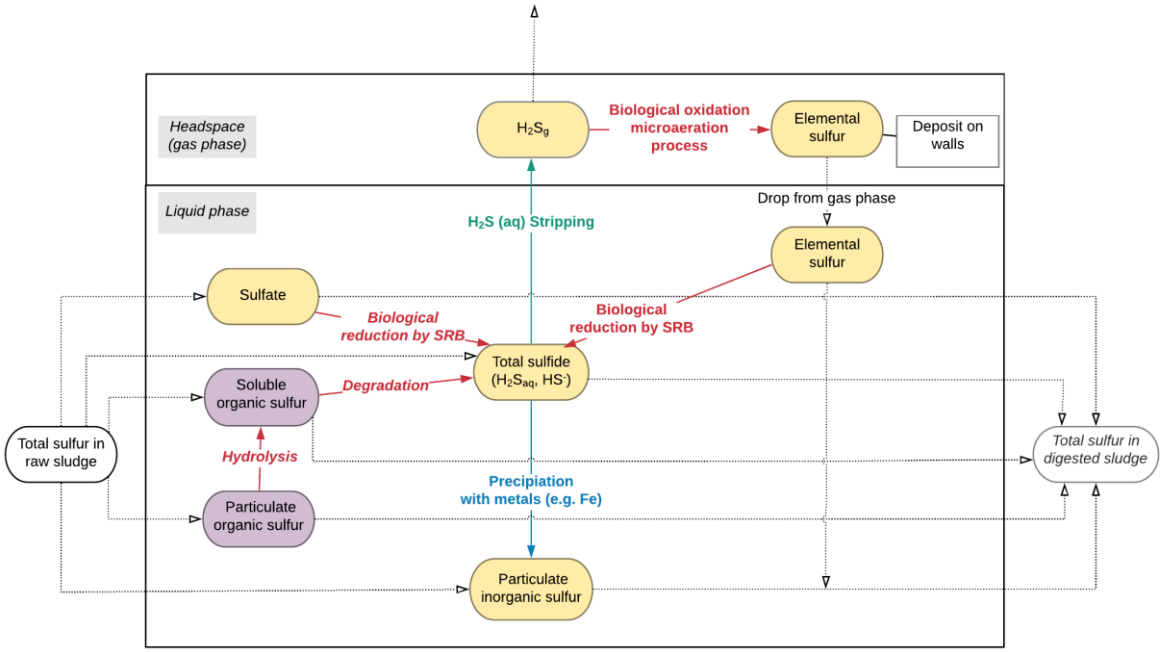


Fig.1: Sulfur species conversions during anaerobic digestion. Biological, chemical and physical reactions are indicated by red, green and blue colours. Organic and inorganic sulfur species are specified by yellow and purple colours. The dashed line shows the distribution of total sulfur in the raw sludge entering anaerobic digestion, and the composition of total sulfur in the digested sludge.

In anaerobic digestion, the formation of H_2S from biological sulfate reduction has been well established. In addition to experimental investigations, the inorganic sulfur reactions have been incorporated into mathematical models of anaerobic digestion process (Barrera et al., 2015; D'Acunto et al., 2011; Fedorovich et al., 2003; Flores-Alsina et al., 2016; Hauduc et al., 2018; Poinapen and Ekama, 2010; Solon et al., 2017). In these studies, the sulfur reactions typically entails microbial kinetics for SRB groups, ionic speciation of sulfate and H_2S and liquid to gas mass transfer of H_2S (Ahmed and Rodríguez, 2018). To include the interaction between sulfur, iron and phosphorus, some models have considered additional reactions such as precipitation of ferrous iron with sulfide as FeS, chemical

reduction of ferric iron to ferrous iron using sulfide as electron donor and release of iron phosphate with sulfide (Flores-Alsina et al., 2016; Hauduc et al., 2018; Solon et al., 2017).

On the other hand, the formation of H_2S originating from degradation of organic sulfur during anaerobic digestion has been given less attention compared to biological sulfate reduction. This could be explained by the fact that majority of experimental and modelling studies focused on sulfate-rich wastewaters (Barrera et al., 2013; Fedorovich et al., 2003; Visser, 1995). On the contrary, sludge originating from municipal WWTPs is composed predominantly of organic sulfur (Sommers et al., 1977). In a recent study, Erdirencelebi and Kucukhemek (2018) observed a strong correlation between the organic solids in primary sludge and H_2S concentration in biogas of full-scale anaerobic digesters over a long period. They suggested that hydrolysis of the proteinaceous matters in primary sludge was the major source of dissolved and gaseous hydrogen sulfide.

The application of sludge pretreatment techniques, as a successful method to increase the biodegradability of sludge, has increased to overcome the main limiting factor of the anaerobic digestion process, i.e. hydrolysis (Appels et al., 2008; Barber, 2016). Thermal hydrolysis can either be applied as a pretreatment step (usually for secondary sludge) or intermediate treatment for the digested sludge (Remy and Diercks, 2016). Recently, the total sulfur mass flow analysis in a municipal WWTP indicated high H_2S mass flows in biogas of anaerobic digester located after thermal hydrolysis (Forouzanmehr et al., 2021) Studying the impact of sludge thermal treatment on the sulfur cycle and formation of H_2S in the subsequent anaerobic digestion is still relatively unexplored in the literature.

