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Sulfur transformations in water resource recovery facilities (WRRFs) : Plant-wide assessment with focus on anaerobic digestion

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**Sulfur transformations in water
resource recovery facilities (WRRFs)
-plant-wide assessment with focus
on anaerobic digestion**

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آب را گل نکنیم
شاید این آب روان، می رود پای سپیداری،
تا فروشوید اندوه دلی

Les's not muddy the water
Perhaps this flowing stream runs by the foot of a poplar tree
and eases some heart's grief
...

Ne rendons pas l'eau boueuse :
Peut-être va-t-elle, cette eau vive,
jusqu'au pied d'un peuplier laver la tristesse d'un cœur
...

S. Sepehri

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**Sulfur transformations in water resource
recovery facilities (WRRFs) -
plant-wide assessment with focus on
anaerobic digestion**



INSA



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List of abbreviations

abbreviation	description
AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model N.1
ASM2d	Activated Sludge Model N.1
BMP	Biochemical Methane Potential
BOD	Biological Oxygen Demand
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DLD	Digestion – Lysis – Digestion
DMDS	Dimethyl Disulfide
DMS	Dimethyl Sulfide
DO	Dissolved Oxygen
DS	Dry Solids
EBPR	Enhanced Biological Phosphorus Removal
EPS	Extracellular Polymeric Substances
EPT	Electrochemical PreTreatment
FISH	Fluorescence In Situ Hybridization
HRT	Hydraulic Retention Time
ICP	Inductively Coupled Plasma
IFAS	Integrated Fixed-film Activated Sludge
MM	Methyl Mercaptan
OAV	Odour Activity Value
ODT	Odour Detection Threshold
ORP	Oxidation Reduction Potential
OUR	Oxygen Uptake Rate
PAO	Phosphate Accumulating Bacteria
P.E.	Population Equivalent
PFD	Process Flow Diagram
SANI	Sulfate Reduction Autotrophic denitrification Nitrification Integrated
SCADA	Supervisory Control And Data Acquisition

SOB	Sulfur-Oxidizing Bacteria
SRB	Sulfate-Reducing Bacteria
SRT	Solids Retention Time
THP	Thermal Hydrolysis Process
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus TP
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VOSC	Volatile Organic Sulfur Compounds
VS	Volatile Solids
VSC	Volatile Sulfur Compounds
VSR	Volatile Solid Reduction
WRRF	Water Resource Recovery Facility
WWTP	Wastewater Treatment Plant

List of symbols

abbreviation	characterization	unit
a	Typical value of the measurement	-
b	Expected uncertainty of the measurement	-
c	Individual cost weighting factor of additional measurement	-
I	Identified/reconciled variables	-
K_S	Half saturation	kg COD.m ⁻³
Q	Flow measurement	m ³ .d ⁻¹
MS	Mass flow of total sulfide	kg.d ⁻¹
S	Sulfur flow variables	-
m	Measurement availability	-
v	Standard error of the key variables	-
y	Measured values	-
sD	Relative standard error	-
i_x	Precision improvement defined	-
μ_{max}	Maximum specific growth rate of microorganisms	d ⁻¹
Δ	Ratio of reconciled value to raw estimate	-

Summary

Sulfur is an often-overlooked but key element in water resource recovery facilities (WRRFs). It undergoes biological and physicochemical conversions in the prevailing unit processes, while its emission as hydrogen sulfide (H_2S) may cause odour nuisance, corrosion and have adverse effects on biogas production. Despite its importance, little information is available concerning the fate of sulfur in WRRFs, notably when it comes to full-scale and plant-wide characterisation. This doctoral research work presents advancements in our understanding of characterisation of total sulfur flow and sulfur transformation during wastewater treatment processes of municipal WRRFs.

A comprehensive literature review (Chapter 2) was performed to identify potential sulfur transformations within individual unit processes of municipal WRRFs and to specify the limitations and knowledge gaps. The review included both experimental and modelling studies. Due to the variety of sulfur species and their conversions, most unit processes are affected by the sulfur cycle. The results revealed that inorganic sulfur species and their transformations have been well-established in experimental and modelling studies, while less attention has been given to organic sulfur conversions. In terms of unit processes, most studies have focused on the anaerobic digestion process, while some processes such as primary thickener and emerging treatment processes (e.g. thermal hydrolysis) have been rarely studied. Given the increased application of sludge pretreatment techniques, especially thermal hydrolysis, their influence on the fate of sulfur species during the anaerobic digestion process deserves more investigation. A major limitation of sulfur studies is the lack of full-scale investigations and validation of experiments and models with actual plant data.

A quantification of total sulfur mass flows was therefore performed at a full-scale municipal WRRF (620 000 p.e.) (Chapter 3). Total sulfur mass flows were quantified on a plant-wide level for all water and sludge streams. The gaseous emission of sulfur as H_2S was also included because of its important influence on process operation. Data availability and quality were optimised by experimental design and data reconciliation. The results identified key sulfur flows and how they are affected by the unit processes. In addition, the

collected data enabled the detailed comparison of sulfur flow distribution in unit processes, e.g. primary thickener vs biological sludge thickener, emissive unit processes, two stages of anaerobic digestion and the possible influence of thermal hydrolysis. Total sulfur flows were successfully balanced over individual process treatment units as well as the plant-wide system, confirming that total sulfur is a conservative quantity. The majority of sulfur mass flow from the influent left the plant through the treated effluent (> 95%). Still, but the sulfur discharge through dewatered sludge and gaseous emissions are critical. The two-stage anaerobic digestion with intermediate thermal hydrolysis led to a decreased sulfur content of dewatered sludge (by 36%). Higher (gaseous) H₂S emissions were obtained in the second-stage digester (42% of total emission) than in the first one, suggesting an impact of thermal treatment on the production of H₂S.

Given the lack of quantitative information about sulfur transformations, full-scale data collection was performed in a municipal WRRF to investigate the speciation and variation of sulfur in incoming wastewater and evaluate the fate of these species in different stages of wastewater treatment (Chapter 4). In this way, the possible transformations identified in the literature review were verified with full-scale experimental data. The sulfur speciation in influent wastewater showed that incoming sulfur consists primarily of sulfate. Sulfide was detected in low concentrations, while sulfite and thiosulfate were not detected. Sulfate accounted for the majority of sulfur in presettled wastewater and treated effluent as well. The variation of sulfate and sulfide concentrations was negligible in different secondary treatment zones; for instance, the reduction of sulfate to sulfide during the anaerobic zone was not noticeable. A low sulfide concentration entering the anoxic zone showed that autotrophic denitrification could not be expected. In general, secondary treatment hardly affected the fate of sulfur species. The decrease of sulfate concentration in the primary thickener and high emission of H₂S from this unit revealed that biological reduction of sulfate was significant during the static thickening process. Despite a slight decrease in sulfate concentration, the biological reduction of sulfate was highly influenced by operating conditions of primary

settling. For instance, the analysis of primary sludge showed that its characteristics were highly variable over time, reflected in a wide concentration range of sulfate concentration, oxidation-reduction potential and solid contents. Higher H₂S emission was observed from the primary thickener than the primary settling tank, consistent with the abovementioned sulfate reduction trends in those units.

Anaerobic digestion is crucial for modern wastewater treatment plants (WWTPs) due to biogas production as a renewable energy source. However, the valorisation of biogas is adversely affected by presence of H₂S in elevated concentrations. The two sources of H₂S production during the anaerobic digestion process are biological sulfate reduction and degradation of sulfur-containing organic matter. While the former has been widely studied in the literature, the role of organic sulfur has been less investigated. The fate of organic and inorganic sulfur was investigated during two-stage anaerobic digestion with intermediate thermal hydrolysis through a seven-week, full-scale measuring campaign complemented with batch experiments (Chapter 5). The full-scale measurement revealed that organic sulfur decreased during the anaerobic digestion process. This reduction was mostly observed for particulate organic sulfur during anaerobic digestion of raw sludge and soluble organic sulfur for thermally treated sludge. The fraction of soluble organic sulfur was noticeable after thermal hydrolysis, indicating the solubilisation of sulfur-containing organic matter such as protein during thermal hydrolysis process. 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. H₂S). The batch tests of anaerobic digestion revealed that sulfate reduction does not explain all the sulfide production during anaerobic digestion, especially for thermally treated sludge. Ignorance of organic sulfur contribution to H₂S formation could lead to underestimating sulfide production in anaerobic digestion processes.

In sum, this doctoral research work provides a comprehensive view of total sulfur distribution in WRRFs and the key transformation of sulfur species within unit processes.

Sommaire

Le soufre est un élément clé, souvent négligé, dans les stations d'épurations des eaux usées (STEP). Il subit des conversions biologiques et physico-chimiques dans les procédés unitaires dominants, tandis que son émission sous forme de sulfure d'hydrogène (H_2S) peut provoquer des nuisances olfactives, de la corrosion et avoir des effets négatifs sur la production de biogaz. Malgré son importance, peu d'informations sont disponibles sur le devenir du soufre dans les STEPs, notamment en ce qui concerne la caractérisation à grande échelle et à l'échelle de l'usine. Ce travail de recherche doctorale présente les avancées dans notre compréhension de la caractérisation du flux de soufre total et de la transformation du soufre pendant les processus de traitement des eaux usées des STEP.

Une revue de la littérature (chapitre 2) a été réalisée afin d'identifier les transformations potentielles du soufre dans les processus unitaires individuels des STEP municipaux et de préciser les limitations et les lacunes dans les connaissances. L'analyse a porté sur des études expérimentales et de modélisation. En raison de la variété des espèces de soufre et de leurs transformations, la plupart des procédés unitaires sont affectés par le cycle du soufre. Les résultats ont révélé que les espèces de soufre inorganiques et leurs transformations ont été bien établies dans les études expérimentales et de modélisation, tandis que moins d'attention a été accordée aux conversions du soufre organique. En termes de procédés unitaires, la plupart des études se sont concentrées sur le processus de digestion anaérobie, tandis que certains processus tels que l'épaississeur primaire et les processus de traitement émergents (par exemple, l'hydrolyse thermique) ont été rarement étudiés. Étant donné l'application croissante des techniques de prétraitement des boues, en particulier l'hydrolyse thermique, leur influence sur le devenir des espèces de soufre au cours du processus de digestion anaérobie mérite d'être étudiée plus avant. L'une des principales limites des études sur le soufre est le manque de recherches à grande échelle et de validation des expériences et des modèles avec les données réelles de les STEP.

Une quantification des flux massiques de soufre total a donc été réalisée dans une station d'épuration municipale à grande échelle (620 000 EH) (chapitre 3).

Les flux massiques de soufre total ont été quantifiés à l'échelle de la station pour tous les flux d'eau et de boues. L'émission gazeuse de soufre sous forme de H_2S a également été incluse en raison de son influence importante sur le fonctionnement du processus. La disponibilité et la qualité des données ont été optimisées par la conception expérimentale et la réconciliation des données. Les résultats ont permis d'identifier les principaux flux de soufre et la manière dont ils sont affectés par les procédés unitaires. En outre, les données collectées ont permis de comparer en détail la distribution des flux de soufre dans les processus unitaires, par exemple l'épaississeur primaire par rapport à l'épaississeur de boues biologiques, les processus unitaires émissifs, les deux étapes de la digestion anaérobie et l'influence possible de l'hydrolyse thermique. Les flux de soufre total ont été équilibrés avec succès sur les unités de traitement individuelles ainsi que sur le système de l'usine, confirmant que le soufre total est une quantité conservatrice. La majorité du flux massique de soufre provenant de l'influent a quitté l'usine par l'effluent traité (> 95%). Néanmoins, le rejet de soufre par les boues déshydratées et les émissions gazeuses sont critiques. La digestion anaérobie en deux étapes avec hydrolyse thermique intermédiaire a permis de réduire la teneur en soufre des boues déshydratées (de 36 %). Les émissions (gazeuses) de H_2S ont été plus élevées dans le digesteur de deuxième étape (42% de l'émission totale) que dans le premier, ce qui suggère un impact du traitement thermique sur la production de H_2S .

Étant donné le manque d'informations quantitatives sur les transformations du soufre, une collecte de données a été effectuée dans une STEP pour étudier la spéciation et la variation du soufre dans les eaux usées entrantes et évaluer le devenir de ces espèces à différentes étapes du traitement des eaux usées (chapitre 4). De cette façon, les transformations possibles identifiées dans la revue de la littérature ont été vérifiées avec des données expérimentales. La spéciation du soufre dans les eaux usées entrantes a montré que le soufre entrant est principalement constitué de sulfate. Le sulfure a été détecté en faibles concentrations, tandis que le sulfite et le thiosulfate n'ont pas été détectés. Le sulfate représentait la majorité du soufre dans les eaux usées

décantées primaire et dans l'effluent traité également. La variation des concentrations de sulfate et de sulfure était négligeable dans les différentes zones de traitement secondaire ; par exemple, la réduction du sulfate en sulfure dans la zone anaérobie n'était pas perceptible. Une faible concentration de sulfure entrant dans la zone anoxique a montré qu'on ne pouvait pas s'attendre à une dénitrification autotrophe. En général, le traitement secondaire a à peine affecté le devenir des espèces de soufre. La diminution de la concentration de sulfate dans l'épaisseur primaire et la forte émission de H₂S de cette unité ont révélé que la réduction biologique du sulfate était importante pendant le processus d'épaississement statique. Malgré une légère diminution de la concentration en sulfate, la réduction biologique du sulfate a été fortement influencée par les conditions de fonctionnement de la décantation primaire. Ainsi, l'analyse des boues primaires a montré que leurs caractéristiques étaient très variables dans le temps, se traduisant par une large gamme de concentration de sulfate, de potentiel d'oxydo-réduction et de teneur en matières solides. Une émission de H₂S plus élevée a été observée à partir de l'épaisseur primaire que du décanteur primaire, ce qui est cohérent avec les tendances de réduction des sulfates mentionnées ci-dessus dans ces unités.

La digestion anaérobie est cruciale pour les stations d'épuration des eaux usées (STEP) modernes en raison de la production de biogaz comme source d'énergie renouvelable. Cependant, la valorisation du biogaz est affectée par la présence de H₂S à des concentrations élevées. Les deux sources de production de H₂S pendant le processus de digestion anaérobie sont la réduction biologique des sulfates et la dégradation de la matière organique contenant du soufre. Si la première a été largement étudiée dans la littérature, le rôle du soufre organique a été moins étudié. Le devenir du soufre organique et inorganique a été étudié au cours de la digestion anaérobie en deux étapes avec hydrolyse thermique intermédiaire par une campagne de mesures en grandeur réelle de sept semaines complétée par des expériences en lots (chapitre 5). Les mesures en grandeur réelle ont révélé que le soufre organique diminuait pendant le processus de digestion anaérobie. Cette réduction a été principalement observée pour le soufre organique particulaire pendant la digestion anaérobie

des boues brutes et le soufre organique soluble pour les boues traitées thermiquement. La fraction de soufre organique soluble était notable après l'hydrolyse thermique, ce qui indique la solubilisation de la matière organique contenant du soufre, comme les protéines, pendant le processus d'hydrolyse thermique. Dans les deux étapes de la digestion, il y avait plus de soufre organique absorbé que de soufre inorganique particulaire (sulfure métallique) produit, indiquant la formation d'autres formes de soufre réduit (par exemple H_2S). Les essais en lots de la digestion anaérobie ont révélé que la réduction des sulfates n'explique pas toute la production de sulfure pendant la digestion anaérobie, surtout pour les boues traitées thermiquement. L'ignorance de la contribution du soufre organique à la formation de H_2S pourrait conduire à une sous-estimation de la production de sulfure dans les processus de digestion anaérobie.

En résumé, ce travail de recherche doctorale fournit une vue d'ensemble de la distribution du soufre total dans les WRRF et de la transformation clé des espèces de soufre dans les processus unitaires.

Samenvatting

Zwavel is een vaak over het hoofd gezien, maar essentieel element in waterterugwinningsinstallaties (WRRF's). Het ondergaat biologische en fysisch-chemische omzettingen in de gangbare eenheidsprocessen, terwijl de uitstoot ervan in de vorm van waterstofsulfide (H_2S) stankoverlast en corrosie kan veroorzaken en nadelige gevolgen kan hebben voor de biogasproductie. Ondanks het belang ervan is er weinig informatie beschikbaar over het lot van zwavel in WRRF's, met name wat betreft karakterisering op volledige schaal en van de hele installatie. Dit doctoraatsonderzoek biedt een beter inzicht in de karakterisering van de totale zwavelstroom en de zwaveltransformatie tijdens afvalwaterbehandelingsprocessen van gemeentelijke WRRF's.

Er werd een uitgebreid literatuuronderzoek (hoofdstuk 2) uitgevoerd om de potentiële zwaveltransformaties binnen afzonderlijke eenheidsprocessen van gemeentelijke WRRF's te identificeren en de beperkingen en kennishiaten te specificeren. Het onderzoek omvatte zowel experimentele als modelstudies. Door de verscheidenheid aan zwavelsoorten en hun omzettingen worden de meeste processen beïnvloed door de zwavelcyclus. Uit de resultaten bleek dat anorganische zwavelsoorten en hun omzettingen goed zijn onderzocht in experimentele en modelstudies, maar er werd wel minder aandacht besteed aan organische zwavelomzettingen. Wat de eenheidsprocessen betreft, hebben de meeste studies zich geconcentreerd op het anaerobe vergistingsproces, terwijl sommige processen zoals primaire indickers en opkomende behandelingsprocessen (bv. thermische hydrolyse) zelden zijn bestudeerd. Gezien de toegenomen toepassing van voorbehandelingstechnieken voor slib, met name thermische hydrolyse, verdient hun invloed op het lot van zwavelsoorten tijdens het anaërobe vergistingsproces meer onderzoek. Een belangrijke beperking van het zwavelonderzoek is het gebrek aan onderzoek op ware schaal en aan validering van experimenten en modellen met feitelijke installatiegegevens.

Daarom werd een kwantificering van de totale zwavelmassastromen uitgevoerd in een gemeentelijke WRRF op ware grootte (620 000 i.e.) (hoofdstuk 3). De totale zwavelmassastromen werden gekwantificeerd op het niveau van de gehele installatie voor alle water- en slibstromen. De gasvormige

emissie van zwavel in de vorm van H_2S werd eveneens in rekening genomen vanwege de belangrijke invloed ervan op de werking van het proces. De beschikbaarheid en kwaliteit van de gegevens werden geoptimaliseerd via experimental design en de afstemming van de gegevens. De resultaten lieten identificatie toe van de belangrijkste zwavelstromen en hoe deze worden beïnvloed door de eenheidsprocessen. Bovendien maakten de verzamelde gegevens een gedetailleerde vergelijking mogelijk van de verdeling van de zwavelstromen in de verschillende processen, bijv. primaire indikkers vs. biologische slibindikkers, emitterende processen, twee fasen van anaerobe vergisting en de mogelijke invloed van thermische hydrolyse. De totale zwavelstromen werden met succes evenwichtig verdeeld over de afzonderlijke procesbehandelingsinstallaties en het systeem voor de gehele installatie, hetgeen bevestigt dat de totale zwavel een conservatieve hoeveelheid is. Het grootste deel van de zwavelmassastroom van het influent verliet de installatie via het behandelde effluent ($> 95\%$). Toch zijn de zwaveluitstoot via het ontwaterde slib en de gasvormige emissies van cruciaal belang. De anaerobe gisting in twee fasen met tussentijdse thermische hydrolyse leidde tot een lager zwavelgehalte van het ontwaterde slib (met 36%). Hogere (gasvormige) H_2S -emissies werden verkregen in de tweede vergistingsfase (42% van de totale emissie) dan in de eerste, wat wijst op een effect van de thermische behandeling op de productie van H_2S .

Gezien het gebrek aan kwantitatieve informatie over zwaveltransformaties werden gegevens op ware schaal verzameld in een gemeentelijke WRRF om de speciatie en variatie van zwavel in inkomend afvalwater te onderzoeken en de lotgevallen van deze soorten in verschillende stadia van afvalwaterbehandeling te evalueren (hoofdstuk 4). Op deze manier werden de mogelijke transformaties die in het literatuuronderzoek waren geïdentificeerd, geverifieerd met experimentele gegevens op ware schaal. De zwavelspeciatie in influent afvalwater toonde aan dat binnenkomende zwavel voornamelijk bestaat uit sulfaat. Sulfide werd in lage concentraties gedetecteerd, terwijl sulfiet en thiosulfaat niet werden gedetecteerd. Het grootste deel van de zwavel in het voorbehandelde afvalwater en het gezuiverde effluent bestond uit

sulfaat. De variatie van de sulfaat- en sulfideconcentraties was verwaarloosbaar in de verschillende secundaire behandelingszones; zo was de reductie van sulfaat tot sulfide in de anaërobe zone niet merkbaar. Een lage sulfideconcentratie in de anoxische zone toonde aan dat autotrofe denitrificatie niet kon worden verwacht. In het algemeen had de secundaire zuivering nauwelijks invloed op het lot van zwavelsoorten. De daling van de sulfaatconcentratie in de primaire indikker en de hoge emissie van H_2S uit deze eenheid toonden aan dat de biologische reductie van sulfaat aanzienlijk was tijdens het statische indikkingsproces. Ondanks een lichte daling van de sulfaatconcentratie werd de biologische reductie van sulfaat sterk beïnvloed door de bedrijfsomstandigheden van de primaire bezinking. Zo bleek uit de analyse van het primaire slib dat de kenmerken ervan sterk varieerden in de tijd, wat tot uiting kwam in een breed concentratiebereik van sulfaatconcentratie, oxidatiereductiepotentiaal en vaste inhoud. Een hogere H_2S -emissie werd waargenomen in de primaire indikers dan in de primaire bezinktanks, hetgeen in overeenstemming is met de bovenvermelde tendensen inzake sulfaatreductie in die eenheden.

Anaërobe vergisting is van cruciaal belang voor moderne afvalwaterzuiveringsinstallaties (RWZI's) omwille van de biogasproductie als hernieuwbare energiebron. De valorisatie van biogas wordt echter ongunstig beïnvloed door de aanwezigheid van H_2S in hoge concentraties. De twee bronnen van H_2S -productie tijdens het anaerobe vergistingsproces zijn de biologische sulfaatreductie en de afbraak van zwavelhoudend organisch materiaal. Terwijl de eerste bron in de literatuur uitgebreid is bestudeerd, is de rol van organische zwavel minder onderzocht. Het lot van organische en anorganische zwavel werd onderzocht tijdens anaerobe vergisting in twee fasen met tussentijdse thermische hydrolyse door middel van een zeven weken durende, grootschalige meetcampagne, aangevuld met batch experimenten (Hoofdstuk 5). Uit de grootschalige metingen bleek dat de organische zwavel tijdens het anaerobe vergistingsproces afnam. Deze afname werd vooral waargenomen voor organische zwaveldeeltjes tijdens de anaerobe gisting van ruw slib en voor oplosbare organische zwavel voor thermisch behandeld slib.

De fractie oplosbare organische zwavel was merkbaar na thermische hydrolyse, wat wijst op de solubilisering van zwavelhoudend organisch materiaal zoals eiwitten tijdens het thermische hydrolyseproces. In beide vergistingsfasen werd meer organische zwavel opgenomen dan er anorganische zwaveldeeltjes (metaalsulfide) werden geproduceerd, wat wijst op de vorming van andere gereduceerde zwavelvormen (b.v. H_2S). Uit de batchproeven van anaërobe gisting bleek dat sulfaatreductie niet de volledige sulfideproductie tijdens anaërobe gisting verklaart, vooral voor thermisch behandeld slib. Het negeren van de bijdrage van organische zwavel aan H_2S -vorming kan leiden tot een onderschatting van de sulfideproductie bij anaerobe vergistingsprocessen.

Kortom, dit doctoraal onderzoek geeft een uitgebreid beeld van de totale zwaveldistributie in WRRF's en de belangrijkste omzettingen van zwavelsoorten binnen eenheidsprocessen.

1

Introduction

1.1 Research context

Wastewater treatment plants are essential infrastructure for protecting human health and the environment from wastewater produced through domestic and industrial activities. Traditionally, the goal of wastewater treatment plants (WWTPs) was to guarantee a certain effluent quality in terms of organic matter and later nutrients (phosphorus and nitrogen). These facilities need to deal with increasingly stringent effluent requirements to protect vulnerable receiving water bodies and drinking water sources. Moreover, they face new sustainability and societal challenges, including energy efficiency and carbon footprint reduction resource recovery, and climate change. Nowadays, wastewater is seen as a valuable resource rather than a pollution source, and consequently WWTPs are regarded as water resource recovery facilities (WRRFs), reflecting the value of water, nutrients, energy and other resources. For example, phosphorus can be recovered from wastewater in various forms and consequently be used as a bio-sourced fertilisers to reduce the need for synthetic alternatives. Strict legislation has been put in place to ensure the quality of treated effluent and other recovered products. The shift in our perception of WRRFs has been a driving force for research activities in academic and industrial sectors to optimise plant operations and develop new processes and technologies. Thus, these days WRRFs answer deliver effluent qualities with reduced energy and chemical demand, lower capital costs and operational costs while achieving some resource recovery (Lizarralde et al., 2015; Soares, 2020; Solon et al., 2019a).

Sulfur is another important element in wastewater that has received relatively limited attention compared to enormous efforts dedicated to abating pollution by organic compounds, nitrogen and phosphorus (Lens, 2020). The sulfur cycle is rather complex due to the multitude of sulfur species with different features, involved in a wide range of possible conversions (Muyzer and Stams, 2008). Sulfur species have a broad range of oxidation states from -2 (completely reduced) to $+6$ (completely oxidised) and are present in organic and inorganic forms. Moreover, sulfur has a multi-phase nature which can be present in liquid, solid and gas phases. The sulfur cycle consists of many

chemical, biological and physical reactions and interacts with other elemental cycles such as carbon, nitrogen, iron and phosphorus.

Sulfur impacts the performance and operation of key processes within WRRFs. Anaerobic digestion (AD) is a major and essential part of a modern WRRF that has been successfully applied to stabilise and reduce sludge from wastewater treatment processes. During the anaerobic digestion process, organic matter is transformed into biogas (containing 60–70 vol% of methane, CH₄) by anaerobic microorganisms. The biogas has a high calorific value and is considered a renewable energy source and a promising option for climate change mitigation. Biogas could be valorised in combined heat and power (CHP) plants to generate electricity and heat on-site. About 50–70% of the total electricity consumption of WRRFs could be satisfied using biogas from anaerobic digestion of primary and biological sludge (Remy and Diercks, 2016). Besides methane, biogas contains 30–40% of carbon dioxide (CO₂), as well as some water vapour (H₂O), siloxanes, and H₂S. The production of H₂S can affect the operational performance and economic feasibility of anaerobic digestion processes (Robles et al., 2020) by (i) the competition for readily biodegradable organic matter (e.g. volatile fatty acids and hydrogen) between sulfate-reducing bacteria and anaerobic microorganisms, (ii) inhibitory effect of unionised H₂S on anaerobic microorganisms and (iii) the presence of H₂S in biogas cause corrosion in compressors, pipelines, gas storage tanks and engines. The sulfur cycle thus affects the quantity and quality of biogas in the anaerobic digestion process. The H₂S concentration ranges typically from 50–3000 ppm and in some extreme cases can reach well over 10,000 ppm (Krayzelova et al., 2015). It is essential to remove H₂S from biogas prior to its utilisation. Limiting H₂S production during anaerobic digestion is preferred over treatment methods to remove H₂S from the produced biogas, such as those based on activated carbon, which are costly. Understanding and controlling H₂S production during anaerobic digestion enables optimisation of the anaerobic digestion process and improves biogas valorisation.

Maximising biogas production during the anaerobic digestion process is the main approach to reaching energy-positive WRRFs (Remy and Diercks, 2016).

This goal can be achieved by applying alternative process configuration and sludge pretreatment techniques to enhance the hydrolysis stage of anaerobic treatment, which is seen as the rate-limiting step of the entire anaerobic digestion process (Appels et al., 2008). The thermal hydrolysis process (THP) is a proven technology to increase sludge degradability through sludge solubilisation by applying heat (160 –180 °C) and pressure (600 to 2500 kPa). Increased degradation of proteins – the main source of organic sulfur in sludge – can impact the fate of sulfur during anaerobic digestion. Due to the increased application of the thermal hydrolysis process in modern WRRFs and its potential impacts on the fate of sulfur species, it is essential to investigate the consequences, impacts and role of this process on the sulfur cycle to reduce the adverse effects of H₂S and other volatile organic sulfur compounds during the anaerobic digestion process.

While the design and operation of process treatment units are experiencing fast development regarding water quality sent back to the environment, air pollution has become a central challenge. A “zero-emission” solution for WRRFs is aimed to be achieved regarding potential atmospheric pollutants. Public complaints about odorous emissions from treatment facilities have grown over the past years since urbanisation has led to a lower distance between residential areas and WRRFs. In addition, uncontrolled odours adversely impact the overall job satisfaction of plant’s employees and could cause a health threat to them in some cases. Odorous emissions from treatment facilities consist of many individual components such as reduced sulfur or nitrogen compounds, organic acids, aldehydes or ketones. Among these odorous compounds, volatile sulfur compounds (VSCs), particularly H₂S, commonly cause such nuisance emissions. Volatile organic sulfur compounds (VOSCs) such as methyl mercaptan and dimethyl sulfide can also contribute to malodour because of their low odour detection thresholds. Odorous compounds could enter the WRRF through incoming wastewater or be generated at (nearly) any step along the treatment train. H₂S is generated from the reduction of sulfate in the wastewater and the degradation of organic matter. The degradation of sulfur-containing amino acids, methionine and

cysteine, can produce methyl mercaptan, dimethyl sulfide and H₂S (Lomans et al., 2002). The control of odour emissions and the design of odour collection and treatment prior to release to the atmosphere have become increasingly essential in modern WRRFs. Understanding the mechanisms of formation and emission of odorous compounds and quantifying the amount of emission from various emission sources are essential for developing better control initiatives and reaching zero-emissive facilities (Byliński et al., 2019; Fisher et al., 2018, 2017; Gostelow et al., 2001; Kulig and Szyłak-Szydłowski, 2019; Lebrero et al., 2011).

Corrosion of sewers networks and WRRFs is a major problem associated with sulfur that causes tremendous economic losses each year, e.g. the rehabilitation costs in the US were estimated to be \$390 billion (10⁹) over about 20 years (Wu et al., 2020). Primarily induced by H₂S, corrosion can cause rapid and extensive damage to concrete structures and metal equipment. The potential consequences of corrosion are poor reliability, increased maintenance and premature replacement of instrumentation, railings, walkways, air conditioning and ventilation units, and other metal or concrete structures within treatment plants. Two mechanisms are responsible for H₂S corrosion: (1) acid attack to concrete structures resulting from the biological conversion of H₂S gas to sulfuric acid in the presence of moisture and (2) the direct attack of metals such as copper, iron, and steel by H₂S (US EPA, 1994). Therefore, a better understanding of H₂S generation within process treatment units and identifying key H₂S emission spots in treatment facilities can inform design, equipment selections and operational strategies to reduce the negative impacts of sulfur-induced corruptions in WRRFs.

A whole spectrum of environmental technologies that rely on sulfur cycle conversions and interactions with other elements has been developed in recent years. An example is simultaneous sulfide oxidation and nitrate reduction, which is a type of autotrophic denitrification, which has been applied in new processes, e.g. the SANI for saline wastewater treatment (Lau et al., 2006; Wang et al., 2009).

Impact of the sulfur cycle in WRRFs demands more thorough investigations. Stimulated by needs identified through an industry (Veolia Research and Innovation) and university (Ghent University and INSA-Lyon) partnership, this dissertation presents advancements in our understanding of sulfur transformations in municipal water resource recovery facilities. The acquired fundamental knowledge could be applied to better operate and control WRRFs in order to reduce the negative impacts related to sulfur components or even beneficially exploit features of the sulfur cycle.

1.2 Overview of the dissertation

The objective of this dissertation was to understand the fate of sulfur in municipal water resource recovery facilities. Chapter 2 presents a background of sulfur transformations in such facilities. It comprehensively covers the conversions of sulfur species and their interactions with other elements (e.g. nitrogen, iron, organic matter and phosphorus) under various redox environments within water and sludge process treatment units of a typical municipal WRRF. Moreover, an overview of sulfur studies in literature has been provided to identify reactions and process treatment units that have attracted more attention and highlight the research gaps. In particular, Chapter 2 addresses whether all sulfur compounds have received sufficient attention in the literature and whether the consequences of these conversions are well described and known from available studies. Both organic and inorganic sulfur conversions have been included. In addition, unit processes of a typical WRRF are discussed separately for possible sulfur conversions to demonstrate the processes which have been well investigated and those for which the knowledge is more limited. Chapter 2 also discusses the modelling of sulfur transformations in activated sludge and anaerobic digestion processes. The review evaluates whether experimental and modelling studies have been validated with data collected from full-scale installations.

In Chapter 3, full-scale data collection was performed to investigate the distribution of total sulfur mass flows in water, sludge and gaseous streams of a municipal WRRF. Quantification of sulfur flows allows identifying and

comparing key sulfur streams and evaluating the impacts of process treatment units on sulfur distributions, knowing that this information is relatively scarce in the literature. The sulfur flows are quantified in a municipal WRRF with a capacity of 620,000 P.E., consisting of a relatively complex and innovative configuration, especially in the sludge treatment line. The specificity of the sludge treatment line is the usage of two-stage anaerobic digestion with intermediate thermal hydrolysis. Data availability and quality were optimised by following a mass balanced-based experimental design and data reconciliation procedures applied for the first time in sulfur studies.

Chapter 4 and Chapter 5 explore the transformations of sulfur species in the water treatment line and sludge treatment line, respectively, on the basis of the experimental results presented in Chapter 3 and additional measurements performed on the studied facility. Chapter 4 presents sulfur speciation and hourly variation in the influent wastewater.

Chapter 5 provides a comprehensive study about sulfur transformations during anaerobic digestion and evaluates the impact of the thermal hydrolysis process on hydrogen sulfide production during anaerobic digestion by using full-scale data and lab-scale investigations. As highlighted in Chapter 2, degradation of organic sulfur and its contribution to hydrogen sulfide production in biogas is less investigated than biological sulfate reduction; therefore, Chapter 5 explores the fate of organic sulfur during anaerobic digestion of raw sludge and thermally treated sludge. The results from Chapter 5 show that the organic sulfur content of sludge decreases during the anaerobic digestion process. The observed decrease in organic sulfur was related to particulate organic sulfur in the first digester receiving a mixture of primary and secondary sludge and soluble organic sulfur (produced through thermal hydrolysis) during the second digester stage. In Chapter 6, the impact of these findings and areas of future research are presented.

2

Literature review: Transformation of sulfur species in municipal WRRFs

2.1 Abstract

Sulfur cycle greatly influences water resource recovery facilities (WRRFs) in various aspects, including design, operation, performance and economics. Perhaps generation and emission of volatile sulfur compounds (VSCs) is the most well-known issue leading to odorous nuisance, corrosion and adverse effects on anaerobic digestion performance. Nevertheless, the sulfur cycle is rather complex. The complexity of the sulfur cycle originates from various sulfur compounds occurring in multi phases, a diverse network of biochemical conversions under different oxidative conditions and interactions with other elements, including carbon, nitrogen, iron and phosphorus. Consequently, various aspects of the sulfur cycle have been subject to research in literature. Based on the published studies, an extensive review of sulfur transformation at municipal WRRFs is presented. Focus is placed on state of the art within individual unit processes and plant-wide level to cover the evolution of sulfur compounds throughout the treatment line and establish a comprehensive picture for sulfur management. Knowledge gaps are pointed out and discussed. In summary, not all sulfur mechanisms and process treatments have received attention. Inorganic sulfur mechanisms within anaerobic digestion and activated sludge process are well established, while research on organic sulfur pathways and some process treatment units such as primary settling and thickening are limited. More work should be performed to understand the influence of emerging sludge pretreatment techniques prior to anaerobic digestion on the fate of sulfur compounds. The review of existing literature revealed lack of full-scale investigation and calibration/validation of models with actual data.

2.2 Introduction

Wastewater treatment plants (WWTPs) are essential infrastructures for protecting human health and the environment through the removal of pollutants from wastewater. The goals of WWTPs have evolved over the years, and the performance of these facilities are currently being evaluated based on environmental, commercial, economic, and social aspects. In order to improve environmental protection, the quality levels of treated effluent imposed by regulation are continuously increasing, leading to recent advances in wastewater treatment processes and technologies for the removal of nutrients, e.g. nitrogen and phosphorus (Palatsi et al., 2021). In addition, resource limitation in nature has been a key driving force for a paradigm shift where WWTPs are now being considered as potential water resource recovery facilities (Solon et al., 2019a). Examples of the latter include the production of biogas during anaerobic digestion (AD), production of a wide range of recovered products (e.g. struvite, bioplastics, syngas, heavy metals) as well as usage of final biosolids (e.g. in agriculture and in building and producing combustible pellets) (Cieřlik et al., 2015; Soares, 2020). WRRFs are also energy-consuming, so the production of energy from biogas (methane) through sludge digestion could yield economic benefits for these facilities (Nowak et al., 2015). The produced biogas is either used to generate electricity and heat in combined heat and power (CHP) units or purified for direct injection into the natural gas grid.

At WRRFs, hydrogen sulfide (H_2S) may be present at different stages leading to potential negative impacts including (i) corrosion issues, (ii) odorous compounds production and safety issues, (iii) biological reactions inhibition or (iv) biogas purity problems in relation to its usage. Corrosion of concrete and carbon steel are caused primarily by the activity of sulfur-related bacteria, e.g. sulfate-reducing bacteria (SRB) and sulfur-oxidizing bacteria (SOB) (Wu et al., 2020a, 2020b), imposing great economic challenges for sewer networks and WRRFs.

The odorous nuisance problem in WRRFs is mainly caused by volatile sulfur compounds (VSCs) (Frechen, 1988; Gostelow et al., 2001; Lebrero et al., 2013). The generation of nuisance odour has become a rising concern mainly due to urban spread around these facilities. In addition, it leads to low quality of the working environment and safety risks for personnel at WRRFs (Bazemo et al., 2021; Lebrero et al., 2011; Zhang et al., 2008). Therefore, WRRFs must be required to collect and treat odorous compounds prior to release to the atmosphere, among which sulfur species. The high concentration of sulfur can also pose a serious problem during incineration of biosolids due to the release of sulfur dioxide (SO₂) that may cause a series of environmental issues, including acid rain (Dewil et al., 2008; Shao et al., 2013).

Sulfur cycle influences both energy recovery and resource recovery. It has been reported that the sulfur cycle in sludge AD could adversely affect methane production due to micro-organisms inhibition by H₂S (Appels et al., 2008; Chen et al., 2008; Harada et al., 1994; Visser, 1995). In addition, a high concentration of H₂S in biogas necessitates further processing before co-generation due to its corrosive properties (Tchobanoglous et al., 2003); hence lowering biogas valorisation and profitability. In France, for instance, the concentration of H₂S for injection of biomethane into gas grid should be as low as 3.3 ppmv while its content can be initially higher than 5000 ppmv in raw biogas. Higher H₂S content of up to 200 ppmv is possible when biogas is used for power generation, but a removal treatment is commonly required (Gasquet et al., 2020; Sarperi, 2014).

Positive impacts of sulfur compounds have also been demonstrated. In recent years, the interactions of the sulfur cycle with other elements have been used for the development of new processes (e.g. SANI process) and lowering the dosage of chemicals (e.g. for chemical phosphorus removal in activated sludge process and H₂S removal in AD) (Ge et al., 2013; Gutierrez et al., 2010; Lau et al., 2006; Roussel and Carliell-Marquet, 2016; Wang et al., 2009).