At present, there is a lack of quantitative information on the formation of H_2S in full-scale municipal anaerobic digesters. In this study, first the operational performance of a full-scale Digestion – Lysis – Digestion (DLD) process configuration was evaluated. Next, total sulfur content and fractionation of sulfur species in feed and digested sludge of both digestion stages were obtained using long-term collected data. The influence of intermediate thermal hydrolysis on the solubilisation of organic matter and sulfur was especially examined. Furthermore, the contribution of biological sulfate reduction to the formation of H_2S was monitored in lab-scale anaerobic digestion experiments. The latter were also used to analyse the profile of H_2S production and methane yield for the two stages of sludge treatment.

2 Material and methods

2.1 WWTP under study

The municipal WWTP under study has a capacity of 620,000 P.E. and comprises primary treatment and secondary treatment. The secondary treatment is based on an integrated fixed-film activated sludge (IFAS) process for the removal of carbon, nitrogen and phosphorus. During intense rain events, the potential surplus influent wastewater flow is directed towards the rain treatment line which is based on chemically enhanced primary treatment. The raw sludge is composed mainly of primary sludge and secondary sludge and a smaller contribution (~6%) from sludge produced during the rain treatment line. The latter contains iron due to the usage of iron chloride for chemical phosphorus removal in the rain treatment line. The sludge treatment is performed in a Digestion – Lysis – Digestion (DLD) process configuration. The first stage of anaerobic digestion takes place in two parallel units (D1a and D1b). The first-stage digested sludge is then dewatered in a centrifuge and sent to a thermal hydrolysis unit (165°C, 8 bars, 30 minutes). The thermally treated sludge is diluted and cooled by adding some treated WWTP effluent. The subsequent second digestion stage (D2) is performed in a single unit. All three mesophilic digester tanks have the same volume (6100 m^3) and are equipped with air injectors to the headspace for the removal of hydrogen sulfide from the biogas through microaeration. The process flow diagram of the whole plant under study are presented in Supplementary Information (section A1).

2.2 Measurement campaign

2.2.1 Sampling strategy

The operational data for the anaerobic digesters including sludge flow rates, sludge dry solids (DS) and volatile solids (VS) measurements, biogas flow rate and methane concentrations were obtained on a daily basis from historical data between January 2018 to November 2020. These data were used to assess long-term overall performance of the anaerobic digesters in terms of hydraulic retention time (HRT), daily volatile solids load, volatile solids reduction, biogas production and methane yield.

In addition to the routine data, dedicated measurement campaigns were performed. The first measurement campaign (C1) was conducted over seven weeks between May and July 2018 to determine

the various sulfur fractions throughout the sludge treatment line. Grab samples were taken from first stage and second digestion stage. Approximately 1-3 samples per week were taken. Samples were analysed for total sulfur, DS and VS. The second measurement campaign (C2) took place over two weeks in June 2019. Grab samples were taken from the same sampling points as in C1, and were analysed for total sulfur and dry solids. The third measurement campaign (C3) was done on October 22nd 2020. Grab samples were collected from inlet and outlet of first stage digestion, thermal hydrolysis and second digestion stages. Anaerobic digestion batch experiments were performed on these samples (except outlet of the first digester) in order to assess and quantify the methane and the H_2S production (section 2.3). The collected samples were also analysed for total sulfur, sulfate, soluble iron, soluble and total COD and VFAs. The overview of these measurement campaigns including sampling points, type and number of measurements are provided in Supplementary Information (section A2).

2.2.2 Measurement protocols

DS and VS were measured by mass difference after drying (105°C) and calcination (550°C) of the samples. Total sulfur and iron were measured using ICP method. Sulfate was measured by ion chromatography. Reactor digestion method (Hach® method) was used to measure soluble COD and the total COD in C1 and C2, while the analysis of total COD in C3 was done using an internal method based on standard NF U 44-161 and NF ISO 142352, which is described as acid digestion with H_2SO_4 in the presence of $K_2Cr_2O_7$ and the reading by UV at 585nm. VFAs were measured by ion chromatography.

Total sulfur was measured on raw sample, while the soluble and particulate fractions were determined after centrifugation and filtration. Inorganic sulfur was obtained by performing total sulfur analysis on the residuals of calcination of the raw and particulate samples at 550°C. From these measurements other sulfur fractionation was calculated by following equations:

$$\text{Organic sulfur fraction (OSF)} = (S_{Total} - S_{Inorganic})/S_{Total} \quad \text{Eq. (1)}$$

$$\text{Particulate organic sulfur fraction (POSF)} = (S_{Particulate} - S_{Particulate\_Inorganic})/S_{Particulate} \quad \text{Eq. (2)}$$

$$S_{Particulate\_Organic} = POSF \times S_{Particulate} \quad \text{Eq. (3)}$$

$$S_{Soluble\_Organic} = OSF \times S_{Total} - S_{Particulate\_Organic} \quad \text{Eq. (4)}$$

147 $S_{Soluble\_Inorganic} = S_{Soluble} - S_{Soluble\_Organic}$ Eq. (5)

148 In this characterisation, total sulfur is divided into soluble ($S_{Soluble}$) and particulate ($S_{Particulate}$) fractions.
149 Further, each fraction is divided into organic ($S_{Particulate\_Organic}$ and $S_{Soluble\_Organic}$) and inorganic
150 ($S_{Particulate\_Inorganic}$ and $S_{Soluble\_Inorganic}$) fractions. It is assumed that particulate inorganic sulfur consisted of
151 heavy metal sulfides. Particulate organic sulfur was assumed to be sulfur bound in particulate organic
152 matter. Soluble sulfur was assumed to consist of dissolved and colloidal sulfur-containing compounds
153 such as soluble proteins, amino acids, sulfide and sulfate Du and Parker (2013).