This chapter summarizes the present knowledge on the transformation of sulfur species in municipal WRRFs. For this purpose, typical processes in the water line and sludge line are scrutinised. A focus is made on providing a plant-wide

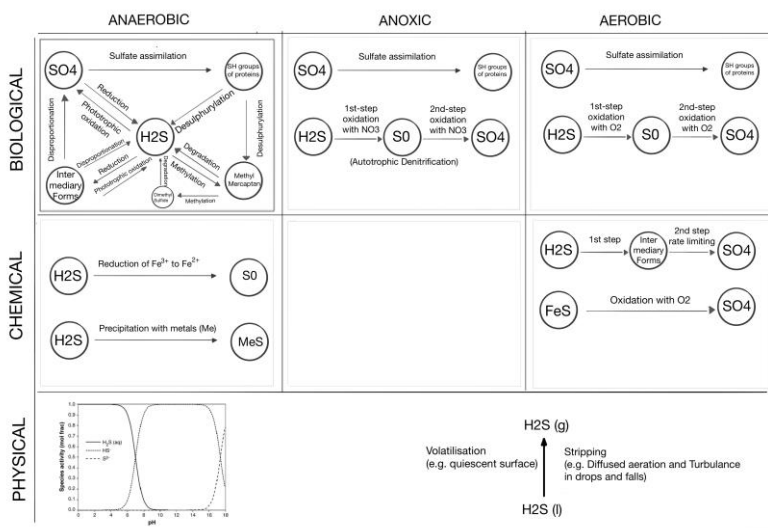
overview by considering the influence of process treatment units on sulfur transformations and subsequent process treatment units. Additionally, interactions of sulfur transformations with other processes, such as C, N, P, heavy metals, are reviewed. Mathematical models developed to incorporate sulfur reactions are finally presented, and their limitations are discussed.

2.2.1 General mechanisms involving sulfur compounds

Sulfur is a highly reactive element that is present in numerous forms in the environment. Sulfur is present in organic and inorganic forms (Table 2.1). Being an essential nutrient for protein synthesis (Huxtable, 2013), sulfur is present in the amino acids methionine and cysteine which contain sulfur in its lowest oxidation stage (-2). Important VOSCs associated with odour emissions at WRRFs are methyl mercaptan (MM), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) (Fisher et al., 2018; Higgins et al., 2006). Inorganic sulfur compounds have a wide range of oxidation states with sulfide (-2) and sulfate (+6) at the end of this range. The most stable form of sulfur in the presence of oxygen is sulfate, while the reduced inorganic sulfur forms of sulfur are quite common in anaerobic environments (Lens and Pol, 2015). Important compounds at intermediate oxidation states are sulfite (+4), thiosulfate (+2) and elemental sulfur (0) which are biologically and chemically active (Huxtable, 2013). Most of sulfur forms play a role in aqueous systems in which redox reactions occur either as a result of microbiological activity or simply following the thermodynamics of the system in non-enzymatic reactions (Stuedel, 2020). Biological, chemical and physical mechanisms involving sulfur species are depicted in the following paragraphs.

Table 2.1 Inorganic sulfur compounds and their oxidation states as well as important VSCs associated with WRRFs.

	Compounds	Formula	Oxidation state
Inorganic sulfur	Sulfide	H ₂ S, HS ⁻ , S ²⁻	-2
	Elemental sulfur	S	0
	Thiosulfate	S ₂ O ₃ ²⁻	+2
	Dithionite	S ₂ O ₄ ²⁻	+3
	Disulfite	S ₂ O ₅ ²⁻	+4
	Sulfite	SO ₃ ²⁻	+4
	Dithionate	S ₂ O ₆ ²⁻	+5
	Sulfate	SO ₄ ²⁻	+6
	Compounds	Formula	Character
Odorants associated with WRRFs	Hydrogen sulfide	H ₂ S	Rotten eggs
	Methyl mercaptan (MM)	CH ₃ SH	Decayed cabbage, garlic
	Diethyl sulfide (DMS)	(CH ₃) ₂ S	Decayed vegetables, garlic
	Dimethyl disulphide (DMDS)	(CH ₃) ₂ S ₂	Putrifaction

**Figure 2.1 Biological, chemical and physical sulfur conversions under anaerobic, anoxic and aerobic conditions**

2.2.1.1 Biological reactions

Microorganisms play an important role in sulfur transformations. Under anaerobic conditions, sulfate functions as an electron acceptor of anaerobic respiratory processes and is reduced to sulfide by Sulfate-reducing bacteria (SRB), referred to as “dissimilatory sulfate reduction” (Figure 2.1). Sulfate reducers can grow on a large range of substrates including hydrogen and organic compounds like short and long-chain fatty acids and amino acids. SRB can also reduce intermediary sulfur compounds (i.e. thiosulfate, sulfite and sulfur) to sulfide (Muyzer and Stams, 2008). In turn, sulfide serves as an

electron donor and is biologically oxidised to elemental sulfur or sulfate by phototrophic and chemotrophic sulfide oxidising bacteria (SOB). During the first reaction that occurs under anaerobic conditions, phototrophic SOBs utilise sulfide as an electron donor for CO₂ fixation in the light. In this photosynthetic reaction, sulfide is first oxidised to elemental sulfur and then further to sulfate given sufficient energy (Lin et al., 2018). Chemotrophic SOBs, commonly referred to as colourless sulfur bacteria, oxidise sulfide and other reduced sulfur compounds while using oxygen or nitrate and nitrite as terminal electron acceptors to fix carbon dioxide (Tang et al., 2009). Sulfur disproportionation is an energy-generating process carried out by specialised groups of microorganisms in which elemental sulfur and thiosulfate function both as electron donor and electron acceptor generating sulfate and sulfide (Bak and Pfennig, 1987).

As a nutrient, sulfate is assimilated by microorganisms to generate reduced sulfur compounds required for the biosynthesis of amino acids and proteins. Assimilatory reduction normally produces no more sulfide than is required for sulfur amino acid biosynthesis and does not lead to direct excretion of sulfide (Huxtable, 2013). The hydrolysis and degradation of proteinaceous materials, sulfur-containing amino acids, leads to the release of sulfide, methyl mercaptan (MM) and dimethyl sulfide (Lomans et al., 2002). The predominant amino acids in proteins that contain sulfur are cysteine and methionine. The degradation of cysteine under anaerobic conditions has been reported to yield only H₂S, while methionine degradation has been observed to lead to the formation of methyl mercaptan, which is further methylated to form dimethyl sulfide (Du and Parker, 2012). Both methyl mercaptan and dimethyl sulfide are degraded under anaerobic conditions due to the activity of methanogens resulting to end products of mainly sulfide and methane (Higgins et al., 2006; Parker and Wu, 2006). The biological aspect of the sulfur cycle such as reaction pathways, microbiology and biokinetics are dealt with in specialised literature (Hao et al., 2014; Huxtable, 2013; Lin et al., 2018; Muyzer and Stams, 2008; Tang et al., 2009).

2.2.1.2 *Chemical reactions*

Chemical oxidation of sulfide occurs in the presence of oxygen is a two-step process which leads to the formation of elemental sulfur and sulfate. The biological oxidation of sulfide generally prevails over abiotic oxidation (Luther et al., 2011; Yavuz et al., 2007). Besides the reaction with oxygen, sulfide reacts chemically with metals, in particular with ferrous iron (Fe^{2+}), to form insoluble iron sulfide (FeS) (Lewis, 2010). Under aerobic conditions, FeS is oxidised to form ferric iron (Fe^{3+}) and sulfate (Schippers and Jørgensen, 2002). The formation of Fe^{3+} can precipitate phosphate, showing the interactions between sulfur, iron and phosphorus (Gutierrez et al., 2010). Under anaerobic conditions, Fe^{3+} oxidises sulfide chemically to elemental sulfur while itself being reduced to Fe^{2+} (Firer et al., 2008; Nielsen et al., 2005). Because sulfide has a high affinity for oxidised iron, it may cause the release of iron phosphate to precipitate as FeS (Batstone et al., 2018; Ge et al., 2013). Depending on the quantities and forms of S, Fe and P that are present, their interactions can result in advantages (e.g., promote chemical phosphorus removal in activated sludge processes, H_2S removal during AD and prevention of struvite formation) and disadvantages (e.g., self-heating of dried sludge) in WRRFs which will be discussed later in this article.

2.2.1.3 *Physical reactions associated with hydrogen sulfide*

In aqueous solutions, H_2S is a weak acid, and sulfide can be present in three different forms: H_2S , HS^- , and S^{2-} , which are in equilibrium. Their distribution is a function of pH, temperature, and ionic strength to a lesser extent (Boon, 1995). For instance, at pH 7.0 and 25 °C, 50% of the total sulfide is in its unionised H_2S form. Only this form is emitted into the gaseous phase. This occurs when the concentration of H_2S exceeds the saturation given by the thermodynamic equilibrium (Henry's law). Under this condition, the emission of H_2S is governed by liquid to gas mass transfer, and the most important factors are the interfacial area and the hydrodynamic conditions in the liquid phase (Carrera et al., 2016; Springer et al., 2020). This phenomenon is termed "volatilisation" when it occurs at the open surface, such as the surface of primary settlers and in drop structures such as weir. The term "stripping" is

used when it occurs due to diffused aeration in which the injected air in the liquid phase carries the contaminant and releases it to the atmosphere when it reaches the free surface.

2.3 Sulfur transformations in process treatment units

2.3.1 Overview of sulfur studies in literature

The reactions involved in the sulfur cycle that have been studied in process treatment units are given in Table 2.2. Water and sludge treatment lines of municipal WRRFs are operated under anaerobic, anoxic or aerobic conditions, and as such provide the environment for some of the biological, chemical and physical transformations of sulfur species presented in the previous section. The complexity of species and reactions in the sulfur cycle, many process treatment units and extensive literature body on sulfur necessitated an overview and classification of available research on sulfur transformation in process treatment units (Table 2.2 and Appendix A). From Table 2.2, it can be seen that research has been focused mainly on inorganic sulfur reactions, namely biological sulfate reduction under anaerobic conditions (e.g. AD) and biochemical oxidation of sulfide to elemental sulfur and sulfate under aerobic conditions (e.g. aeration zone in activated sludge systems and microaeration process in AD). Another interest in inorganic sulfur was the mass transfer of reduced sulfide compounds (particularly H_2S) from liquid to the gas phase. On the other hand, research works on the fate of organic sulfur are less reported. These studies mostly investigated the mechanisms for production and degradation of VOSCs as key odour causing compounds during AD, dewatering and biosolids storage.

Regarding treatment processes, AD attracted most available research due to the importance of the sulfur cycle on the performance of this process, the problems associated with H_2S in biogas, and more recently interaction of sulfur with iron and phosphorus which affects phosphorus recovery. Pretreatment units including inlet, grit and grease removal and primary settling together with primary thickening were of interest for the emission of reduced sulfide compounds. The secondary treatment has been studied for inorganic sulfur

conversions under anaerobic, anoxic and aerobic conditions to identify the fate of sulfur compounds and investigate the effect of the sulfur cycle on other cycles. In more recent studies, new configurations have been proposed and tested using inorganic sulfur features to benefit from the high sulfate content of saline wastewater to treat organics and nitrogen. It is apparent from Table 2.2 that some process treatment units and sulfur reactions have not been studied yet or have not been considered as significant under studied operating conditions.

Table 2.2 An overview of sulfur transformations in process treatment units of a typical WRRF which have been investigated I in literature. Each number corresponds to an article. A brief description of these articles are provided in Tables A in Appendix of Chapter 2.

unit processes → Reactions ↓	Inlet/ pretreatment	Primary settling	Biological tanks	Secondary settling	Primary/secondary thickener	Sludge pretreatment (e.g. Thermal hydrolysis)	Anaerobic digestion (AD)	Sludge Post-treatment (dewatering, drying)
Assimilation sulfate reduction								
Dissimilatory sulfate reduction	1	10	11-13-14-15-16-17-18-19-20-21-22-23-24-25-26-31-59	17-20-21		54	23-25-26-31-36-37-38-41-43-45-46-47-48-49-50-51-52-53-54-55-56-59	
Desulphurylation of organic sulfur						60-64-65	23-55-60-64-65-68	67-72-74-75
Phototrophic sulfide oxidation								
Disproportionation of elemental sulfur and thio-sulfate								
Biological sulfide oxidation by O ₂ and NO ₃			12-13-14-15-16-19-23-25-26-31-59		30		-36-37-38-39-40-41-56-57-	
Chemical oxidation of sulfide to elemental sulfur by Fe ³⁺			23-26-31				-23-25-26-31-49-58-59-	
Precipitation of metal sulfide	4	8	23-25-26-31-59		62-63		-23-25-26-27-28-31-32-33-34-35-49-58-59	
Chemical oxidation of sulfide by O ₂	2	5	12-16-23-25-26-31				26	76-77-78
Oxidation of iron sulfide under aerobic condition			8-23-25-26-27-28-31-59					76-77-78
Transformation of volatile organic sulfur compounds -VOSCs)		10-29	29	29		64-65	42-43-44)	69-70-72-74-75
Emission of H ₂ S and VOSCs	3-4	6-7-9-10-29	7-9-25-26-29	7-9-29	-30-62-63	61-64-65	23-25-26-31-32-33-35-41-43-44-46-47-49-51-52-53-56-59-64-65-66	32-33-67-69-70-71-72-73-74-75

electron donors; sulfate concentration in wastewater; temperature, and electron acceptors such as oxygen. Higher organic matters as well as sulfate content, higher temperature and longer hydraulic retention time in sewer networks result in higher sulfide production (Sharma et al., 2008). Applying control methods in sewer networks to minimise sulfide formation and H₂S emission such as addition of metal salts and nitrate plays an important part in both the concentration and form of sulfide in influent wastewater. The reported values of organic sulfur compounds from proteinaceous material is about 3–6 mg·L⁻¹ (Gostelow et al., 2001).

Odour and corrosion are common problems in the inlet and pretreatment units (i.e., screening, grit and grease removal), which are mostly caused by reduced sulfur compounds. Among the reduced sulfur forms, H₂S is more likely to be responsible for these problems compared to VOSCs (Chen and Szostak, 2013). According to Gostelow et al. (2001), inlet and pretreatment units promote the emission of H₂S which was previously formed in the sewer network, making them important emission zones when the incoming wastewater is oxygen-depleted (Frechen, 2004). Volatilisation and stripping are the two mechanisms responsible for the emission of H₂S from these units. Inlets, non-aerated grit removal units and channels promote the emissions through volatilisation, while aerated grit removal causes the emission through the stripping. Emissions can also occur due to the turbulence created as wastewater falls over drop structures and from the surface area of the wastewater at the weir (Tata et al., 2003).

2.3.3 Primary settling

Primary settling separates the readily settleable organic and inorganic solids contained in the wastewater from the liquid phase and discharges them through the primary sludge. FeS is among the inorganic solids that partly precipitate in this unit. Gutierrez et al. (2010) reported that FeS precipitation in primary settling is dependent on the travelling time of FeS in the sewer network, meaning that a longer distance between dosing location of iron and downstream plant results in larger aggregates which settle more easily in the primary settling tank.

At low discharge rates, the accumulation of settled solids and increase of sludge blanket height creates anaerobic conditions at the bottom of the primary settling tank. The prevailing anaerobic condition (Carrera-Chapela et al., 2014) and active biomass seeding from sewer network and fermentation product availability (Bazemo et al., 2021) are the main drivers for the production of H₂S and VOSCs. Quantification of volatile sulfur compounds in off-gas of primary settling at a municipal WRRF indicated that H₂S and methyl mercaptan were the dominant forms (Bazemo et al., 2021). In another study, H₂S, methyl mercaptan, dimethyl sulfide, and dimethyl disulfide emissions of 0.39, 0.19, 0.17, and 0.83 $\mu\text{g S m}^{-2} \text{ s}^{-1}$, respectively, were found from the measurements on kraft mill primary settling tank (Catalan et al., 2009). As reported by Bazemo et al. (2021), H₂S emission in primary settling tanks is mainly the result of the emission of dissolved sulfide (64%) generated in the sewer network, while methyl mercaptan emission is attributed to its production (84%) in the primary settling tank. Atmospheric emission from primary settling tanks occurs through volatilisation from the quiescent surface and stripping when the wastewater falls over the weir (Tata et al., 2003). It is reported that emissions from the quiescent surface could account for 10–30% of total emission from settling tanks, whereas weir drops account for the remaining emission (Pincince, 1991).

Both sulfur content and fractionation (i.e. soluble/particulate and organic/inorganic) are highly variable in primary sludge. Fisher et al. (2017) reported a wide range of sulfur concentrations of 4551–9958 $\text{mg S} \cdot (\text{kg dry solid})^{-1}$ in the primary sludge of 6 WRRFs. This variability was attributed to municipal vs industrial wastewater emissions, heavy metal levels producing insoluble metal sulfides. In another study, Du and Parker (2013) fractionated sulfur in primary sludge. The soluble fraction of total sulfur was between 2–13%, indicating a high variation. It is important to note that, besides the soluble sulfur present in the influent wastewater, the hydrolysis of particulate organic sulfur to soluble forms in the primary settler influences this fraction. The organic fraction of sulfur in primary sludge accounted for 57–76% of total sulfur. The organic fraction of sulfur is particularly important in AD, as high

degradation of organic sulfur from primary sludge was observed during AD (Du and Parker 2013).

2.3.4 Biological treatment

Biological treatment units commonly employ anaerobic, anoxic and aerobic stages or zones for the removal of organic carbon, nitrogen and phosphorus. Several studies investigated the transformation of sulfur species and their possible effects on the performance of the biological treatment.

2.3.4.1 Anaerobic zone

SRB are present in a broad range of biological treatment processes, including conventional activated sludge and biofilm-based systems (Lens et al., 1995). The relative presence of these bacteria in the activated sludge process is below 8% (Manz et al., 1998; Nielsen and Nielsen, 2002; Schramm et al., 1999), is constant over the year (Ingvorsen et al., 2003) and is independent of reactor configurations, organic loadings and influent sulfate concentrations (Lens et al., 1995). Due to small growth yield of $0.3 \text{ g VSS} \cdot (\text{g COD})^{-1}$ (Wang et al., 2009), the presence of SRB in biological treatment units rely on re-inoculation from the influent wastewater and return sludge from settler and reject water from anaerobic digester (Schramm et al., 1999; Van Den Brand et al., 2015).

Whether biological sulfate reduction occurs or not in the anaerobic zone of activated sludge does not have a consistent answer in the literature. While some researchers (Baetens et al., 2001; Ingvorsen et al., 2003; Lens et al., 1995; Wanner et al., 1987; Yamamoto et al., 1998, 1994, 1991) have detected this reaction, despite a generally low sulfate reduction rate $0\text{--}3.1 \text{ mgSO}_4^{2-} \cdot (\text{g VSS} \cdot \text{h})^{-1}$ (Lens et al., 1995), others did not detect any reduction in the anaerobic zone (De Beer et al., 1998; Schramm et al., 1999). Ingvorsen et al. (2003) investigated the biological sulfate reduction in activated sludge of a municipal WRRF. They found that the sulfate reduction rate was linear for the first 5 h with a low sulfate reduction rate, followed by an exponential phase. Given the much lower hydraulic retention time (HRT) of the anaerobic zone in activated sludge, this result supports the low sulfate reduction in this zone.

Another study of sulfate reduction in the anaerobic zone of the Enhanced Biological Phosphorus Removal (EBPR) process showed that biological sulfate reduction and phosphate release could occur simultaneously (Baetens et al., 2001). SRB and phosphate accumulating bacteria (PAO) do not compete for substrate, instead PAO utilise the acetate which is produced by SRB (Yamamoto et al., 1998). However, elevated concentrations of sulfide severely affect the anaerobic and aerobic metabolisms of PAO. Saad et al. (2017) observed that the specific acetate uptake rate of PAO was inhibited by 50% at around H_2S concentration of $60 \text{ mg S}\cdot\text{L}^{-1}$. Although this concentration is not expected in domestic wastewater, it is important in case of saline sulfate-rich wastewaters (Rubio-Rincón et al., 2017a).

2.3.4.2 Anoxic and aerobic zones

Under anoxic and aerobic conditions, sulfide is biologically oxidised by SOB. Investigation of sulfide oxidation rate by Lens et al. (1995) showed high sulfide removal rates (11.6 to $131.7 \text{ mg HS}^- \cdot (\text{g VS}\cdot\text{h})^{-1}$) in both biofilms and mixed liquor activated sludge. As observed by Luther et al. (2011), the biological sulfide oxidation rates are three or more orders of magnitude higher than chemical oxidation rates, suggesting that in activated sludge process the chemical oxidation is negligible (Hauduc et al., 2018; Lens et al., 1995). High degradability of sulfide in the biological treatment results in low emission of H_2S from activated sludge system, which has been supported by full-scale emission data (Frechen, 2004). It is important to note that excessive growth of filamentous SOB such as *Beggiatoa* in activated sludge processes can cause poor settling (Baetens et al., 2001; Yamamoto et al., 1998), a condition known as filamentous bulking (Williams and Unz, 1985).

There is also an increasing interest in the application of sulfide-driven autotrophic denitrification for the treatment of carbon-deficient wastewater contaminated with sulfide and nitrogen oxides (Manconi et al., 2006; Wang et al., 2009; Xu et al., 2016). In municipal wastewater, however, the high carbon content and low sulfide concentration hinders the application of this process in municipal WRRFs. As stated by Pokorna et al. (2013), heterotrophic and autotrophic denitrification do not co-occur, but the organic substrate is used

preferably and only after its exhaustion will autotrophic denitrification with sulfide proceed.

The effect of sewer iron dosing on the downstream WRRF was investigated in batch test (Gutierrez et al., 2010) and continuously-operated laboratory systems (Rebosura et al., 2018), which showed that iron sulfide present in pre-settled wastewater was rapidly oxidised in aerobic conditions, resulting in phosphate precipitation. Implementation of these strategies under real-life conditions through year-long full-scale investigations has been recently demonstrated by Salehin et al. (2019).

2.3.5 Secondary clarifier

After exposure to oxygen in the aerated zone of the activated sludge process, SRB might recover in the secondary clarifier due to anaerobic conditions at the bottom of the tank (Kjeldsen et al., 2004). The activity of SRB in the activated sludge blanket lead to the difference in dissolved sulfide concentration between the top (0.73 ppb) and bottom (98 ppb) of a secondary clarifier tank (Sekyiamah et al., 2008). In the same study, the authors reported dimethyl sulfide and dimethyl disulfide formation in the sludge blanket at the bottom of the settling tank and further established a strong correlation between their concentration with sludge blanket height.

The stability of sludge flocs decreases under anaerobic conditions and is reported to be highly correlated to the content of oxidised iron (Ingvorsen et al., 2003). The reduction of Fe^{3+} to Fe^{2+} that occurs by microbial respiratory processes or by reaction with sulfide is demonstrated to significantly reduce the strength of sludge flocs (Caccavo et al., 1996; Nielsen and Keiding, 1998; Wilén et al., 2000). Laboratory studies showed that sulfide concentration of higher than 1mM is necessary for significant disintegration (Nielsen and Keiding, 1998). Ingvorsen et al. (2003) reported that production of milimolar concentration of sulfide in settled activated sludge would occur in less than 48 h, indicating that the storage of sludge for a couple of days could cause the deflocculation of sludge particles.

Fisher et al. (2017) reported the sulfur content of secondary sludge to be in the range of 0.8–1.1% on a dry solid basis, which is in accordance with the previously reported range of 0.3–2.3% (Sommers et al., 1977; Dewil et al., 2006). Sulfur in secondary sludge is in inorganic and organic forms due to the presence of sulfate and sulfur-containing amino acids present in the protein structure of biomass. According to Du and Parker (2013), the organic sulfur fraction accounts for 69% to 74% of total sulfur in secondary sludge.

2.3.6 Thickening process

Primary thickening is typically performed in gravity thickeners, which have a similar structure to primary settling tanks (Tambo et al., 1982). In addition, the sulfur reactions in primary thickeners are similar to those occurring in the primary settlers (section 3.3). Nevertheless, the longer hydraulic retention time of gravity thickeners (~24 hours) and anaerobic conditions promote the biological reduction of sulfate and hydrolysis of sulfur-containing organic matter. As a result, primary thickeners are a main source for the production of reduced sulfur forms causing odour emissions (Frechen, 2004). The elimination of conditions leading to anaerobic conditions of primary sludge, such as dosing of nitrate and iron salts, will likely reduce emissions of H₂S and other VOSCs from primary thickeners (Einarsen et al., 2000; Fisher et al., 2018).

The thickening of secondary sludge directs an important fraction of incoming sulfur (50–68%) to the filtrated streams, which is likely due to the high levels of soluble sulfur in the form of sulfate formed in the activated sludge process (Dewil et al., 2008; Fisher et al., 2017). Directing the sulfur flow towards filtrate rather than thickened sludge makes the thickening of secondary sludge an efficient unit for reducing the sulfur flows reaching the subsequent stage of sludge treatment.

2.3.7 Sludge buffer tank prior to anaerobic digestion

The reactions involving sulfur species under anaerobic conditions continue in the storage tank. Nielsen and Keiding (1998) observed significant sulfate reduction in storage tanks where the sludge was stored anaerobically for a few

days before dewatering. As mentioned in Section 2.3.5, the presence of sulfide would have deteriorating effects on the activated sludge floc stability by reducing Fe^{3+} to Fe^{2+} as FeS .

2.3.8 Sludge pretreatment

The application of sludge pretreatment methods to improve the performance of AD has increased. The goal of sludge pretreatment is to make more organic matters available to microbial consumption for digestion, resulting in a lower final amount of sludge to be disposed of and increasing methane production. Sludge pretreatment techniques can be categorized into mechanical, chemical, biological, thermal and a combination of these processes (Shrestha et al., 2020). These methods may increase degradability extent and increase degradation kinetics (Carrère et al., 2010).

Pretreatment studies have mainly focused on sludge solubilisation and enhancement of anaerobic digestibility, while the effects of pretreatment methods transformation of sulfur compounds during pretreatment and AD have been less investigated. It has been demonstrated in lab-scale studies that chemical pretreatment (H_2O_2 and FeCl_2) (Dhar et al., 2011) and electrochemical pretreatment (EPT) (Zeng et al., 2019) cause the elimination of dissolved sulfide from sludge through precipitation with metals, leading to lower emission of H_2S into biogas. In electrochemical pretreatment, the disintegration of sludge flocs and subsequent release of metals (i.e., Fe, Mn, and Ni) from their residuals and organically bound fractions was responsible for removing produced sulfide via metal precipitation.

Among pretreatment methods, the thermal hydrolysis process (THP) is widely used to increase the removal efficiency of organic components and the recovery of energy (Xue et al., 2015). Recent studies reported the fate of sulfur compounds in this process and its impact on the formation of VSCs in subsequent AD. Li et al. (2020) reported the presence of typical VSCs in the gas produced by THP at 160 °C for 30 min, indicating the effect of THP on producing VSCs. The detected concentration for H_2S , methyl mercaptan, dimethyl sulfide and dimethyl disulfide were 2000 ppm, 500 ppm, 40 ppm and

100 ppm, respectively. However, it is important to mention that the observation of Liu et al. (2015) showed that the formation and release of VSCs during sludge pyrolysis occurred at temperatures higher than 150 °C (250 °C–450 °C).

The results reported by Li et al. (2020) showed a reduction in levels of cysteine (24.0%) and methionine (15.5%) after THP, indicating that high temperature and high pressure of THP promoted the hydrolysis of proteins into cysteine and methionine, which were further converted into VSCs. In addition, the authors reported an increase (4%–10%) of sulfate after THP, whereas sulfite and thiosulfate were not detected before or after this unit (Li et al., 2020b). Liu et al. (2015) reported that sulfate was relatively stable with no significant change during the pyrolysis of raw sludge at temperatures between 150 °C and 450 °C. The variation of total sulfur, dissolved sulfide and particulate sulfide is reported negligible in THP based on the results reported by Han et al. (2017).

Several studies have reported that THP increases the production of VSCs in AD (Drennan and DiStefano, 2010; Han et al., 2021; Li et al., 2020). Li et al. (2020) observed a significantly higher amount of VSCs during AD of thermally treated sludge. The results reported by Li et al. (2020b) suggested THP promotes conversions of VSCs by reducing sludge particle size and subsequently increasing the interaction between sulfur compounds and enzymes. In general, recent developments indicate that THP plays a major role in promoting VSCs production in AD.

2.3.9 Anaerobic digestion

AD is a microbially-catalysed process involving the breakdown and fermentation of organic matter in the absence of oxygen (Ahmed and Rodríguez, 2018). AD is a key unit for the reduction of sludge mass and volume, stabilisation of sludge and generation of biogas as a valuable energy source (Ge et al., 2013). The transformation of sulfur species during AD leads to the formation of VSCs, which are the main hazard components in biogas of AD (Montebello et al., 2012). Typical ranking of VSCs released during AD are hydrogen sulfide > methyl mercaptan > dimethyl sulfide > dimethyl

disulphide (Han et al., 2021; Novak et al., 2006). The formation of VSCs during AD is problematic because most of VSCs are toxic and cause corrosion to pipes, combustors, and other metallic instruments. Moreover, the high concentration of H_2S in the biogas might necessitate the pretreatment of biogas prior to its utilisation (Charles et al., 2006).

2.3.9.1 *Biological sulfate reduction*

Studies of AD have frequently addressed the quantification and transformation of biological sulfate reduction, and have covered different aspects of this reaction, namely kinetics and stoichiometry (Cassidy et al., 2015), the inhibition effect of the sulfide produced on microorganisms (Paulo et al., 2015) and competition of SRB with other anaerobes (e.g. acetogens and methanogens) for the available substrates (Chen et al., 2008). In AD, SRB reduce sulfate into sulfide while utilising volatile fatty acids (VFAs) and/or hydrogen as electron donors. Utilising similar substrates creates a potential competition between SRB with several microbial groups in AD. These competitions do not occur in the hydrolysis and acidogenesis step of AD since SRB are not capable of degrading biopolymers, instead they depend on the degradation products of the fermentation step (Appels et al., 2008). Nevertheless, SRB effectively compete in the acetogenesis step with acetogens for VFAs and ethanol and in the methanogenesis step with methanogens for acetate and hydrogen (Cassidy et al., 2015). The literature regarding the outcomes of competition is sometimes contradictory, as in practice several factors such as COD/SO_4^{2-} , sulfide toxicity and relative population of SRB and other anaerobes influence the competition. Previous works show that in sulfate-limiting conditions, like municipal WRRFs, acetogens and acetoclastic methanogenesis can effectively compete with SRB for VFAs and acetate, respectively. Having a higher affinity for H_2 , SRB outcompete hydrogenotrophic methanogens in mesophilic AD (Chen et al., 2008). Sulfide in non-dissociated form is toxic for microorganisms due to facilitated diffusion through the cell membranes which lead to denaturation of proteins and interfering with the sulfur assimilation. The reported inhibitory levels for non-dissociated H_2S ranges from 50 to 400 $mg\ S \cdot L^{-1}$, a value more elevated than

what can be found in municipal AD. The most inhibited groups by sulfide is reported to be methanogens followed by acetogens and fermentative microorganism (Appels et al., 2008; Cassidy et al., 2015; Chen et al., 2008).

2.3.9.2 *Organic sulfur conversions*

Organic sulfur is the most abundant form of sulfur in sludge (Liu et al., 2015; Sommers et al., 1977). Its main source is sulfur-containing proteins (Du and Parker, 2013; Sommers et al., 1977). The hydrolysis of proteins to amino acids and further uptake of methionine and cysteine are precursors of VSCs produced by AD (Figure 2.1). Cysteine and methionine are degraded during the hydrolysis/acidogenesis step of AD in which amino acids are converted to VFAs. Degradation of cysteine and methionine during AD were investigated in lab-scale experiments and reported values ranged between 34%–61% and 42%–48%, respectively (Chen et al., 2019; Li et al., 2020a; Park et al., 2014).

The pathways of organic sulfur degradation, however, is not well established in literature. Du and Parker (2012) investigated the AD of methionine to characterise the generation and degradation of VOSCs and obtain the kinetics of the reactions. The observation at both mesophilic and thermophilic temperatures supported the pathway presented in Figure 2.1 that methyl mercaptan was the direct product of anaerobic methionine degradation. The generated methyl mercaptan was methylated to form dimethyl sulfide. Both methyl mercaptan and dimethyl sulfide were degraded to H₂S by the methanogenic population. As stated before, cysteine directly transforms to H₂S under anaerobic conditions in AD. Proposed VSCs pathways proposed in earlier studies have been recently supported through lab-scale AD of municipal sludge (Li et al., 2020a).

Du and Parker (2013) studied the reduction of organic sulfur in the AD of primary sludge and secondary sludge, separately. The result indicated a dramatic reduction of organic sulfur in digested primary sludge, whereas a lower degradation was observed for digested secondary sludge. The observation was explained by lower degradability of protein in secondary sludge (Novak et al., 2003) which consists mainly of biomass that build up

extracellular polymeric substances (EPS) for active protection against enzymatic attacks (Aichinger et al., 2019). The impact of organic sulfur originated from proteins on H₂S production was demonstrated at full-scale AD (Erdirencelebi and Kucukhemek, 2018). The authors observed a strong correlation between H₂S production with volatile solids of primary sludge. It was concluded that the degradation of proteins was the major source for H₂S formation.

2.3.9.3 *Sulfur, iron and phosphorus interactions*

As AD is becoming more phosphate-rich environments due to the increasing legislative drive to remove phosphorus from wastewater, the interactions of S, P and Fe in AD has attracted more attention in recent years. The preferential binding of iron with sulfide to form iron sulfide is the first reaction controlling the behaviour of iron during AD. Moreover, the lower solubility of iron sulfide in comparison with ferric phosphate precipitates can lead to the release of iron-bound phosphate, which were formed due to iron addition in activated sludge process for chemical phosphorus removal. Recently, the feasibility of multiple reuses of iron salts which are dosed for sulfide control in sewer networks for reuse in H₂S control in the biogas from AD has been demonstrated in batch test (Ge et al., 2013), continuous laboratory-scale urban wastewater system (Rebosura et al., 2018), and full-scale sewer network and treatment plant (Salehin et al., 2019). Supporting the previous findings, the full-scale investigation of Salehin et al. (2019) indicated ~43% reduction in the concentration of H₂S in biogas when FeCl₂ was dosed in the sewer network. Following precipitation with sulfide, iron reacts with phosphate to precipitate as vivianite and various ferrous-(hydroxyl)-phosphate compounds (Roussel and Carliell-Marquet, 2016).

2.3.9.4 *Microaeration process*

Microaeration can be used to reduce H₂S concentration in the biogas through the biological oxidation of sulfide to elemental sulfur by SOB. It is a low-cost method to remove sulfides through the addition of small amounts of air or oxygen into the gas or liquid phases of the anaerobic digester. Empirical

observations on anaerobic digesters operated under microaerobic conditions have shown that microaeration takes place almost solely in the reactor headspace through the growth of SOB in biofilms. The produced elemental sulfur creates yellowish-white deposits in the headspace, including the reactor wall and ceiling (Kobayashi et al., 2012; Ramos et al., 2014a; Rodríguez et al., 2012). It can also partly fall to the liquid phase (Díaz et al., 2011; Krayzelova et al., 2014; Ramos et al., 2014b) which might accumulate in the digester and be biologically reduced back to sulfide or dragged out with the digested sludge.

The majority of literature on microaeration process has dealt with lab-scale (van der Zee et al., 2007) and pilot-scale experiments (Díaz et al., 2011; Ramos et al., 2014b). Nevertheless, Jeniček et al. (2017) evaluated the application of microaeration in full-scale anaerobic digesters, where air was injected to the recirculation stream of the digester. The long-term evaluation showed that H₂S removal efficiency of about 99% could be achieved.

2.3.10 Sludge management

2.3.10.1 Dewatering and storage of dewatered sludge

Dewatering processes such as belt filter presses and centrifuges produce dewatered cake sludge with high solid content. The main purpose for sludge dewatering is sludge volume minimisation and cost efficiency for disposal handling. Dewatering and sludge storage tanks are known to be emissive units (Frechen, 2004). Major odour compounds produced from dewatered sludge are VSCs which are degradation products of sulfur-containing amino acids in proteins (Erdal et al., 2008; Higgins et al., 2006; Novak et al., 2006). AD does not eliminate the odour potential of digested sludge, and the centrifuge dewatering releases proteins making them bioavailable (Chen et al., 2011; Novak et al., 2006). Greater shearing of sludge during dewatering results in a higher release of proteins resulting in elevated VSCs generation during dewatered sludge storage (Muller et al., 2004).

Reducing the bioavailability of proteins by complete degradation of them during AD and using metal binding (Fe and Al) could lower the formation and emission of VSCs (Chen et al., 2007; Gruchlik et al., 2017, 2013; Higgins et

al., 2008). In addition, increasing the storage time (20–30 days) of dewatered sludge decreases the emission of VOSCs due to their degradation to sulfide by methanogens (Chen et al., 2005, 2011; Novak et al., 2006).

2.3.10.2 *Thermal drying and self-heating of dried sludge*

Different technologies have been developed to address the need for the disposal of sewage sludge (Bixio et al., 2006; Fernandez-anez et al., 2014). Among these is thermal drying that is a widely-used technology providing an opportunity for resource recovery as the thermally dried sludge, which contains high organic content, is reduced to ashes and its energetic content is used as refuse-derived fuel (Della Zassa et al., 2019; Poffet et al., 2008; Spinosa and Vesilind, 2001).

The storage of dried sludge can lead to spontaneous temperature increases known as “self-heating” (Bertani et al., 2016; Della Zassa et al., 2019, 2013; Fernandez-anez et al., 2014; Poffet et al., 2008; Zerlottin et al., 2013). This self-heating is sometimes followed by a thermal runaway and a consecutive fire if the dried sludge silo is not emptied rapidly enough (Poffet et al., 2008). This is an important issue as the increasing restriction for disposing of organic material in landfills has led to a rise of the number of incinerators for solid waste in many countries which required the storage of dried solids, including dried sludge, prior to incineration (Hogland and Marques, 2007; Poffet et al., 2008).

The self-heating of dried sludge from municipal WWTPs has been only recently investigated (Bertani et al., 2016; Díaz et al., 2019; Poffet et al., 2008). According to Poffet et al. (2008), a cascade of exothermic chemical events between iron, sulfur and oxygen is responsible for the sudden temperature increase which is initiated first by the oxidation of Fe^{2+} , representing a significant part of the total iron. The heat release of this oxidation combined with the catalytic effect of the resulting Fe^{3+} triggers the autoxidation of the organic matter. In parallel, the oxidation of sulfide, principally captured as FeS , liberates locally a significant amount of heat. Water absorption by the sewage granules also produces further heat because exothermic hydration is taking

place on the dried material. According to Biasin et al. (2014), the best prevention practice for self-heating problem would be the reduction or removal of S and Fe from the dried solid matrix.

2.4 Modelling sulfur transformations

Sulfur transformations considered in state of the art models are reported in Table 2.3 and further described in the following paragraphs.

Table 2.3 Sulfur transformations included in activated sludge and anaerobic digestion modelling studies.