154 ## 2.3 Batch tests

155 Anaerobic digestion batch tests were performed on samples taken from inlet and outlet of the anaerobic
156 digesters and thermal hydrolysis process. These tests were carried out in 1-L glass bottles at 35 °C to
157 measure the methane yield and evaluate the contribution of biological sulfate reduction to sulfide
158 production.

159 The tests were performed according to the biochemical methane potential (BMP) guidelines provided
160 by a dedicated international working group (Holliger et al., 2016). Substrate to Inoculum ratio (S/I) was
161 0.5 on a VS basis. The substrates were collected from the inlet of the first stage digester, inlet and outlet
162 of thermal hydrolysis unit, and outlet of second stage digester. Each reactor was flushed with nitrogen
163 for at least 3 minutes to ensure anaerobic conditions. For all samples, the test was performed in
164 triplicates. Three blank tests containing only inoculum were incubated simultaneously to correct for the
165 methane and H_2S produced by the inoculum. The digestion experiments were run for approximately 30
166 days. The biogas production was determined with the manometric method (Amodeo et al., 2020). The
167 biogas composition was measured by gas chromatography using an Agilent 3000 micro gas
168 chromatograph, equipped with a thermal conductivity detector (GC-TCD). Molsieve 5A (14 m length;
169 pore size: 5 Å) and PoraPlotA (10 m length; 0.320mm ID) columns were used as stationary phases for
170 GC-TCD, with Argon and Helium as carrier gases, respectively. The micro-GC was calibrated for H_2,
171 H_2S, CO_2, CH_4, O_2 and N_2. Methane and hydrogen sulfide production were calculated in STP conditions
172 (0°C, 101325 Pa) after correction for moisture. At the end of each batch test, the digested sludge was
173 analysed for total sulfur, soluble sulfur, and sulfate.

The contribution of biological sulfate reduction to formation of H_2S was calculated by the difference between initial and final sulfate concentrations. Produced sulfide in these experiments was the sum of H_2S in biogas and precipitated sulfide as FeS. Precipitated sulfide as FeS was estimated based on the difference between initial and final soluble iron concentrations. It is important to bear in mind that other forms of sulfide (e.g. soluble sulfide remained in effluent and precipitated sulfide with other metals) were not included; therefore, produced sulfide value could be lower than the total sulfide.

3 Results

3.1 Long-term operation of anaerobic digesters

The two parallel first-stage digesters (D1a and D1b) were operated under similar conditions (Table 1): an HRT of 21 days and a VS load of 11011 and 11278 kg VS/day for D1a and D1b, respectively. Their operational performance was also very comparable: a VS reduction of 39% and 41% and a mean methane yield of 279 ± 54 and 316 ± 65 mL CH_4/g VS_{in}, for D1a and D1b, respectively. These methane yield values were in agreement with the value obtained from batch experiments, which was performed on a grab sample of $D1_{feed}$ taken in 2020. As the main operational variables of D1a and D1b indicate similar operating conditions and performance, only one of them – in this case D1a - was considered for the study of sulfur transformations.

Table 1: Summary of overall mean values and standard deviations of operational parameters of the first stage digesters (D1a and D1b) and the second stage digester (D2), obtained from daily measurements between January 2018 to November 2020.

Parameter		First stage		Second stage
		D1a	D1b	D2
HRT	day	21 ± 2	21 ± 3	31 ± 6
Daily VS load	kg VS/day	11011 ± 1708	11278 ± 1774	9610 ± 1798
VS reduction (VSR)[1]	%	39 ± 5	41 ± 5	32 ± 5
Daily biogas production	Nm^3/d	5163 ± 1185	6012 ± 1302	6162 ± 1585
Methane yield	mL CH_4/g VS_{in}	279 ± 54	316 ± 65	379 ± 66
Methane yield of BMP test[2]	mL CH_4/g VS_{in}	310 ± 6	-	226 ± 2
Total volatile solids reduction[3]	%		55 ± 7	

[1] Volatile solid reduction was calculated as $(VS_{in}-VS_{out})/VS_{in}$

[2] BMP tests were performed on grab samples from the feed of D1a and D2 taken in October 2020

[3] VSR_{Total} = (VSR_1st stage + VSR_2nd stage)/VS_{in}_1st stage

The values for Daily VS load, VS reduction and methane yield of second-stage digester (D2) are affected by underestimation of volatile solids content of thermally treated sludge. Previous studies have indicated that VS measurements in thermally treated sludge underestimate the actual volatile solids content of sludge due to evaporation of VFAs, ammonia and other volatile short-chain products at drying temperature (105°C) (Kreuger et al., 2011; Panter, 2008). The mean methane yield of D2 was 379 ± 66 mL CH_4/g VS_{in}, which was higher than that obtained from BMP test 226 ± 2 mL CH_4/g VS_{in}. The latter is similar to the value of 236 mL CH_4/g VS_{in} obtained for anaerobic digestion of thermally treated digested sludge reported by Filer (2019). The overestimation of the biogas flow rate can also be caused by deposits of elemental sulfur on the flowrate sensors generated from microaeration, estimated by operators in the range of ~20%. The latter explanation might be more plausible because the same VS measurement procedure was followed in the plant and for the BMP test.