Modelling studies	Unit process / Modelling approach	State variables	Reactions	Main hypotheses and results
(Flores-Alsina et al., 2016)	Anaerobic digestion	SO_4^{2-} S^{2-}	Redissolution of iron phosphate with sulfide Biological reduction of sulfate (Kinetics are selected as such that SRB outcompete traditional microorganisms)	Sulfur in the inlet of AD in form of sulfate ($110 \text{ g S}\cdot\text{m}^{-3}$) H_2S in biogas = 68% (3565 ppm) of influent sulfate Dissolved sulfide = 21% ($23 \text{ g S}\cdot\text{m}^{-3}$)
	Extended ADMI	S^0 FeS	Fe^{3+} reduction with sulfide Precipitation of FeS Dissociation of total sulfide H_2S stripping	Remained sulfate = 11% pH of AD = 6.9
(Solon et al., 2017)	Anaerobic digestion		Redissolution of iron phosphate with sulfide Biological reduction of sulfate by SRB utilizing H_2	Sulfur in the inlet of AD in form of sulfate ($128 \text{ g S}\cdot\text{m}^{-3}$) H_2S in biogas = 65% (5400 ppm) of influent sulfate
	Extended ADMI		Fe^{3+} reduction with sulfide Precipitation of FeS Dissociation of total sulfide H_2S stripping	Dissolved sulfide = 25% ($32 \text{ g S}\cdot\text{m}^{-3}$) Remained sulfate = 10%
	Activated sludge	SO_4^{2-} S^{2-} S^0 FeS	Biological reduction of sulfate with acetate and fermentable substrate as electron donor Chemical oxidation of sulfide and elemental sulfur by NO_3 and O_2 FeS precipitation H_2S stripping	
	Extended ASM2d		Chemical reduction of Fe^{3+} by sulfide under reducing conditions (Firer et al., 2008; Nielsen et al., 2005) FeS precipitation Oxidation of FeS in aerated tank (Gutierrez et al., 2010; Schippers and Jørgensen, 2002)	Total sulfur in the inlet of AD $20 \text{ mg}\cdot\text{L}^{-1}$ and a S:COD ratio $0.004 \text{ g S}\cdot\text{g}^{-1} \text{ COD}$ H_2S in biogas = 5000 ppm
(Hauduc et al., 2018)	Activated sludge and anaerobic digestion	SO_4^{2-} S^{2-} S^0 FeS	Biological reduction sulfate (Barrera et al., 2015) Two-step biological sulfide oxidation using NO_3 and O_2 (Mannucci et al., 2012) Two-step chemical oxidation with O_2 (Nielsen et al., 2005) Degradation of organic sulfur content of COD and biomass results in the formation of	
	Supermodel approach*			

			SO ₄ ²⁻ (not mentioned in the paper but is part of Gujer matrix) Oxidation of FeS in aerated tank Two-step biological sulfide oxidation using NO ₃ and O ₂ Two-step chemical oxidation with O ₂
(Guo et al., 2016)	Anaerobic digestion Extended ADM1 Activated sludge Extended ASM2d	SO ₄ ²⁻ S ²⁻ S ⁰ FeS Organic sulfur	Biological reduction of sulfate Fe ³⁺ reduction with sulfide FeS precipitation Hydrolysis of organic sulfur Oxidation of FeS in aerated tank Chemical oxidation of sulfide and elemental sulfur FeS precipitation
(Parker and Wu, 2006)	Anaerobic digestion Extended ADM1	SO ₄ ²⁻ S ²⁻ MeS MT (methyl mercaptan) DMS (dimethyl sulfide) MAC (methoxylated aromatic compounds)	Biological reduction of sulfate Hydrolysis of MAC Uptake of amino acids leads to formation of H ₂ S, MT and DMS Uptake of MAC and H ₂ S to produce MT Uptake of MAC and H ₂ S to produce DMS Degradation of DMS Uptake of MT to produce H ₂ S
(Donoso-Bravo et al., 2018)	Anaerobic digestion Extended ADM1	Additional state variable S ⁰ , O ₂ , SOB	Sulfide oxidizing bacteria as the only microbial community that consumes oxygen Biological conversion of H ₂ S to S ⁰

*In supermodels a unique set of state variables in both aerobic and anaerobic environments is used

2.4.1 Activated sludge models

In recent years, part of sulfur cycle reactions have been incorporated into activated sludge models by extending ASM2d (Guo et al., 2016; Solon et al., 2017) or through a supermodel approach (Hauduc et al., 2018). These models introduce the following inorganic sulfur compounds as state variables: sulfate, sulfide, elemental sulfur, and FeS. The main reactions incorporated in the activated sludge models are biological sulfate reduction under anaerobic conditions, biological and chemical sulfide oxidation with nitrate and oxygen, redissolution of FeS under aerobic conditions, FeS precipitation, and H₂S stripping. As shown in Table 2.3, reported models consider the fate of inorganic sulfur species while the organic sulfur present in influent wastewater or incorporated into biomass during the activated sludge process has not been considered.

Simulation using activated sludge models showed a small reduction of sulfate to sulfide by SRB in anaerobic zones, followed by reoxidation of sulfide in anoxic and aerobic zones. Simulations with different sulfur concentrations and sulfide fractions in influent wastewater by Hauduc et al. (2018) showed the impact of sulfide oxidation on oxygen uptake rate (OUR), emphasizing the need to consider sulfur reactions for accurate modelling of the oxygen demand.

2.4.2 Anaerobic digestion models

The modelling of sulfur conversions during AD started with the inclusion of the sulfate reduction process in existing AD models, such as ADM1 (Barrera et al., 2015; Fedorovich et al., 2003; Kalyuzhnyi and Fedorovich, 1998; Knobel and Lewis, 2002; Lizarralde et al., 2010; Poinapen et al., 2014). The sulfate reduction process described in these models typically entails microbial kinetics for SRB groups, inhibition due to the unionized form of H₂S, ionic speciation of sulfate and sulfide and liquid to gas mass transfer of H₂S (Ahmed and Rodríguez, 2018). Several groups of SRB have been suggested for modelling the sulfate reduction process. The majority of models, which have been developed for AD of sulfate-rich wastewater denoted by a high S:COD ratio, consider hydrogen and different organic acids as electron donors.

AD of municipal wastewater sludge receives a much lower sulfate concentration where the S:COD ratio is closer to 0.001 g S·(g COD)⁻¹ under most circumstances (Flores-Alsina et al., 2016). Up to the S:COD ratios of 0.06–0.10, Batstone (2006) and Flores-Alsina et al. (2016) have recommended considering H₂ as the only electron source for SRB. This is because when the concentration of sulfate is low, electrons come only from H₂ due to faster bacterial growth (higher μ_{\max}) and higher uptake substrate rate (lower K_S) by hydrogenotrophic SRB. On the other hand, calibration of the model developed for describing AD of sulfate-rich waste stream indicated that considering only hydrogen and acetate as electron donors for SRB activities was a good compromise between model performance and complexity in terms of prediction errors against experimental data (Ahmed and Rodríguez, 2018).

Recently, the modelling of sulfur reactions in AD of municipal wastewater sludge has gained interest due to its interactions with the phosphorus and iron cycles (Table 2.3) (Flores-Alsina et al., 2016; Guo et al., 2016; Hauduc et al., 2018; Solon et al., 2017). Anaerobic sulfur transformations that have been incorporated in the AD model are the biological reduction of sulfate, the release of ferric phosphate with sulfide, reduction of Fe^{3+} with sulfide and precipitation, and gas transfer of H_2S . Although these models have been developed for municipal WWTPs and have not been validated with experimental datasets, the simulations in most cases addressed elevated sulfate concentration (i.e., up to $100 \text{ mg S}\cdot\text{L}^{-1}$) to highlight the capability of predicting H_2S formation.

The modelling of the microaeration process in AD has been done by extending the ADM1 to incorporate the biological oxidation of H_2S to elemental sulfur by SOB. The developed model was calibrated using lab-scale data from anaerobic treatment of sulfate-rich wastewater (Donoso-Bravo et al., 2018; Pokorna-Krayzelova et al., 2017).

Contrary to the sulfate reduction process, modelling the formation, conversion, and degradation of VOSCs in AD have been given less attention. Guo et al. (2016) introduced a state variable for organic sulfur in the ADM1 model to consider the generation of sulfide due to hydrolysis of organics (protein). Parker and Wu (2006) extended the ADM1 model to incorporate the formation of VOSCs due to the degradation of cysteine and methionine. The model did not directly address the concentration of cysteine and methionine; rather the yield was based upon the typical composition of proteins with respect to these amino acids. This model was not validated by experimental data. Du and Parker (2012) further developed a mathematical model for the generation of VOSCs from anaerobic digestion of methionine in AD.

2.5 Summary and perspectives

Sulfur is present under various forms and oxidative states; all of them being reactive depending on the different environmental conditions encountered in WRRF unit processes. A wide range of sulfur forms and their multiphase

nature (i.e. solid, liquid and gaseous state), various biochemical reactions under common environments in unit processes, and interactions with other elements such as nitrogen, iron and organic matter have resulted in a wide range of research topics in literature.

The review of literature shows that not all mechanisms of sulfur transformations are well established. While inorganic sulfur reactions have been widely investigated in several experimental and modelling studies, the knowledge on the organic sulfur pathways is only limited to a few research groups and thus should draw attention. Mathematical models (e.g. ADM1 extensions) are good indicators of this “state of the art” about the mechanisms knowledge. Most modelling studies mainly overlook organic sulfur reactions contrary to inorganic sulfur reactions that have been fully incorporated into available models such as ADM1. Nevertheless, recent studies have highlighted the role of organic sulfur on the formation VSCs in several process treatment units, including primary settling and sludge treatment line. Considering the AD process, there is a need to better understand the mechanisms associated with sulfide formation (sulfate reduction vs organic sulfur hydrolysis). This is even more important since thermally treated sludge may release higher amounts of sulfur-containing amino acids.

Some treatment units have received less attention. Primary settling and primary thickening are major sources of VSCs emission; however, few quantitative experiments were found that elucidate the formation and emission of these compounds VSCs from these units. Developing mathematical models that include both biochemical reactions for the generation of VSCs and emission mass transfer may provide better tools to accurately estimate atmospheric emission from these units. Sludge pretreatment techniques have steadily grown in modern WRRFs in order to improve the performance of the AD process. More work should be performed to investigate further the influence of sludge pretreatment on the fate of sulfur species during AD. Indeed, a variety of pretreatment techniques may lead to different influences on the fate of sulfur. Furthermore, no mathematical models were found that consider the influence of sludge pretreatment on sulfur transformations.

One major limitation concerning sulfur studies would be the lack of full-scale investigations. Except for the monitoring of VSCs emissions from unit processes that have been performed in WRRFs, transformations of sulfur species have been primarily addressed in lab-scale experiments. The lack of calibration and validation of mathematical models with full-scale data is also highlighted for modelling studies. To our knowledge, no published work has performed full-scale calibration of sulfur transformations in municipal AD.

It can be concluded from this extensive review that sulfur management in WRRFs requires a plant-wide approach. In other words, sulfur transformations and interactions with other elements should be evaluated within each unit processes, but also their evolutions throughout the whole treatment line should be considered, which otherwise would result in inaccurate decisions. An example would be the interaction of iron, sulfur, and phosphorus in several unit processes. On the one hand, these interactions reduce the iron needed for chemical phosphorus removal in the activated sludge process and further H₂S removal in the AD process. On the other hand, the interaction of iron, sulfur, and oxygen has been shown to cause self-heating problems in the storage of dried sludge, which have led to the complete replacement of iron with aluminium within plants.

3

Plant-wide investigation of sulfur flows in a water resource recovery facility (WRRF)

3.1 Abstract

Even though sulfur compounds and their transformations may strongly affect wastewater treatment processes, their importance in water resource recovery facilities (WRRF) operation remains quite unexplored, notably when it comes to full-scale and plant-wide characterization. This contribution presents a first-of-a-kind, plant-wide quantification of total sulfur mass flows for all water and sludge streams in a full-scale WRRF. Because of its important impact on (post-treatment) process operation, the gaseous emission of sulfur as hydrogen sulfide (H_2S) was also included, thus enabling a comprehensive evaluation of sulfur flows. Data availability and quality were optimized by experimental design and data reconciliation, which were applied for the first time to total sulfur flows. Total sulfur flows were successfully balanced over individual process treatment units as well as the plant-wide system with only minor variation to their original values, confirming that total sulfur is a conservative quantity. The two-stage anaerobic digestion with intermediate thermal hydrolysis led to a decreased sulfur content of dewatered sludge (by 36%). Higher (gaseous) H_2S emissions were observed in the second-stage digester (42% of total emission) than in the first one, suggesting an impact of thermal treatment on the production of H_2S . While the majority of sulfur mass flow from the influent left the plant through the treated effluent (> 95%), the sulfur discharge through dewatered sludge and gaseous emissions are critical. The latter are indeed responsible for odour nuisance, lower biogas quality, SO_2 emissions upon sludge combustion and corrosion effects.

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3.2 Introduction

Wastewater treatment plants (WWTPs) are no longer viewed solely for protecting the aquatic environment and ensuring the required effluent quality in terms of chemical oxygen demand and nutrients (nitrogen and phosphorus), but instead, they are increasingly regarded as WRRFs with growing interest for energy and resource recovery (Hao et al., 2019; Solon et al., 2019b). The energy recovery is mostly in the form of methane-containing biogas produced from anaerobic digestion (Guest et al., 2009) that can be combusted on-site for heat and electricity generation or cleaned-up and sold (Puchongkawarin et al., 2015). The resource recovery in WRRFs typically relates to phosphorus and nitrogen recovery which are of the interest due to limited resource of phosphorus and substantial energy requirement for nitrogen production and greenhouse gas emission, respectively (Galloway and Cowling, 2002; Marti et al., 2008; Mihelcic et al., 2011; Puchongkawarin et al., 2015).

Sulfur cycle influences both energy recovery and resource recovery, in addition to safety concerns. The methane production can be negatively affected by the competition of sulfate-reducing bacteria and methanogens for hydrogen and acetate (Harada et al., 1994; Muyzer and Stams, 2008; Visser et al., 1993). Moreover, sulfide, especially in un-dissociated form, has an inhibitory effect on anaerobes (e.g. methanogens and acetogens) which are involved in the anaerobic digestion of sludge (Appels et al., 2008; Chen et al., 2008; Guerrero et al., 2016; Yang et al., 2016). Generation of high concentration of H₂S in biogas necessitates further processing of biogas before co-generation due to its corrosive properties (Tchobanoglous et al., 2003); hence lowering the profitability of produced biogas.

The sulfur cycle is strongly linked to that of other elements such as nitrogen and phosphorus through various biological and (geo)chemical processes (Lomans et al., 2002; Puyol et al., 2017; Solon et al., 2019a). These interactions include the reoxidation of iron sulfide in the aeration tank (Schippers and Jørgensen, 2002) and subsequent precipitation of phosphate with released iron (Ge et al., 2013; Gutierrez et al., 2010), effects on the performance of enhanced

biological phosphorus removal (EBPR) process as a result of SRB activity (Baetens et al., 2001; Wanner et al., 1987; Yamamoto-Ikemoto et al., 1994) and exposure to high sulfide concentration (Rubio-Rincón et al., 2017a, 2017b; Saad et al., 2017), the release of ferric ions from ferric phosphate precipitates to precipitate with the sulfide formed in anaerobic digester (due to higher affinity of sulfide for iron than for phosphate) which ultimately lowers H₂S emission from digesters (Ge et al., 2013; Roussel and Carliell-Marquet, 2016), and the simultaneous sulfide oxidation and nitrate reduction known as autotrophic denitrification which has been applied in the development of new processes e.g. SANI (Lau et al., 2006; Wang et al., 2009).

The potential emission of volatile sulfur compounds (VSCs) has given rise to public concern about WRRFs in the vicinity of residential areas (Frechen, 1988; Gostelow et al., 2001; Lebrero et al., 2013). The main volatile sulfur compounds are H₂S, methyl mercaptan (MM), dimethyl sulfide (DMS) and dimethyl disulphide (DMDS) (Bazemo et al., 2021). H₂S has traditionally been used as a surrogate for odour emissions (Gostelow et al., 2001). In a recent study on 6 WRRFs (Fisher et al., 2018), H₂S was confirmed to be the most important volatile sulfur compound in terms of concentration as well as the most dominant odorant based on odour activity value (OAVs). In the latter study, methyl mercaptan was also found to be sensorially important due to low odour detection threshold (ODT). Consequently, the odour collection and treatment are becoming more and more required in WRRFs. Odorous emission occurs in two categories of unit processes (Gostelow et al., 2001); unit processes that only promote emission of previously formed odorous compounds e.g. wastewater inlet works and unit processes in which both formation and emission occur e.g. primary settling/thickening. Based on information from literature, the two important sources of emission are primary treatments and sludge treatment units (Dincer and Muezzinoglu, 2008; Jiang et al., 2017; Lebrero et al., 2011; Ras et al., 2008), whereas the biological treatment units (aerobic, anoxic tanks and secondary settling) were shown to be less emissive (Frechen, 2004), since hydrogen sulfide is biologically and chemically oxidised to sulfate in these oxidative environments.

Despite its importance, less attention has been given to the sulfur cycle when it comes to full-scale studies. Full-scale sulfur studies are challenging because of the multiphase nature of sulfur (i.e. liquid, solid and gaseous states), the wide range of sulfur species and redox states, ranging from sulfide (-2) to sulfate (+6), interrelated conversions and transformations, difficulties in monitoring gaseous emissions from unit processes (e.g. open surface of settling tanks) and challenges in sulfur analysis in liquid samples because of the loss of H₂S due to volatilisation during sample collection.

A key step towards enhancing sulfur studies in WRRFs is quantifying the distribution of sulfur in a plant-wide level as it is helpful for: (i) identifying key sulfur flows, (ii) enabling quantitative comparison of sulfur flows in different streams, (iii) identifying the influence of unit processes on sulfur flows, (iv) identifying key spots for sulfur recovery and/or reducing the negative effects of sulfur and (v) facilitating the correct design of odour collection and treatment facilities as data is typically scarce.

In literature, several studies partly addressed the distribution of sulfur flows in the sludge treatment units, providing valuable information in regard to the effect of unit processes on the sulfur flows (Dewil et al., 2008, 2009; Fisher et al., 2017; Yoshida et al., 2015). The role of secondary sludge thickening in reducing sulfur flows towards successive process treatment was highlighted by Dewil et al. (2008, 2009), who traced flows of total sulfur through four WWTPs. By mapping the sulfur flows in the sludge processing of six WWTPs with different configurations, Fisher et al. (2017) noted higher sulfur recovery in sludge at sites with a combination of primary and secondary treatments, attributing it to the incorporation of sulfate into the biomass and capturing and sending more solids to the sludge processing. Other factors increasing sulfur recovery were higher efficiency of the primary settling tank, thickening and dewatering units, as well as higher iron content in the digesters. Yoshida et al. (2015) traced mass flows of 32 elements including sulfur throughout a WRRF. In their study, gaseous emissions were not measured but instead calculated from mass balances, leading to high uncertainties of gaseous streams, often above 100%. Nevertheless, the comprehensive sulfur management in WRRFs

requires a plant-wide level approach as most unit processes are more or less affected by the sulfur cycle and because sulfur can be distributed to water, sludge and gaseous streams. To our knowledge, quantification of total sulfur mass flows in plant-wide level with simultaneous measurements in water, sludge and gaseous streams has not yet been performed at WRRFs.

In the process of tracking sulfur flows, Fisher et al. (2017) mentioned the limitations in data availability and quality, which highlights the importance of thorough experimental design and data reconciliation for tracking substance flows (Behnami et al., 2016; Meijer et al., 2002, 2015; Puig et al., 2008). To address this issue, Le et al. (2018) developed a mass balanced-based experimental design procedure which provides Pareto-Optimal solutions (i.e. measurement layouts) in terms of their cost and accuracy of key variables. These solutions guarantee the subsequent reconciliation of the collected dataset through data reconciliation. Reconciliation, in this context, means that the value of variables, regardless of being measured or not, would be calculated from other measurements based on the constraints in the form of mass balances. This methodology is applied to a WRRF to trace sulfur flow to ensure the quality of data but also prevent trivial measurements.

This study aims to quantify the distribution of sulfur flows in a municipal WRRF, addressing all unit processes in the water line and sludge line as well as taking into account water, sludge and gaseous streams. The case study plant had an innovative configuration for sludge treatment by having two-stage digestion with intermediate thermal treatment, which enabled the evaluation of sludge post-treatment processes on sulfur flow distributions. The total sulfur mass flows were obtained following three steps: experimental design, data collection and data reconciliation. The sulfur flows throughout the plant were compared, identifying key flows and how they are influenced by unit processes.

3.3 Materials and methods

3.3.1 WRRF under study

The municipal WRRF under study (Figure 3.1) has a capacity of 620000 P.E. (average daily flow during the measuring campaign: $137151 \pm 12573 \text{ m}^3 \cdot \text{d}^{-1}$) and comprises pre-treatment, secondary treatment and sludge treatment. Pre-treatment process consists of screening and grit and grease removal, followed by primary settling equipped with lamella plates. The primary settling effluent is sent to secondary treatment in an integrated fixed-film activated sludge (IFAS) process for the removal of carbon, nitrogen and phosphorus. The secondary treatment is realized in seven compartments: a pre-anoxic reactor, an anaerobic reactor, an anoxic reactor, an aerated reactor with carriers, a de-oxygenation reactor without aeration, a post-anoxic reactor with methanol addition and a post-aeration tank with aluminium chloride addition for chemical phosphorus removal. Effluent from the secondary clarifier passes through filtration as a tertiary treatment before final discharge. 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.

Primary and secondary sludge are pumped to the gravity thickener and dynamic thickener (rotary drums), respectively. The thickened primary and secondary sludge as well as a small fraction of the thickened sludge originating from the rain treatment line are mixed in a sludge buffer tank. Mesophilic anaerobic digestion is performed in two stages, the first of which takes place in two parallel units. The first-stage digested sludge is pre-dewatered by a centrifuge and sent to a thermal hydrolysis unit (165 °C, 8 bar, 30 minutes). The thermally treated sludge is diluted and cooled by adding some treated effluent. The subsequent second stage digestion is performed in a single unit. All three digester tanks have the same volume (6100 m^3). The average sludge retention time of the first stage and second stage are 20 and 30 days, respectively. These digesters are equipped with air injection to the headspace for biological removal of hydrogen sulfide from biogas (microaeration

process). The second-stage digested sludge is sent to a storage tank and then dewatered by a centrifuge. The filtrate of primary and secondary thickening, centrate of pre-dewatering and post-dewatering as well as the reuse water, which is used for internal usages e.g. cleaning, return to the upstream of pre-treatment.

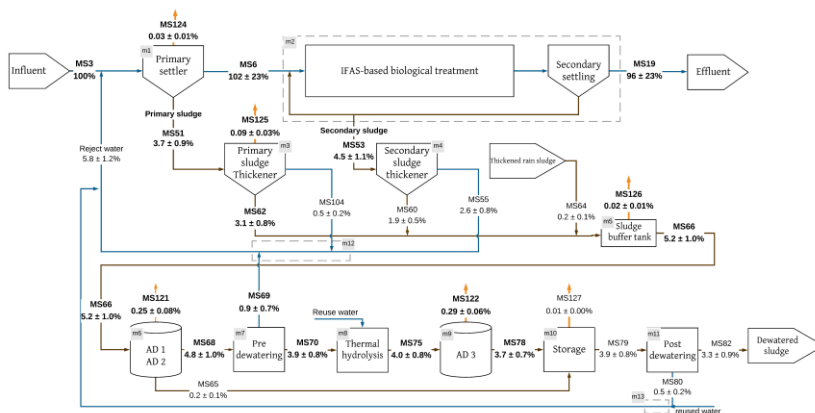


Figure 3.1 Simplified process flow diagram of the WRRF under study. The total sulfur mass flows in the streams are given as a percentage of the total sulfur mass flow in the influent. Water streams, sludge streams and gas streams are shown in blue, brown and yellow lines, respectively. The key variables which need to be reconciled to fulfil the main goal of the measuring campaign are given in bold. The mass balances (#m) were derived around individual and/or combined unit processes; in the latter case, the boundaries are shown by dashed boxes.

3.3.2 Measurement campaign

The measurement campaign was performed during two weeks in June 2019. The measurement layout in this campaign was selected by following an experimental design procedure (Section 3.3.3.1). The sampling method in the water line and sludge treatment lines were composite samples and grab samples, respectively. The water and sludge samples were analysed for total sulfur ($\text{g S}\cdot\text{m}^{-3}$) using inductively coupled plasma optical emission spectrometry (ICP-OES) following NF EN ISO 11885 standard. An overview of the measurements including the sampling points and the number of samples is provided in Appendix C2.

Determination of gaseous sulfur from unit processes where high emission levels were expected was done by monitoring the emitted hydrogen sulfide as the dominant gaseous sulfur species. Other volatile sulfur compounds in the gas phase were assumed negligible, such that hydrogen sulfide was considered as an approximation of total sulfur in the gas phase. This assumption is in accordance with previous studies that hydrogen sulfide is the most dominant and prevailing gaseous sulfur species in WRRFs (Fisher et al., 2018; Gostelow et al., 2001).

The studied unit processes included primary settler, primary thickener, sludge buffer tank, anaerobic digesters and digested sludge storage tank. The selection of these units was based on literature review of emissive units in WRRFs, consulting with practitioners on site about units with odour and corrosion issues and previous experiences with other WRRFs. Although some emission could occur from other unit processes, their contribution is expected to be negligible compared to the emission from the seven most emissive units covered in this study. Sulfur mass flow data for the biogas of anaerobic digesters were collected from the supervisory control and data acquisition system (SCADA), which was programmed to provide sulfur mass flow data based on the measured airflow rate and H₂S concentration of biogas. The H₂S concentration of biogas was analysed by AwiFlex gas analyser (Awite Bioenergie GmbH, Germany). For the other unit processes, which were completely covered, the H₂S concentration was measured in the ventilation pipes. Two types of H₂S meters were installed in parallel to ensure measurement reliability: a myKlearSens H₂S meter (standard range 0–200 ppm, also covering peaks up to 1000 ppm) (Klearios, France) and an OdaLog® H₂S meters (0–200ppm) (App-Tek, Australia). Continuous measurements of gaseous H₂S were performed with a frequency of 5 minutes over two weeks. In order to obtain the mass flow, airflow rates in the ventilation pipes were measured on the first day of the campaign using pitot tube, hot-wire and helix anemometers. The flow rates of the water and sludge streams were collected from the SCADA system.

3.3.3 Quantification of total sulfur mass flows

The main goal of this study was the quantification of the total sulfur flows in the WRRF. Experimental design was applied to select sampling points that guaranteed obtaining a reliable and adequate data set through subsequent data reconciliation. The experimental design and data reconciliation procedures were based on the principle of mass conservation, the conservative quantity being total sulfur.

3.3.3.1 *Experimental design*

Experimental design was applied to choose the measurement layout to obtain the required information with a minimal number of measurements and maximum accuracy. The step-wise experimental design procedure of Le et al. (2018) (see Appendix B1) was followed to this end. This experimental design procedure evaluates possible measurement layouts through redundancy analysis and identifies the list of optimum solutions in terms of cost and accuracy that guarantee the reconciliation of key variables, and thus fulfil the main goal of the measuring campaign. Key variables may be measured or not. Reconciliation means that the value of key variables is calculated from other, measured variables, based on the constraints in the form of mass balances. The applied experimental design procedure consists of 7 steps, which are detailed below.

As a first step, the main goal was translated into key variables, in this case 17 total sulfur mass flows (see Appendix B3). The key variables covered water, sludge and gas lines and were selected as streams which were expected to contain significant sulfur loads and/or to be involved in important sulfur conversions, based on information from literature and expert knowledge. Gas streams which were expected to contain a significant amount of sulfur were added a priori as measured variables (6 streams, see Figure 3.1 or Appendix B5). Five of them were taken up as key variables (see Appendix B3). Second, mass balances for total sulfur flows were defined around individual and/or combined unit processes (see Appendix B4). Third, data inventory was done based on historical data and expert knowledge to estimate the mean values,

expected uncertainties and the measurement cost of potential additionally measured variables (see Appendix B5). The potential additional measured variables were limited to total sulfur concentrations. The flow rate measurements were limited to ones already installed in the plant (30 out of 33 flows were measured). The experimental design procedure then performed a redundancy analysis (step 4–6 of Figure B1 in Appendix B1) and solved a multi-objective optimization problem (step7), minimizing the cost (number of additional measurements) and maximizing the accuracy (precision improvement of key variables). The results were visualised in a Pareto-optimal front that was used to select the measurement layout, i.e. the set of additional measured variables.

3.3.3.2 Data reconciliation

The collected dataset was subject to the mass balance-based bilinear data reconciliation procedure of Le (2019, see Appendix C1). Provided there is sufficient redundancy in the dataset, this procedure provides better estimates for the key variables in terms of their mean value and uncertainty. In addition, the applied procedure performs several gross error detection techniques. The procedure can be divided into three steps, input preparation, data reconciliation and gross error detection, which are detailed below.

The data reconciliation procedure required three input information, namely, key variables, mass balances and pre-processing of the raw measurements. The key variables and mass balances were the same as for the experimental design step. Pre-processing of the raw dataset involves listing the mean values and uncertainty of the measured variables. Determination of variable uncertainties was done taking into account the measurement errors, standard error and sampling method (see Appendix C2).

The extent of data reconciliation was assessed based on two indicators, namely the correction factor and the precision improvement. The correction factor (Δ_x) reflects the accuracy of measurements and is defined as the ratio of the difference between the mean of the measurement ($mean(x)$) and the mean of

the reconciled value ($mean(\bar{x})$) to the mean of the measurement, expressed in percentage.

$$\Delta_x = \frac{mean(x) - mean(\bar{x})}{mean(x)} \quad \text{Eq.3.1}$$

The precision improvement (i_x), also known as the effect of balancing (van der Heijden et al., 1994), is defined as the ratio of the difference between the variance of the measurement ($var(x)$) and the variance of the reconciled value ($var(\bar{x})$) to the variance of the measurement, expressed in percentage. The higher the precision improvement, the more accurate the value of the key variable is known after data reconciliation.

$$i_x = \frac{var(x) - var(\bar{x})}{var(x)} \times 100 \quad \text{Eq.3.2}$$

Furthermore, the dataset was checked for gross errors by testing the dataset against alternative hypotheses: (1) the null hypothesis, H_0 , that no gross error is present, and (2) the alternative hypothesis, H_1 , that gross errors are present. Three tests were incorporated in the data reconciliation procedure, namely, global test, nodal test and measurement test. The global test provides a general signal if there is a potential gross error in the data set, the nodal test narrows this down to individual constraints and the measurement test suggests potential suspected measurements with gross errors. Details of these tests can be found in Le (2019).

3.4 Results and discussion

3.4.1.1 *Experimental design- selection of measurement layout*

Key variables were selected from the water line, sludge line and gas streams (see Appendix B3). The experimental design evaluated whether or not the main goal, subsequent reconciliation of the key variables, was achievable for the given set of measurements and potential additionally measured variables (see Appendix B6). Based on the process flow sheet and the initially available measurements, 17 potentially additional sampling points for the measurement

of total sulfur were identified, corresponding to $2^{17} = 131,072$ potential measurement layouts, i.e. combinations of potential additional sampling points. All measurement layouts were evaluated by the experimental design procedure. From all evaluated combinations, 3534 measurement layouts resulted in the reconciliation of key variables and thus were considered as solutions, seven of them were optimal solutions, lying on the Pareto-front (Figure 3.2, details of Pareto-Optimal solutions including the sampling points of each solution are given in Appendix B6). Each of these solutions is a measurement layout (= combination of possible additional measurements) that guarantees the improvement of defined key variables and is Pareto-optimal in terms of cost and accuracy.

The Pareto-front groups the Pareto-optimal solutions meaning that a lower cost of measurements can only be obtained at the expense of lower accuracy of key variables and vice versa, higher accuracy can be obtained at the expense of a higher cost. Solution 1 was the cheapest measurement layout with 11 additional sampling points for the measurement of total sulfur concentration. Solution 7 with 17 additional measurements provided the highest accuracy and was selected as the measurement layout in this study.

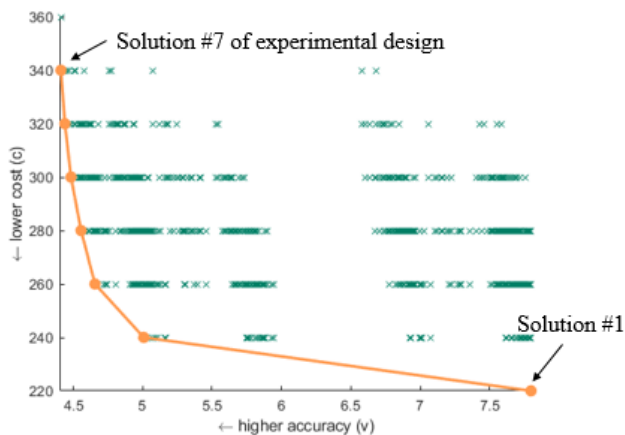


Figure 3.2 Solutions of experimental design. Solutions are expressed in terms of cost (c) and accuracy (v). Each x represents a solution: the line filled circles represent the Pareto-front, containing all optimal solution.

Overall, the experimental design procedure proved successful in identifying Pareto-optimal measurement layouts, balancing the number of measurements and their accuracy, despite handling the given relatively complex process configuration including many possible sampling points.

3.4.1.2 Data Reconciliation- Quality check

A data quality check was performed for the water and, sludge streams, as well as for the gas streams. For each of them correction factor, precision improvement, gross error detection were analysed (for details of these criteria see Section 3.3.3.2 and Appendix C1).

3.4.1.2.1 Water and sludge streams

Data reconciliation results in reconciled (i.e. improved) values for key variables, which could be measured or unmeasured variables. The measured key variables (total sulfur mass flows) in the water and sludge streams had correction factors (Eq. 3.1) ranging from 1% to 15% (Figure 3.3a). The maximum correction factors in the water line and sludge line were seen in the influent wastewater (MS3) with an 8.7% increase in the value of raw measurement and thickened primary sludge (MS62) with a 14.9% decrease in the raw measurement, respectively. This value for the feed and digested sludge

of the first stage digestion (MS66 and MS68) and second stage digestion (MS75 and MS78), which have high retention time, was below 13%. Overall, the correction factors were low, reflecting that all imposed constraints (mass balances) were met by small changes in the values of raw measurements. This indicates good reliability of the raw measurements.

The total sulfur mass flow in the centrate of pre-dewatering (MS69) was an unmeasured key variable (i.e. both flow and total sulfur concentration were unmeasured) for which the calculated mass flow from raw data was 15 ± 92 kg S·d⁻¹. Thanks to data reconciliation, the estimate of this total sulfur mass flow could be improved to 50 ± 40 kg S·d⁻¹, which clearly implies a more precise value (data is given in Appendix C3).

The precision improvement (Eq. 3.2) of the key variables through data reconciliation, quantifying the reduction in measurement uncertainty, is summarized in Figure 3.3b. The average precision improvement of the key variables in the water line and the sludge line was 72%. Total sulfur mass flow in the primary sludge (MS51) had the highest precision improvement (96%). The uncertainty of the total sulfur mass flow in the primary sludge, which was defined as the ratio of the standard error to the mean, was reduced significantly: from 79% in raw measurements to 19% in reconciled value (Appendix C3). Raw measurements from full-scale WRRFs bear uncertainties for various reasons, e.g., influent dynamics, sampling method and measurement errors. For instance, the significant variations in the total sulfur concentrations in the primary sludge during the measurement campaign could be attributed to the different solid content of the grab samples.

The precision improvement of measured variables by data reconciliation is beneficial for further data handling. This improvement relies on redundancy in the measured data set that allowed variables to be estimated in several independent ways from separate sets of constraints imposed by mass balances. The improvement of the standard deviation of raw measurements by data reconciliation techniques was also reported for flow, COD and phosphorus in literature (Behnami et al., 2016; Puig et al., 2008).

The global test, nodal test and measurement test detected no gross errors in the total sulfur mass flows in the water line and the sludge line. It is important to note that the high uncertainty of raw measurements (average of 26%) may influence the detection of gross errors. However, in this case, there is a good agreement between measured values and reconciled values, as expressed through a low correction factor (15%). Given that the latter is lower than the 20% threshold applied by Fisher et al., (2017), it was concluded that there was a good agreement between measured and reconciled values. This constitutes an additional quality check on top of the substantial precision improvement for the water and sludge streams, and the gross error tests.

It can be noted that any error in mass balances could come from flow rate measurement or concentration measurement or from both. In this study, the availability of historical flow rate data over a long period permitted to perform several plant-wide flow balance reconciliations in order to check the quality of flow rate measurement prior to conducting an intensive measurement campaign for obtaining total sulfur mass flows. These pre-evaluation steps, which are not reported in this paper, did not detect any gross error in the flow rate data. This was confirmed during data reconciliation, which allowed to reconcile both the flow rate and total sulfur concentration data. The average uncertainty of sulfur concentration measurements was higher (29%) than the one for flow rate data (9%) (Appendix C2).

3.4.1.2.2 *Gas streams*

The values of the six measured gas streams are given in Appendix C2. Five of them were key variables (MS121, MS122, MS124, MS125, MS126), which means that their value was also reconciled, i.e., calculated from other variables. However, their mean values and uncertainties hardly improved upon data reconciliation, the value of correction factor and precision improvement being less than 1% (Figure 3.3a, b). The very low precision improvement indicates that data reconciliation did not really reconcile ('improve') these variables. The latter behaved as non-redundant variables in the sense that their values could not be estimated from other sets of measurements in the mass balances, even though they were indicated as redundant through the (theoretical) redundancy

analysis. Such variables are referred to as “practically non-redundant” (Narasimhan and Jordache, 2000). The reason why the total sulfur mass flows in the gas streams were practically non-redundant in this study is because of their small mass flows compared to those in the water and sludge streams. In case of primary settling, for instance, the sulfur flow in the gas stream (MS124) and influent wastewater (MS3) were $2.0 \pm 0.5 \text{ kg S}\cdot\text{d}^{-1}$ and $5817 \pm 947 \text{ kg S}\cdot\text{d}^{-1}$, respectively (for more detail see Appendix C3). Indeed, the sulfur mass flows in gas streams were much smaller than the variance of the total sulfur mass flows in water and sludge streams, which for the mentioned example is 2 orders of magnitude smaller.

Because of their practical non-redundancy, gross error detection could not be applied to the measurements of total sulfur in the gas phase. Still, the measurements in these gas streams were considered to be quite reliable because of the nature of the measurement: the units were covered and the air was extracted through a ventilation pipe, in which the H_2S analyser and the flow meter were installed. The reliability of the measurement is illustrated by the relatively low uncertainty range. For instance, the measured value of the total sulfur mass flow in the biogas of second stage digestion (MS122) was $16.9 \pm 2.5 \text{ kg S}\cdot\text{d}^{-1}$ (Appendix C3). For comparison, if this variable would not have been measured but calculated from the available measurements and prevailing mass balances, its value would have been $58.5 \pm 50.1 \text{ kg S}\cdot\text{d}^{-1}$, showing 85% uncertainty. Such high uncertainties, often above 100%, were also reported by Yoshida et al. (2015) when calculating the gaseous emissions from some unit processes in a conventional wastewater treatment plant were calculated from other measurements using mass balances. Similar results were also reported by Fisher et al. (2017) when calculating the total sulfur mass flows in the biogas of an anaerobic digester from total sulfur measurements in the feed and outlet of the digester.

Despite representing smaller mass flows, gaseous sulfur streams are very important due to the problems associated with emitted sulfur even in small quantities. They therefore need to be accurately determined by direct measurements rather than calculated from other measurements through mass

balances. Indeed, it was shown in this study that gaseous sulfur streams cannot be accurately calculated through data reconciliation (i.e., do not qualify as key variables) because their values are very small compared to those of sulfur loads in the liquid and sludge streams.