3.2 Total sulfur content and fractionation in and out anaerobic digesters

The total sulfur concentrations of anaerobic digesters (D1a and D2) are shown in Table 2. During the seven-week measurement campaign C1 (2018), the total sulfur concentrations in the inlet and outlet of D1a were 9.5 ± 2.6 mg S/g DS and 11.6 ± 2.7 mg S/g DS, respectively. The increase of sulfur concentrations (mg S/kg Dry solids) after anaerobic digestion is linked to decrease of organic matter that is converted into biogas in the anaerobic digester, causing the decrease of total solids (Dewil et al., 2006). The few replicates of total sulfur measurements performed in 2019 and 2020 fall within the standard deviation of the measurements performed in 2018. The total sulfur concentration in the digested sludge of D1a was similar to those reported by Fisher et al. (2017). The total sulfur concentration measurements in D2 were relatively similar, with lower standard deviation compared to D1a, which can be attributed to more stable sludge characteristics and sulfur content.

Table 2: Mean and standard deviation of total sulfur concentrations as mg S/g of dry solids (DS) in sludge treatment line. C1, C2 and C3 refer to the measurement campaigns in 2018, 2019 and 2020, respectively. Values in parentheses represent the number of analyses in each period.

	$D1a_{feed}$ mg S/g DS	$D1a_{outlet}$ mg S/g DS	$D2_{feed}$ mg S/g DS	$D2_{outlet}$ mgS/g DS
C1 (2018)	9.5 ± 2.6	11.6 ± 2.7	12.3 ± 1.0	12.4 ± 1.5

	(n=22)	(n=7)	(n=6)	(n=6)
C2 (2019)	7.9 ± 0.5 (n=3)	9.8 (n=2)	10.9 ± 0.3 (n=4)	12.9 ± 0.6 (n=4)
C3 (2020)	7.2 (n=2)	11.7 (n=2)	12.7 (n=2)	12.7 (n=2)

TPS: thickened primary sludge; TWAS: thickened waste activated sludge

The total sulfur mass flow decreased during both first stage (D1a) and second stage (D2) digestion (Table 3). In D1a, total sulfur flows decreased from 139 ± 12 kg S/d in the inlet to 117 ± 13 kg S/d in digested sludge. The total sulfur flow in the biogas (as H_2S) accounted for 6.3 ± 1.2 kg S/d. It is important to note that recorded H_2S in the biogas is smaller to the actual total amount of H_2S emitted because part of H_2S is oxidised to elemental sulfur through microaeration. Based on sulfur mass flows in D1a, the gap in sulfur balance was ~11% (16 kg S/d), which could be attributed to elemental sulfur deposits in the headspace and accumulated sulfur in the reactor. In addition, this value is within the standard deviation of the measurements. Given the complexity of sampling from full-scale anaerobic digesters and system fluctuations during measurement campaigns, the mass balances could be considered as closed within acceptable range. In case of D2, sulfur flow in the feed decreased from 165 ± 12 kg S/d to 149 kg S/d in digested sludge and 9.2 ± 0.8 kg S/d H_2S in biogas, implying a 4% gap in sulfur mass flows.

Table 3: Average and standard deviation of total, particulate, and soluble sulfur mass flow as kg S/d in the inlet, outlet and biogas of D1a and D2. The organic fraction of sulfur in the total sample and particulate fraction is also given for D1a and D2. Values in parentheses represent the number of samples analysed, n.

		D1a$_{feed}$	**D1a$_{outlet}$**	**D1a$_{biogas}$**	**D2$_{feed}$**	**D2$_{outlet}$**	**D2$_{biogas}$**
Total sulfur (S_{Total})	kg S/d	139 ± 12*	117 ± 13	6.3 ± 1.2	165 ± 12	149 ± 9	9.2 ± 0.8
	mg S/L	501 ± 42	422 ± 43	908 ± 107 (ppm)	1146 ± 58	1035 ± 33	1533 ± 68 (ppm)
Particulate sulfur ($S_{Particulate}$)	kg S/d	119 ± 13	107 ± 13		110 ± 8	111 ± 10	
	mg S/L	431 ± 45	385 ± 44		762 ± 42	770 ± 56	
Soluble sulfur ($S_{Soluble}$)	kg S/d	19 ± 2	10 ± 0.4		55 ± 4	38 ± 6	
	mg S/L	70 ± 6	37 ± 1		384 ± 19	266 ± 40	
Fractionation (%)							
$S_{Organic}/S_{Total}$ (OSF)		76 ± 3 (n=22)	68 ± 6 (n=6)		72 ± 3 (n=6)	58 ± 6 (n=5)	
$S_{Particulate\_organic}/S_{Particulate}$ (POSF)		77 ± 4	66 ± 5		60 ± 6	54 ± 6	

	(n=6)	(n=5)	(n=7)	(n=6)