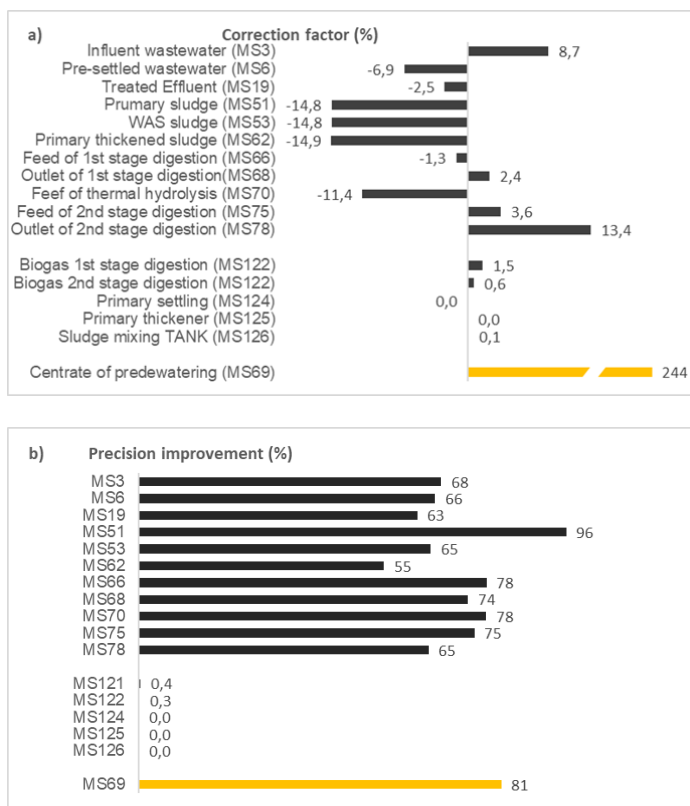


Figure 3.3 The indicators of data reconciliation a) Correction factor b) Precision improvement for quality check of the key variables. Measured key variables (black) and unmeasured key variables (yellow).

3.4.1.2.3 Effectiveness of experimental design and data reconciliation

In this work, the effectiveness of combining mass balance-based experimental design and data reconciliation procedures was demonstrated for the first time for reliable quantification of total sulfur flows and for a relatively complex WRRF. The Pareto-optimal measurement layouts put forward by the experimental design procedure showed valid for the identification of key variables. Data reconciliation and gross error detection provided better

estimates for the total sulfur mass flows in the water line and the sludge line, fitting the total flow and total sulfur mass balances and characterized by a relatively high accuracy.

A balanced data set is a prerequisite for performing any type of process evaluation of full-scale WRRFs. Raw measurements are never fully accurate so the mass balances would not close perfectly without proper data reconciliation and gross error detection. For instance, model calibration and validation on erroneous data would lead to laborious and unjustified model calibrations of kinetics and stoichiometric parameters (Meijer, 2004). It is therefore, essential to reconcile the raw measurements to verify (gross) errors and improve their accuracy before being implemented.

3.4.1.3 *Distribution of total sulfur mass flows*

The plant-wide distribution of total sulfur mass flows using the reconciled data set is summarized in Figure 3.1. In order to facilitate the comparison of streams, the total sulfur mass flows of the streams are expressed as the percentage of the total sulfur mass flows in the influent wastewater (the absolute values are given in Appendix C3). In what follows, the total sulfur distribution in the water line, sludge line and gas streams is discussed consecutively.

3.4.1.3.1 *Sulfur distribution in the water line*

The influent wastewater (MS3, 100% total sulfur mass flow) is combined with the reject water from the sludge treatment line $5.8 \pm 1.2\%$ and then enters the primary settling process (Figure 3.1). Most of the total sulfur mass flow entering primary settling remained in the water line with the pre-settled wastewater (MS6) accounting for $102 \pm 23\%$ of the total sulfur present in the influent. The high mass of sulfur in the pre-settled wastewater indicates that sulfur in the influent wastewater is mostly in soluble form. This is in agreement with the findings of Dewil et al. (2008), who reported that sulfate accounts for 99% of the sulfur in the influent wastewater.

After secondary treatment, comprising IFAS-based biological treatment (including anaerobic/anoxic/aerobic zones) and secondary settling, the water

stream (MS19) still comprised $96 \pm 23\%$ of the total sulfur mass flow in the incoming wastewater (MS3). This is within the range of 78–98% reported by Fisher et al. (2017), including observations from six WWTPs with different process configurations and influent wastewater flow rate. Overall, the amount of total sulfur in the water line is hardly affected by conventional (secondary) water treatment processes. Still, sulfur discharges through the dewatered sludge and gaseous emissions are critical because of their important impact on (post-treatment) process operation.

3.4.1.3.2 *Sulfur distribution in the sludge line*

The primary sludge (MS51) and secondary sludge (MS53) streams contained about equal amounts of total sulfur mass flows, amounting to $3.7 \pm 0.9\%$ and $4.5 \pm 1.1\%$ of the influent wastewater, respectively. In contrast, the distribution of sulfur during thickening was different for primary and secondary sludge, despite their similar performance in retaining total solids in the sludge line vs the filtrate - both the primary and secondary thickeners had an average thickened solid content of $60 \text{ g}\cdot\text{L}^{-1}$. In the primary thickener, the majority of sulfur was directed to the thickened sludge (MS62, $3.1 \pm 0.8\%$) rather than filtrate (MS104, $0.5 \pm 0.2\%$), while the secondary thickener resulted in a higher release of sulfur (57%) to the filtrate (MS55, $2.6 \pm 0.8\%$) compared to thickened sludge (MS60, $1.9 \pm 0.5\%$). The long HRT in the primary thickener (gravity thickening) promoted the biological formation of sulfide, which may be emitted to the hydrogen sulfide or react with present metals and form metal sulfide; hence reducing the soluble sulfur in the filtrate. As for the secondary sludge, soluble sulfur is expected to be in the form of sulfate due to the redox conditions in the aeration zone as the last step of biological treatment. Moreover, no significant microbial activity is expected in secondary thickener (rotary drums), in which physical separation between liquid and solid forms would take place. Of the total sulfur mass flow entering secondary thickening, 57% was directed to filtrate that is in agreement with the 50–68% values reported by Fisher et al. (2017). A lower value (38%) was reported in the gravity belt thickener by Dewil et al. (2008). The direction of sulfur to filtrate rather than the thickened sludge makes the dynamic thickener an important

unit for reducing sulfur mass flows to the subsequent sludge treatment units (e.g., anaerobic digestion) as was also concluded by (Dewil et al., 2008). Besides thickened primary sludge and secondary sludge, the sludge buffer tank receives a fraction of thickened rain sludge (MS64, $0.2 \pm 0.1\%$).

The contributions of different sludge sources, namely primary thickened sludge, secondary thickened sludge and thickened rain sludge in terms of total sulfur flow to sludge buffer tank were about 60%, 36% and 4%, respectively. A correlation between the primary sludge volatile solids content – as a source of organic sulfur in the form of proteinaceous matter - and the hydrogen sulfide concentration in the biogas has been suggested by Erdirencelebi and Kucukhemek (2018). This study strengthens their findings through the quantification of the total sulfur flow in the primary thickened sludge, which clearly represents a considerable contribution to anaerobic digestion.

During the first stage digestion, the total sulfur mass flow decreased from $5.2 \pm 1.0\%$ in the feed to $4.8 \pm 1.0\%$ in the digested sludge, implying that sulfur mostly remained in the digested sludge. Note that the H_2S loss to biogas is reduced by microaeration, which converts H_2S to elemental sulfur. The elemental sulfur produced may partly attach to the walls and ceilings, or leave the reactor with digested sludge. Other studies have not detected significant flows of sulfur in the biogas during the studying of sulfur flows in anaerobic digestion (Dewil et al., 2008; Du and Parker, 2013; Yoshida et al., 2015). During the digestion process, the formation of metal sulfide and hydrogen sulfide are expected due to the reduction of sulfate and also degradation of organic sulfur especially from primary sludge source (Du and Parker, 2013). Sulfur removed in the centrate following the pre-dewatering of the anaerobically digested sludge (MS69) was $0.9 \pm 0.7\%$ of the sulfur in the influent wastewater, showing that 18% sulfur entering pre-dewatering process being removed in the centrate. Fisher et al. (2017) reported the range of 0.5–23.1% and the range was attributed to different solid separation efficiencies of the dewatering processes. On the other hand, other studies noted minimal sulfur in the centrate of the dewatering process that was explained by

the formation of insoluble metal sulfide complexes in the anaerobic digester (Dewil et al., 2008; Yoshida et al., 2015).

Despite a small increase in the mass of sulfur during thermal hydrolysis, which was due to the addition of reuse water containing sulfur, the total sulfur mass flow during the thermal hydrolysis remained the same with $3.9 \pm 0.8\%$ and $4.0 \pm 0.8\%$ sulfur mass flow in the feed (MS70) and outlet (MS75) of this unit, respectively. Liu et al. (2015) studied the release of sulfur-containing odorants during the pyrolysis of sewage sludge and noted that the formation of H_2S , the predominant odorant, at $150\text{ }^\circ\text{C}$ was insignificant. Although not yet addressed in the literature, the solubilisation of organic sulfur during thermal hydrolysis may promote the formation and emission of hydrogen sulfide to the biogas of subsequent anaerobic digestion.

Sulfur mass flow in the second stage of anaerobic digestion following thermal hydrolysis decreased from $4.0 \pm 0.8\%$ in the feed (MS75) to $3.7 \pm 0.7\%$ in the digested sludge (MS78). This implies that 7% of the sulfur in the feed of second-stage digestion unit ends up in the biogas, which is considerable. The further reduction in the total sulfur flow in the sludge line through second-stage digestion is particularly interesting knowing that the degradation of sulfur species already occurred during the first-stage digestion, which indicates a possible effect of sludge thermal treatment. The hydrolysis of organic material during thermal hydrolysis especially in case of organic sulfur present in the protein of biomass. According to Du and Parker (2013), a small fraction of protein in secondary sludge, which are likely the major contributors of organic sulfur in these streams, are not very well biodegradable in anaerobic digestion, so it could be that this source of sulfur was not degraded during the first stage digestion. While proteins are protected from the enzymatic hydrolysis during anaerobic digestion by the cell wall, thermal pre-treatment destroys the cell walls and makes the proteins accessible for biological degradation (Neyens and Baeyens, 2003). In another study, Bougrier et al. (2008) noted strong solubilisation of protein (95%) during thermal hydrolysis at $170\text{ }^\circ\text{C}$ and Brooks (1970) reported 40–60% solubilisation of organic material. Therefore, elimination of the rate-limiting factor in anaerobic digestion, i.e. hydrolysis,

(Wilson and Novak, 2009) and the cell destruction might explain the increasing removal of sulfur in the second stage digestion.

The total sulfur mass flows removed through the centrate of post-dewatering (MS80) units was $0.5 \pm 0.2\%$, showing 13.6% sulfur removal through centrate of post-dewatering. This high sulfur removal was due to the solubilisation of sulfur happening during thermal hydrolysis. Overall, the total sulfur mass flow in the reject water i.e., the centrates of primary sludge and secondary sludge thickening, pre-dewatering and post-dewatering processes, accounted for $5.8 \pm 1.2\%$ of the sulfur mass flow in incoming wastewater. The amount of total sulfur mass flow in the post-dewatered sludge (MS82) was $3.3 \pm 0.9\%$ of the total sulfur in the influent wastewater.

The unique configuration of the two-stage anaerobic digestions with intermediate pre-dewatering, thermal hydrolysis and post-dewatering in the case study plant caused a 36% decrease in the total sulfur mass flow. Lower sulfur content in the dewatered sludge is desirable especially when the sludge is further used for co-combustion with other fuels in power stations or cement kilns, or incineration in dedicated sludge combustors because lower sulfur dioxide (SO₂) is emitted with the fuel gases (Van de Velden et al., 2008; Werther and Ogada, 1999).

3.4.1.3.3 Gaseous sulfur streams

The amount of H₂S gas streams monitored in this study accounted for $0.7 \pm 0.1\%$ of the total sulfur mass flows in the influent wastewater (Figure 3.1). Despite representing only small fractions of the total sulfur load, H₂S emissions are critical because they cause odour nuisance, lower biogas quality and corrosion. Of the total gaseous sulfur emissions, 78% were related to the biogas of the first stage and the second stage digestion and 22% from fugitive emissions during primary settling, primary thickening, sludge buffer tank and digested sludge storage tank. Note that only the H₂S emissions from these unit processes were measured, the H₂S emission from other unit processes such as biological treatment was assumed negligible. No significant odour was detected in the biological treatment and clarifiers during the measurement

campaign which supports previous studies that showed the lowest emission from these units (Frechen, 2004) and justifies the decision on the 7 most important emissive units in this study. This study enabled the full quantification of gaseous sulfur emissions and their comparison.

The majority of H₂S mass flow was detected in the second stage digestion (MS122, 0.29 ± 0.06%) and first stage digestion (MS121, 0.25 ± 0.08%), which accounted for 78% of the total mass of emitted H₂S. During the measurement campaign, the range of H₂S concentration in the biogas of first stage and second stage digestion was 356–1035 ppm and 1299–1555 ppm, respectively. The high mass flow of gaseous H₂S in the second-stage digestion is in line with the above mentioned potential effect of sludge thermal treatment on H₂S formation. Two other emissive unit processes were primary thickener (MS125) and primary settling (MS124) with 0.03 ± 0.01% (13–187 ppm) and 0.09 ± 0.03% (0–22 ppm) of the total sulfur mass flows in the influent, respectively. The extent of H₂S emission from the primary settling tank depends upon both the concentration of dissolved sulfide in the incoming wastewater and the production of sulfide within the settler. Bazemo et al. (2021) estimated that majority (64%) of H₂S emission from the primary settling tank is related to dissolved sulfide formed in sewer network and present in incoming wastewater. The long HRT of the primary thickener and reductive conditions promotes the formation and subsequent emission of gaseous sulfur which was also accompanied by a drop in the ORP and pH of the samples taken from thickened sludge. The mass of emitted sulfur from sludge buffer tank (MS126) was 0.02 ± 0.01% (60–283 ppm). It was expected to have more emission due to the presence of biomass from thickened secondary sludge that could increase the reduction of remained sulfate and organic sulfur. One explanation could be the presence of iron in the thickened rain sludge which might lead to the formation of FeS and thus less sulfur in gas streams. The lowest sulfur flow to the gas streams was detected in the digested sludge storage tank (MS127) with 0.01% (1–21 ppm).

3.4.1.4 *Implications for research and practice*

In this work, a thorough investigation of total sulfur flows was performed, identifying key sulfur flows over individual unit processes in a plant-wide context and pointing out their relative importance. The large majority of sulfur mass flow in the influent ($96 \pm 23\%$) left the plant through the treated effluent. The sulfur in dewatered sludge accounted for $3.3 \pm 0.9\%$, while gaseous emissions amounted to $0.7 \pm 0.1\%$. Despite forming a small portion of total sulfur flow, the gaseous sulfur flows are highly undesirable and therefore require collection and treatment prior to release to the atmosphere or utilisation of biogas in co-generation.

The detailed quantification of gaseous streams showed that most (gaseous) H_2S emissions, namely 42% of total H_2S emission, were produced in the second-stage anaerobic digester, ending up in the biogas. The higher H_2S emissions from the second-stage digester compared to first stage (35.7%) suggests an impact of thermal treatment on the production of H_2S . Further investigation on the mechanisms and conversion of sulfur species –organic and inorganic species– are required for understanding this trend and applying control techniques as high H_2S flows reduce the quality of biogas and cause corrosion problems on downstream equipment. H_2S was also produced in the primary thickener (13.5%) and the primary settler (4.9%). The relatively high H_2S emissions from the primary (gravity) thickener suggests the formation and emission of hydrogen sulfide during relatively long hydraulic retention time. In view of accurately modelling H_2S emissions, H_2S formation under anaerobic conditions thus needs to be considered to overcome the limitation of currently available H_2S emission models (Santos et al., 2013).

When the treated sludge is used for combustion, it is beneficial to have low sulfur content in view of SO_2 formation upon combustion. In this regard, the secondary (drum) thickener proved effective for limiting sulfur flows towards the subsequent sludge treatment by directing majority of entering sulfur flows towards the centrate. Sludge treatment through two-stage digestion configuration with intermediate thermal hydrolysis and pre and post

dewatering also considerably reduced (by 36%) the total sulfur content remained in dewatered sludge.

The sulfur mass flows obtained for the WRRF in this study could be used as an indication of plant-wide sulfur distribution in other WRRF facilities and configurations. Nevertheless, several specificities of WRRF in this study should be taken into account. For instance, the WRRF under study has two-stage digestion with intermediate thermal hydrolysis whereas most WRRFs have only one digester, in some cases preceded with thermal hydrolysis. As a result, the sulfur content in the final sludge stream is lower in this study.

3.5 Conclusions

Sulfur mass flows in a full-scale WRRF were quantified for the first time on a plant-wide level, assessing liquid, sludge and gas streams simultaneously.

- Total sulfur was demonstrated to be a conservative quantity, allowing the reliable quantification of total sulfur flows through mass balance-based experimental design and data reconciliation procedures.
- Data reconciliation and gross error detection provided better estimates for the total sulfur mass flows in the water line and the sludge line, fitting the total flow and total sulfur mass balances and characterized by a relatively high accuracy.
- The water treatment line hardly affected the incoming sulfur flows as sulfur was mostly removed through the treated effluent as sulfate. Amounting to about 8% of the sulfur in the influent wastewater, the sulfur flows in the primary and secondary sludge caused high H₂S emissions in primary thickener and in both stages of anaerobic digestion. In particular, the relatively higher H₂S emissions from the second-stage digester suggested an impact of the thermal treatment resulting in increased H₂S production.
- Gaseous sulfur loads representing a relatively low mass are non-redundant in practice, which means that their values cannot be accurately determined from measured variables and mass balances

through data reconciliation. Gaseous H₂S emissions therefore need to be measured directly for obtaining reliable data.

4

Evolution of sulfur compounds throughout water treatment line of a municipal WRRF

4.1 Abstract

This study quantifies sulfur speciation in domestic wastewater and investigates the evolution of sulfur species through the water treatment line of a municipal water resource recovery facility (WRRF). Results show that total sulfur in influent wastewater was mainly in the soluble fraction (80%, 28 ± 2.8 mg S·L⁻¹) and in the form of sulfate (ca. 80–100% of the total soluble sulfur). Analyses performed on grab samples of influent wastewater gave a range of sulfide concentration between 0–3.6 mg S·L⁻¹, while sulfite and thiosulfate were not detected. The speciation of sulfur in primary effluent and treated effluent remained similar to influent wastewater. No significant change in concentration of sulfate was detected in anaerobic, anoxic and aerobic zones of secondary treatment. Moreover, the concentration of sulfide through secondary treatment remained low (<0.2 mg S·L⁻¹), indicating that conversions of sulfate and sulfide during secondary treatment were not significant. Part of the influent total sulfur was directed towards the sludge treatment line via the primary and secondary excess sludge that accounted for $3.7 \pm 0.9\%$ and $4.5 \pm 1.1\%$, respectively. The analysis of primary sludge revealed that its characteristics were highly variable over time, reflected in a wide concentration range of sulfate (26 ± 10 mg S·L⁻¹) and oxidation-reduction potential (-167 ± 135 mV) as well as solid contents in grab samples. This variation, which was attributed to influent dynamics and rain events during the measurement campaign, affected the retention time of settled sludge in the settling tank and prevented the evaluation of biological sulfate reduction in the primary settling tank. Nevertheless, a decrease in the concentration of sulfate and an increase in sulfide concentration was observed in the primary sludge. Significant biological sulfate reduction and hydrogen sulfide formation occurred in the primary thickening unit due to longer hydraulic retention time and more reducing conditions, leading to low sulfate concentration and high sulfide concentration in thickened sludge. The average concentration of H₂S in the headspace of the primary settling tank and primary thickener were 3.4 ppm and 68 ppm, respectively. Higher H₂S emission from the primary thickener was in accordance with more substantial reactions in this unit.