* Mean ± standard error of the mean

The fractionations of soluble and particulate sulfur were different for the inlet of D1a and D2 (Table 3). The majority of sulfur in raw sludge entering D1a was in particulate fraction (~85%), while in thermally treated sludge ($D2_{feed}$) the particulate fraction of sulfur was lower (66%) resulting in elevated soluble fraction (34%). The elevated fraction of soluble sulfur after thermal hydrolysis was also observed in the measurements performed during C2 (41%, see Fig. A3 in SI). In addition to sulfur, elevated soluble fraction in thermally treated sludge was detected for COD (47% and 31% for C2 and C3, respectively, see Fig. A3 in SI). The measurement of organic and inorganic sulfur revealed that total sulfur in raw sludge was mostly in organic fraction (76%). The lowest organic sulfur fraction was observed in the final stage of treatment (i.e. $D2_{digested}$ = 58 ± 6%).Based on sulfur fractionation in Table 3, the fate of soluble/particulate organic/inorganic sulfur in D1a and D2 can be deduced (Fig. 2). From Fig. 2, it is apparent that the mass of total organic sulfur (i.e. sum of soluble and particulate organic sulfur) decreased in both stages of digestion. The decrease in D1a and D2 are equal to 27 kg S/d and 33 kg S/d, respectively. In D1a, the uptake of particulate organic sulfur was significant (21 kg S/d), while in D2 the uptake of soluble organic sulfur was more pronounced (27 kg S/d).

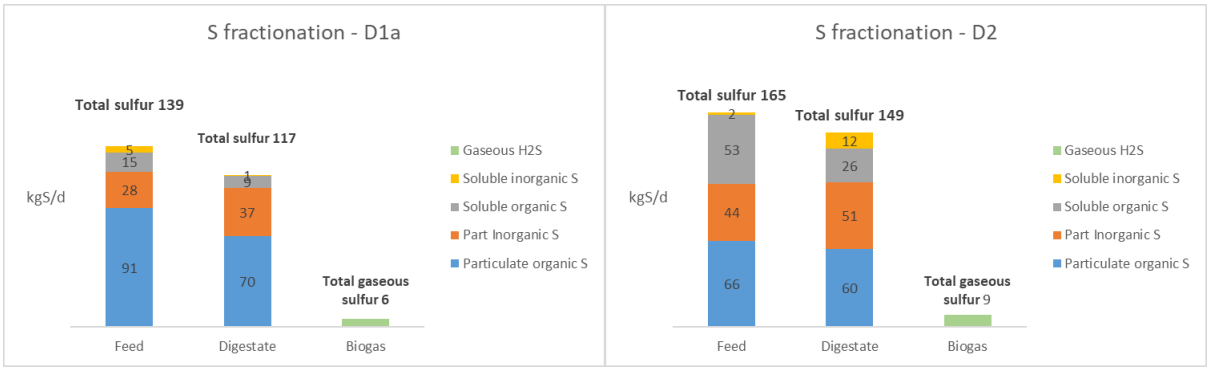


Fig 2: Fate of soluble/particulate organic/inorganic sulfur in D1a and D2, calculated from the fractionations given in Table 3.

Particulate inorganic sulfur increased after D1a and D2 with +9 and +7 kg S/d, respectively. Soluble inorganic sulfur decreased in D1a (–4 kg S /d) but increased in D2 (+10 kg S/d). The evaluation of

soluble inorganic sulfur in anaerobic digestion is complex. First, accounting for the lowest fraction of sulfur, soluble inorganic sulfur lies within the standard deviation of other fractions, thus these data have to be interpreted with caution. Moreover, the behaviour of soluble sulfur species are different in anaerobic digestion. For instance, while sulfate concentration is generally reduced due to the activity of SRBs, the concentration of soluble sulfide might experience increase or decrease in effluent according to several factors such as pH of the reactor and presence of soluble metals.

3.3 Contribution of biological sulfate reduction to H_2S formation

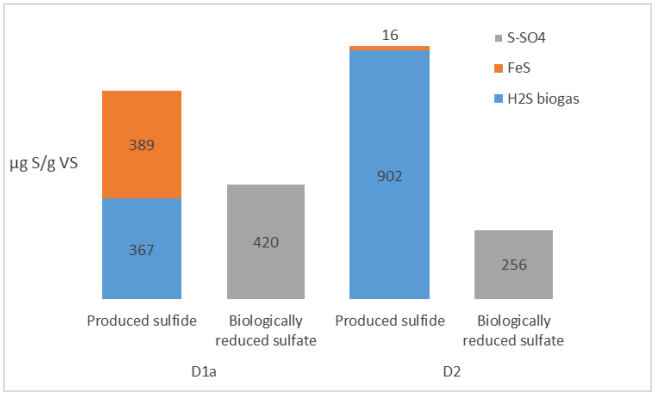
To estimate the contribution of biological sulfate reduction to sulfide production, anaerobic digestion batch experiments were performed on the samples taken from inlet of D1a and D2. The initial and final concentrations of sulfate and soluble iron as well as cumulative concentration of H_2S in biogas are provided in Table 4. Sulfate concentrations were used to estimate the contribution of sulfate reduction to the formation of sulfide. Sulfide formation were the sum of sulfide precipitated with soluble iron as FeS and H_2S emitted to biogas.

Table 4: Concentration for sulfate, soluble iron and gaseous H_2S in batch experiments.

	$D1a_{feed}$	**$D1a_{outlet}$**	**$D1a_{biogas}$**	**$D2_{feed}$**	**$D2_{outlet}$**	**$D2_{biogas}$**
Sulfate (mg S/L)	12.9	1.4		12.5	6.0	
Soluble Fe (mg /L)	21	0.5		1.7	0.5	
Gaseous H_2S in biogas (mL at STP*)			0.434 ± 0.044			1.185 ± 0.102

*Standard temperature and pressure

In anaerobic digestion batch experiments (Fig. 3), sulfide produced from the biological reduction of sulfate accounted for 56% (420 µg S/g VS_{in} /756 µg S/g VS_{in}) and 28% (256 µg S/g VS_{in}/918 µg S/g VS_{in}) of total sulfide in D1a and D2, respectively. This result indicates that sulfate reduction would not be the only mechanism contributing to sulfide production in D1a. The contribution of biological sulfate reduction was much lower for the thermally treated sludge, since sulfate reduction only accounts for 28% of sulfide production. It is important to bear in mind that other forms of sulfide (e.g. soluble sulfide remained in effluent and precipitated sulfide with other metals) were not included; therefore, produced sulfide could be lower than the actual total sulfide.



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280 Fig 3: Comparison between sulfide production (either as H_2S in biogas or precipitated FeS) and
281 biological sulfate reduction, for both first stage (D1a) and second-stage (D2) digestion. Values obtained
282 from batch tests.

283 Based on these results, it was then assessed whether the degradation of sulfur-containing amino acids
284 (cysteine and methionine) could explain the remaining difference between sulfide production and
285 biologically reduced sulfate. Because methionine and cysteine were not analysed in this study, their
286 concentrations in raw sludge and degradation rates in anaerobic digestion that were reported by Chen et
287 al. (2019) were used (See SI section A4). Indeed, these authors have reported the content of hydrolytic
288 cysteine and methionine in raw sludge as 0.46 ± 0.01 mg/g dry sludge and 3.60 ± 0.01 mg/g dry sludge,
289 respectively. In addition, the reported removal rate of cysteine and methionine in lab-scale anaerobic
290 digestion was 34.78 ± 7.87% and 48.06 ± 0.77%, respectively. With these values, the contribution of
291 cysteine and methionine to the formation of sulfide were calculated, as 62 µg S/ g VS_{in} and 542 µg S/ g
292 VS_{in}, respectively, leading to a total potential sulfide formation of 1023 µg S/g VS_{in}. Although the
293 calculated potential sulfide formation is higher than the measured sulfide (756 µg S/g VS_{in}), these values
294 are in the same order of magnitude. The difference could be explained by the fact that we did not measure
295 all sulfide (remaining soluble sulfide and sulfide precipitated with other metals). Nevertheless, this result
296 supports our previous statement that the degradation of organic sulfur is a major mechanism for the
297 formation of H_2S in anaerobic digestion.

3.4 Profile of methane yield and H_2S in biogas of batch anaerobic digestion

H_2S formation and methane yield during anaerobic digestion batch experiments of samples taken from various stages are given in Fig. 4. The yield of H_2S dramatically increased from 153 µg H_2S/g VS_{in} to 921 µL H_2S/ g VS_{in} because of thermal hydrolysis. The increase is partially caused by the sulfate content (20-40 mg S/L) of the treated effluent which was added to the thermally treated sludge for dilution and cooling. Interestingly, the H_2S yield of digested sludge ($D2_{outlet}$) remained noticeable (352 µg H_2S/g VS_{in}). The methane yield also increased considerably from 53 mL CH_4/g VS_{in} in the inlet to 226 mL CH_4/g VS_{in} in thermally treated sludge.

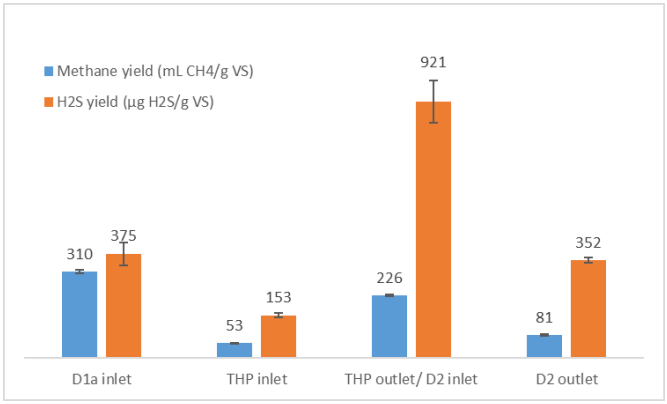

Fig 4: Profile of gaseous methane yield (mL CH_4/g VS) and hydrogen sulfide yield (µg H_2S/ g VS) in the different stages.

4 Discussion

4.1 Operational assessment of two-stage anaerobic digestion

In a two-stage anaerobic digestion with intermediate thermal hydrolysis (also referred to DLD configuration), the first digestion stage should have similar operational and performance behaviours to typical one-stage anaerobic digestion. It was confirmed by the calculated methane yield of D1a using long-term dataset which corresponded to typical methane yields reported for mesophilic anaerobic digestions (Bachmann et al., 2015). On the other hand, the literature on second digestion stage located after thermal hydrolysis is relatively scarce. The performance evaluation of second digestion stage, in particular the parameters related to biogas flow rate and VS measurements (i.e. VS reduction, methane

yield) was complex. A number of authors have reported that the assessment of volatile solids by standard weight loss after drying is often difficult for samples containing a large fraction of soluble organic material (Beall et al., 1998; Kreuger et al., 2011; Panter, 2008), due to the volatilisation of soluble components during solids drying at 105°C that would otherwise be considered volatile solids (e.g. VFA and ammonia). This loss results in an artificially low sludge dry solid content in hydrolysed sludge, hence low volatile matter content. According to Panter (2008), this underestimation is more intensified in case of thermally treated sludge, which can account for up to a loss of 1% DS, i.e. 10% DS measured is actually 11% total solids, and a solution would be DS and VS measurement in the raw cake (i.e. inlet of thermal hydrolysis). In this study, the long-term comparison of dry solids in the inlet and outlet of thermal hydrolysis showed an average of 15 ± 7% lower DS in the thermally treated sludge. When using the measurement of the dry solids in the inlet of thermal hydrolysis, the calculated methane yield of D2 decreased from 379 ± 66 mL CH/ g VS_{in} to 314 ± 58 mL CH_4/ g VS_{in}, and VS reduction increased from 32 ± 5% to 43 ± 6%. Further research is needed to assess the emission of volatile organic compounds in the off-gas stream of the thermal hydrolysis process.

4.2 The effect of intermediate thermal hydrolysis on organic matter solubilisation, methane production, and H_2S production

Intermediate thermal hydrolysis focuses on the solubilisation of hard to digest fraction of sludge during first anaerobic digestion, making them more degradable in the second stage digester (Abu-Orf and Goss, 2012; Shana et al., 2015). The results obtained from full-scale thermal hydrolysis in this study demonstrated the efficiency of this process unit in solubilising organic matter, which is typically measured by the degree of solubilisation determined as soluble COD relative to the total COD. The soluble fraction of COD in thermally treated sludge obtained in this study (47% and 31% for C2 and C3, respectively) was similar to the prior findings in lab-scale experiments (Han et al., 2017; Wett et al., 2009; Xue et al., 2015), although these authors obtained the values for thermal treatment of raw sludge. The biodegradability improvement due to thermal treatment is supported by the results of BMP tests, where a 327% (i.e. from 53 ± 1.6 to 226 ± 1.9 ml CH_4/ g VS_{in}, Fig. 4) increase in methane yield of the digested cake was obtained after thermal hydrolysis.

Similarly, the elevated soluble fraction of sulfur in thermally treated sludge (34% and 41% of total sulfur for C1 and C2, respectively) could be attributed to the solubilisation of protein as the largest fraction of wastewater organic material (Wilson and Novak, 2009), which is also the major contributor to organic sulfur (Du and Parker, 2013). The organic origin of soluble sulfur in thermally treated sludge is also supported by sulfur fractionations given in Table 3. It is also consistent with the findings of Han et al. (2017) that reported minor variation of inorganic sulfur (i.e. sulfate, soluble sulfide, and particulate sulfide) during thermal hydrolysis. Solubilisation of sulfur-bearing organics, likely protein, during thermal hydrolysis resulted in an increase in the biodegradability of organic sulfur, which could be clearly seen by comparing the H_2S production in anaerobic digestion batch experiments of sample taken from thermally treated sludge to that of digested cake entering thermal hydrolysis.

4.3 Influence of organic sulfur on the formation of H_2S

In municipal anaerobic digestion, H_2S is generated from the biological sulfate reduction and organic sulfur degradation. Sulfur containing amino acids (Cysteine and methionine) are the main source of organic sulfur in sludge (Sommers et al., 1977) which are reported to be source of H_2S and other volatile organic sulfur compounds (e.g. methyl mercaptan, dimethyl sulfide and dimethyl disulphide). Cysteine is considered as an organic precursor of only H_2S under anaerobic conditions, while methionine is reported to be degraded through different pathways under different conditions to produce either methyl mercaptan, dimethyl sulfide or H_2S. VOSC concentrations in digesters are reduced by methanogens that mediate the degradation of VOSC to H_2S (Du and Parker, 2012). While a considerable amount of literature has been published on biological sulfate reduction, focusing on sulfate-rich wastewater, the influence of organic sulfur fraction on the formation of H_2S and other volatile organic sulfur compounds has been rarely reported. The results obtained in this study enabled to elucidate the fate of organic sulfur in two-stage anaerobic digestion with intermediate thermal hydrolysis.

Organic sulfur fraction accounted for the majority of total sulfur in mixed primary and secondary sludge entering first anaerobic digestion stage (Table 3). In the first digestion stage the uptake of organic sulfur was 25% (Table 3, calculated as relative difference of organic sulfur in the inlet and outlet of D1a), mostly affected by the particulate organic sulfur. This behaviour could be explained by the low fraction

of soluble organics in raw sludge due to preceding thickening process units. The increase of particulate inorganic sulfur was as expected because of precipitation of sulfide with metals (e.g. Fe^{2+}) and the presence of elemental sulfur in digested sludge. Interestingly, the increase in particulate inorganic sulfur (i.e. metal sulfide) was inferior to organic uptakes in D1a, indicating the role of organic sulfur uptake in production of sulfide, which could be emitted as H_2S or remained in the liquid phase as soluble sulfide given the condition of anaerobic digestion (i.e. pH).

Further investigation of total sulfide formation in anaerobic digestion batch experiments of raw sludge demonstrated the importance of organic sulfur uptake in the formation of sulfide as biological sulfate reduction only accounted for 56% of the total amount of sulfide produced (Fig. 3). The literature on the fate of organic sulfur in anaerobic digestion is relatively scarce, however, from the recent published works, it can be hypothesised that organic sulfur mostly from primary sludge and sulfate contributed to the formation of H_2S in the first digestion stage. According to Du and Parker (2013) the sulfur-containing organic matter in primary sludge are more degradable during anaerobic digestion than that of secondary sludge. The higher degradation of organics in primary sludge is consistent with recent findings that observed a strong correlation between the volatile solids in primary sludge and concentration of H_2S in biogas of full-scale municipal anaerobic digestion (Erdirencelebi and Kucukhemek, 2018).

In the second digestion stage, the role of organic sulfur in the total sulfide production is even more pronounced. Indeed, the uptake of soluble organic sulfur was substantial, with a 50% reduction in mass flows (Table 3). Moreover, anaerobic digestion batch tests of thermally treated sludge showed that biological sulfate reduction only explained 28% of total sulfide formed during the experiment. This result demonstrates the undeniable role of organic sulfur uptake for H_2S formation. It is reported that sulfur-containing organics in secondary sludge present as biomass proteins are not fully degradable in anaerobic digestion due to their large molecular size (Du and Parker, 2013), but become more degradable during thermal hydrolysis due to the disruption of cell walls and even smaller fractions such as amino acids (Remy and Diercks, 2016). Indeed, the majority of sulfur in thickened secondary sludge is in organic form ~90% in this study (data not shown). In our batch tests, the 500% increase of H_2S yield of the samples before and after thermal hydrolysis supported this argument. The data collected

from full-scale digestion and batch experiments are consistent in indicating that the uptake of organic sulfur, especially in the anaerobic digestion of thermally treated sludge (D2) plays an important role in the generation of sulfide.

The above-described fate of organic sulfur needs further investigation to improve speciation of organic sulfur compounds and their transformations in anaerobic digestion by development of measurement techniques. Failing to accurately predict H_2S production in municipal anaerobic digestion causes severe problems including corrosions, lower biogas production, lower biogas profitability due to applying costly H_2S treatment methods (e.g. activated carbons).

4.4 Incorporating sulfur reactions in anaerobic digestion

Several models have been developed to include the transformation of sulfur species during anaerobic digestion process, reported in a number of studies (Barrera et al., 2015; D'Acunto et al., 2011; Fedorovich et al., 2003; Flores-Alsina et al., 2016; Hauduc et al., 2018; Poinapen and Ekama, 2010; Solon et al., 2017). In these models, H_2S is generated solely from biological reduction of sulfate by SRBs, while the contribution of organic sulfur to H_2S is not included. While this assumption could be acceptable for anaerobic digestion of sulfate-rich wastewater, which has been the case for majority of models, the results of this study indicates that biological sulfate reduction leads to underestimation of sulfide production in anaerobic digestion of municipal WWTPs. The result of this study also showed that solubilisation and hydrolysis of organic sulfur during thermal hydrolysis process substantially increased the generation of H_2S during anaerobic digestion process. This effect, to our knowledge, has not yet been addressed into modelling studies. Some software packages such as Sumo© (Dynamita) have incorporated the conversions of organic sulfur during anaerobic digestion, however, their modelling approach, assumptions and kinetics are not well described.

5 Conclusions

The fate of organic and inorganic sulfur compounds during two-stage anaerobic digestion with intermediate thermal hydrolysis was investigated through a seven-week, full-scale measuring campaign, complemented with batch experiments.

- Intermediate thermal hydrolysis effectively improved the solubilisation and thus biodegradability of digested sludge that resulted in significant increase in both methane yield and H_2S production in thermally treated sludge.
- The uptake of organic sulfur during both anaerobic digestion stages was found non-negligible. The converted organic sulfur in the first digester was mostly in particulate form, while converted organic sulfur in the second digester, following thermal hydrolysis, was mostly soluble.
- Sulfate reduction could not explain all sulfide produced during anaerobic digestion. This effect was even more pronounced for thermally treated sludge. Batch digestion experiments indicated that biological sulfate reduction accounted for 56% and 28% of total sulfide (H_2S in biogas and precipitated FeS) produced in the first and second stages of digestion respectively.
- The results dispute sulfate as the single contributor to H_2S formation during anaerobic digestion. H_2S formation from organic sulfur conversion is significant; its share increases through thermal hydrolysis.

6 Acknowledgements

This work was performed within the framework of the EUR H2O'Lyon (ANR-17-EURE-0018) of Université de Lyon (UdL), within the program "Investissements d'Avenir" operated by the French National Research Agency (ANR). The work of Kimberly Solon was supported by the Research Foundation Flanders (FWO) through an ERC runner-up project for Eveline Volcke and by European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie Grant Agreement No. 846316 (WISEFLOW). The authors thank Sylvain Chastrusse, Christophe Renner, Jonathan Coulmin, Vanessa Gromand, Ian Garcia-Fernandez from Veolia for facilitating and carrying out the measurement campaign.

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