4.2 Introduction

Sulfur is a highly reactive element that is present in the environment in numerous forms. In domestic wastewater, sulfur is present in both organic and inorganic forms. Domestic wastewater typically contains organic sulfur. It is predominantly from proteinaceous matter ($3\text{--}6 \text{ mg S}\cdot\text{L}^{-1}$), and can also originate from sulfonates in household detergents (about $4 \text{ mg S}\cdot\text{L}^{-1}$) (Boon, 1995; Gostelow et al., 2001). Inorganic sulfur is in the form of sulfate and/or sulfide depending on the sewer conditions (rising or falling main, retention time) (Batstone et al., 2018). The typical concentration of sulfate in domestic wastewater is $30\text{--}60 \text{ mg S}\cdot\text{L}^{-1}$ (Boon, 1995; Gostelow et al., 2001). The range of sulfide concentration in the inlet wastewater of 11 WRRFs in India was ranged between 1.1 and $14.8 \text{ mg S}\cdot\text{L}^{-1}$ (Prathna and Srivastava, 2021).

The sulfur cycle is complex which arises from a broad range of oxidation states from -2 (completely reduced) to $+6$ (completely oxidised), a network of biological and chemical reactions and a close link between the sulfur cycle and other element cycles such as carbon and nitrogen cycles (Muyzer and Stams, 2008). The biological sulfur cycle consists of oxidative and reductive sides. Under the reductive condition, sulfate is biologically reduced to sulfide by chemolithotrophic sulfate-reducing bacteria (SRBs), while on the oxidative side, sulfide is biologically oxidised to elemental sulfur and sulfate in a two-step process by sulfide-oxidising bacteria (SOB). In addition, microorganisms take sulfate as a nutrient and reduce it to sulfide, which is then incorporated into sulfur-containing amino acids and enzymes (Muyzer and Stams, 2008; Tang et al., 2009), a process known as assimilatory sulfate reduction. The chemical reactions involving sulfur species include chemical oxidation of sulfide with oxygen to elemental sulfur and sulfate, chemical reduction of ferric iron in the presence of sulfide under anaerobic condition, the reaction of sulfide with iron and precipitation as iron sulfide and re-oxidation of iron sulfide to ferric oxide and sulfate under aerobic conditions (Firer et al., 2008; Gutierrez et al., 2010; Lewis, 2010; Nielsen et al., 2005; Schippers and Jørgensen, 2002). A key physical reaction of sulfur species is the emission of hydrogen sulfide (H_2S) from liquid to the gas phase. This transfer occurs when

the concentration of H_2S exceeds the saturation concentration given by the thermodynamic equilibrium (Henry's law). In this situation, the emission of H_2S is governed by liquid to gas mass transfer, and the most important factors are the interfacial area and the hydrodynamic conditions in the liquid phase (Carrera et al., 2016; Springer et al., 2020).

Water resource recovery facilities (WRRFs) are affected by the transformations of sulfur species. The most important influence of sulfur in WRRFs is the generation and emission of H_2S , which is associated with nuisance odours, toxicity, severe corrosion to infrastructures, lower biogas quality and quantity and additional costs for construction of ventilation system, deodorisation unit and biogas treatment. In recent years, on the other hand, the application of sulfur transformation and interactions with other cycles such as carbon, nitrogen, phosphorus and iron has attracted attention in integrated management of sewer network and wastewater treatment plants and the development of new process configurations. After several lab-scale (Ge et al., 2013; Gutierrez et al., 2010) and pilot-scale investigations (Rebosura et al., 2018), Salehin et al. (2019) demonstrated the feasibility of multiple reuses of iron salts dosed as a sulfide control method in sewer network for removal of phosphate from activated sludge and sulfide during anaerobic digestion in the downstream wastewater treatment plant. Another example of a process developed based on sulfur cycle is the Sulfate Reduction Autotrophic Denitrification Nitrification Integrated (SANI®) process, a novel process developed for sulfate removal in wastewater through integration of the sulfur cycle with the treatment of organic carbon and nitrogen (Wang et al., 2009; Wu et al., 2016).

Primary settling can stimulate the emission of substances previously generated in the sewer network. In addition, when the sludge removal is not fast enough, the prevalence of anaerobic condition in the sludge blanket could promote the generation and emission of volatile sulfur compounds (VSCs) (Carrera-Chapela et al., 2014). In a recent study, the mechanisms of formation and emission of VSCs from a primary settling tank of a municipal WRRF was investigated by Bazemo et al. (2021). The authors reported that that H_2S and

methyl mercaptan (MM) concentrations were dominant in the off-gas of primary settling, while dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) were under their odour threshold. Further investigations showed that H₂S emission was mainly the result of stripping of dissolved sulfide (64%) generated in the sewers, while MM emission was more dependent on the conditions in the primary clarifiers (only 16% stripping).

Several studies have investigated the presence and activity of SRBs in activated sludge process. The relative presence of SRB in domestic WRRFs is below 10% obtained from fluorescence in situ hybridization (FISH) technique (Manz et al., 1998; Nielsen and Nielsen, 2002), and is independent of the reactor configuration, the organic loading and the influent sulfate concentration (Lens et al., 1995). According to Schramm et al. (1999) SRBs are unable to grow and multiply in aerated activated sludge, and their presence depends primarily on continuous re-inoculation from other sources such as the sewer network, return sludge from secondary settling tanks and anaerobic digesters. Lens et al. (1995) investigated the activity of SRBs in various systems, and reported an average sulfate reduction rate of 0–3.1 mg SO₄²⁻·(g VSS·h)⁻¹ for activated sludge systems. Other studies have also detected biological sulfate reduction in activated sludge (Baetens et al., 2001; Ingvorsen et al., 2003; Yamamoto et al., 1998, 1991), while some were unable to detect this reaction in activated sludge systems (De Beer et al., 1998; Schramm et al., 1999).

Biological oxidation of sulfide can take place under anoxic and aerobic conditions using either nitrate/ nitrite and oxygen, respectively. In general, at high biomass concentration, the biological oxidation of sulfide is prevailing over chemical oxidation with oxygen (Hauduc et al., 2018). Lens et al. (1995) observed high biological sulfide oxidation rates (11.6–131.7 mg HS⁻·(gVS·h)⁻¹ in biofilms and mixed liquors of activated sludge when 60 mg S·L⁻¹ HS⁻ was supplied.

In the absence of oxygen, the activity of SRBs could recover in the secondary settling tank. According to Sekyiamah et al. (2008), the reduction of sulfate by SRBs were reportedly responsible for different concentrations of sulfide

between the bottom and top layers of secondary settling tank (98 ppb vs. 0.73 ppb).

Anaerobic conditions are inherent to sludge treatment processes including sludge thickeners. Due to the high organic matter concentrations (in the range 5–20% w:w) and the absence of O_2 or NO_3^- , these units often experience high sulfide emission above the odour threshold concentrations (Lebrero et al., 2011). Despite the high emission, studies on the sulfur compounds responsible for emission in such units are relatively scarce.

Treatment of sulfur-induced pollution has received rather limited attention in contrast to other cycles including organic compounds, nitrogen and phosphorus (Lens, 2020). Given the abovementioned challenges and opportunities associated with sulfur cycles, there is a need for more quantitative investigation, for instance, on fractionation of sulfur species in influent wastewater, their transformations within process treatment units of WRRFs. This approach will be beneficial in several ways including identifications of emission sources and possible processes leading to it. Therefore, in this study, the analysis of inorganic sulfur species was performed during a two-week measurement campaign in a WRRF to provide sulfur speciation and evolutions in process treatment units under different oxidation-reduction conditions. The detailed objectives of this work are to investigate:

- a. the speciation of inorganic sulfur the influent wastewater, primary effluent and treated effluent;
- b. the variation of sulfate and sulfide mass flows in influent wastewater;
- c. the occurrence of biological sulfate reduction in primary settling and primary thickening;
- d. the emission of hydrogen sulfide from primary settling and primary thickening; and
- e. the occurrence of sulfate and sulfide transformations under anaerobic, anoxic, aerobic conditions of secondary treatment.

4.3 Material and methods

4.3.1 WRRF under study

The municipal WRRF under study (Figure 4.1) has a capacity of 620,000 P.E. and comprises pretreatment, secondary treatment and sludge treatment. Pretreatment stage consists of screening and grit and grease removal, followed by primary settling equipped with lamella plates. The primary settling effluent is sent to secondary treatment in an integrated fixed-film activated sludge (IFAS) process for the removal of carbon, nitrogen and phosphorus. The secondary treatment is realised in seven compartments: a pre-anoxic reactor, an anaerobic reactor, an anoxic reactor, an aerated reactor with carriers, a de-oxygenation reactor without aeration, a post-anoxic reactor with methanol addition and a post-aeration tank with aluminium chloride addition for chemical phosphorus removal. Effluent from the secondary clarifier passes through filtration as a tertiary treatment before final discharge. Primary and secondary sludge are pumped to the gravity thickener and dynamic thickener (rotary drums), respectively. The filtrate and centrate from sludge treatment are returned to the upstream of pretreatment. The whole plant configuration could be found in Section 3.3.1. 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.

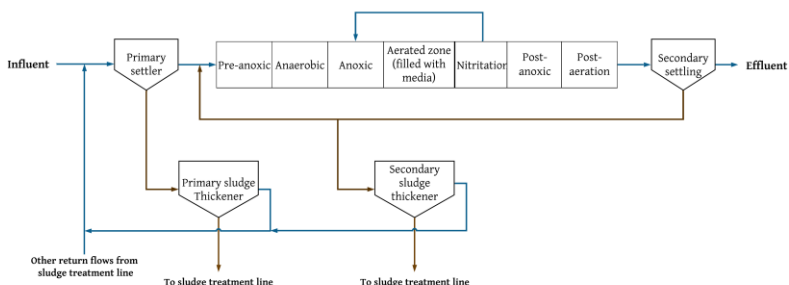


Figure 4.1 Simplified process flow diagram of water treatment line and thickening process of studied plant.

4.3.2 Measurement campaigns

The measurement campaign was performed during two weeks in June 2019. Wastewater influent, primary effluent and treated effluent were sampled (composite) and analysed for total sulfur, soluble sulfur, sulfate, thiosulfate and sulfite. An auto-sampler performed sampling with higher frequency from the inlet of the primary settling tank during 6th and 12th of June. These samples were analysed for sulfate and sulfide. Inlet of primary settling and different secondary treatment zones were sampled (grab sampling) and analysed for sulfate and sulfide. The primary settling, secondary settling and thickened primary sludge were sampled (grab sampling) and analysed for total sulfur, soluble sulfur, sulfate, sulfide, solid content, oxidation-reduction potential (ORP), pH and solids content.

Total sulfur was analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) following NF EN ISO 11885 standard. Sulfate and sulfide were measured using Merck 114548 and Hach-Lange LCK 653 vials, respectively. In order to fix dissolved sulfide and prevent the emission of H₂S, zinc acetate and sodium hydroxide were added to the water and sludge samples, respectively. Sulfite was analysed immediately after sampling by semi-quantitative colorimetric method using Merck test kits (1.10013). Thiosulfate was measured using semi-quantitative iodometric method using chemistry test kits (K-9507). Temperature, pH and ORP were measured at the

sampling time using PHC10103 Hach Lange probe with HQ40d Hach Lange portable meter.

The concentration of hydrogen sulfide in the headspace of primary settling and primary thickener were continuously measured using the myKlearSens H₂S meter (Klearios, France) and OdaLog[®] H₂S meters (App-Tek, Australia). The headspace of these units was covered and the H₂S meters were installed on the ventilation pipe of each unit. Continuous measurements of gaseous H₂S were performed with a frequency of 5 minutes over two weeks.

Additionally, the characteristics of influent wastewater in terms of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand 5 days (BOD₅), Total Suspended Solids (TSS), Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP) were collected from daily measurements performed for plant monitoring between January 2018 and November 2020 (composite samples).

4.4 Results and discussion

4.4.1 Influent wastewater

An overview of influent characteristics in terms of COD, BOD₅, TSS, TP and TKN mass flows obtained from daily data between January 2018 and November 2020 is presented in Table 4.1. The coefficients of variation were between 10% and 14%, indicating relatively stable characteristics of the influent wastewater over time. Additionally, to better understand influent wastewater characteristics, typical ratios of pollutants (e.g. BOD₅/COD) were compared with reported values for municipal wastewater in literature (Pons et al., 2004; Rieger et al., 2012; Filali et al., 2015). While a lower ratio was observed for BOD₅/COD and TP/COD, the TN/COD and TSS/COD ratios were more similar to reported mean values. Given the reported coefficient of variation, all ratios were within the reported ranges. Discharge of industrial effluents to the sewer network may be responsible for the higher COD in influent wastewater, leading to lower values for BOD₅/COD and TP/COD in the studied plant.

Table 4.1 Overview of influent wastewater characteristics for the daily data collected between January 2018 and November 2020, and comparison of typical ratios with those reported for municipal wastewater in literature.

	This study		This study		(Rieger et al., 2012)		(Pons et al., 2004) ²		Fiali et al., 2015) ²	
	Mean kg·d ⁻¹	CV ¹ %	Mean mg·mg ⁻¹	CV %	Mean mg·mg ⁻¹	CV %	Mean mg·mg ⁻¹	CV %	Mean mg·mg ⁻¹	
COD	53015	12								
BOD ₅	18288	13	BOD ₅ /COD	0.34	6	0.48	11	0.43	27	0.43
TSS	24120	14	TSS/COD	0.5	10	0.5	18	0.5	26	0.5
TKN	5838	11	TKN/COD	0.11	8	0.095	17	0.094	70	0.1
TP	677	10	TP/COD	0.013	8	0.016	22	0.016	31	0.011

¹ Coefficient of variation (CV) is calculated as the ratio of standard deviation to mean value.

² These ratios were reported for WRRFs in France.

The characteristics of influent wastewater in terms of total sulfur, soluble sulfur and sulfate obtained over two weeks are presented in Table 4.2. Results show an average total sulfur concentration of 36 mg S·L⁻¹ in the influent wastewater, predominantly in the soluble fraction (81%) in the form of sulfate. Fractionations obtained from other WRRFs in France also revealed the high fraction of soluble sulfur (82%) of which sulfate accounted for lower fraction (80%) compared to this study (~100%) (internal communication). Seven sulfide measurements performed on grab samples ranged between 0 and 3.54 mg S·L⁻¹, with a flow proportional mean value of 0.76 mg S·L⁻¹. Thiosulfate (S₂O₃) and sulfite (SO₃) were not detected in the influent wastewater over the measurement period. A much higher sulfate concentration, as high as 500 mg·L⁻¹ (Lens et al., 1998), could be expected in case of industrial wastewater discharge and seawater infiltration (Van Den Brand et al., 2015).

Table 4.2 Concentrations of total sulfur, soluble sulfur and sulfate in the influent wastewater of the studied plant obtained in 2019.

	4/6	5/6	6/6	7/6	11/6	12/6	13/6	Flow proportional mean
Total sulfur (mg S·L ⁻¹)	46				47	23		36
Soluble sulfur (mg S·L ⁻¹)	32				42	20		28
Sulfate (mg S·L ⁻¹)	32		32		41	20	23	29
Sulfide (mg S·L ⁻¹)	3.5	0.21	0.3	0.8	0.01	0.01		0.76
	Mean ± standard deviation							
Soluble sulfur/total sulfur	0.81 ± 0.1							
Sulfate/soluble sulfur	~1							

4.4.2 Primary settling and primary thickening

4.4.2.1 Hourly variation of sulfate and sulfide

The hourly variation of sulfate and sulfide in the inlet of primary settling are presented in Figure 4.2. The minimum and maximum sulfate mass flows were $94 \text{ kg S}\cdot\text{h}^{-1}$ and $310 \text{ kg S}\cdot\text{h}^{-1}$ for 6th and 12th June, respectively, with most measurements ranging between $100 \text{ kg S}\cdot\text{h}^{-1}$ and $200 \text{ kg S}\cdot\text{h}^{-1}$. The flow diversion of influent wastewater from the main treatment line to the rain treatment line due to rain events on both days led to a decrease of the sulfate mass flows to $100 \text{ kg S}\cdot\text{h}^{-1}$. In terms of sulfate concentration, a different pattern can be seen for 6th and 12th of June. While sulfate concentration gradually increased during 6th of June, the sulfate concentration decreased with a sharp reduction occurring on 12th of June. Lower sulfate concentrations were coinciding with rainy hours, indicating that lower sulfate concentration was linked to dilution effects due to increased influent flow rates. The variation of sulfide mass flows ranged between 0.8 and $3.4 \text{ kg S}\cdot\text{h}^{-1}$, with an average value of $1.5 \text{ kg S}\cdot\text{h}^{-1}$.

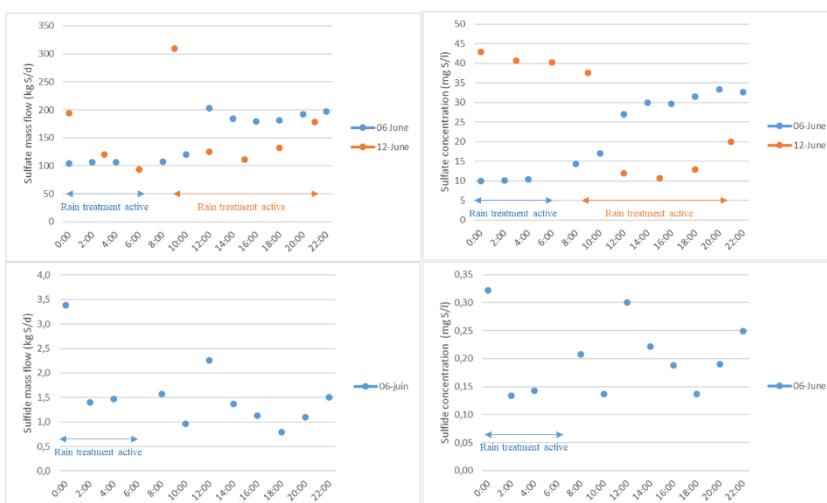


Figure 4.2 Variation of sulfate and sulfide mass flows in the primary inlet.

4.4.2.2 Sulfur speciation

In the primary effluent, the soluble fraction in the form of sulfate accounted for the majority of total sulfur (Table 4.3). This is similar to that of influent wastewater, which indicates that sulfur speciation in the influent wastewater does not change significantly during the pretreatment and primary settling.

Table 4.3 ORP and concentrations of total sulfur, soluble sulfur, sulfate and sulfide in the inlet, effluent, sludge and thickened sludge of the primary clarifier.

	unit	4/6	5/6	6/6	7/6	11/6	12/6	13/6	Mean ± Std*
Primary inlet									
Total sulfide	mg S·L ⁻¹	0.09	0.35					0.27	0.2 ± 0.1
ORP	mV	-104	-18					-25	-49 ± 48
Primary effluent									
Total sulfur	mg S·L ⁻¹	48				44	35		42 ± 7
Soluble sulfur	mg S·L ⁻¹	32				37	32		34 ± 3
Sulfate	mg S·L ⁻¹	37				37	29		34 ± 5
Thiosulfate	mg S·L ⁻¹	ND							
Total sulfide	mg S·L ⁻¹	0.7	0.27		0.35				0.4 ± 0.2
ORP	mV	-180	-120		-183				-161 ± 35
Primary sludge									
Total sulfur	mg S·L ⁻¹		47			47	480	40	153 ± 218
Soluble sulfur	mg S·L ⁻¹		30			40		28	33 ± 6
Sulfate	mg S·L ⁻¹	25	24		37	40	15	16	26.2 ± 10.4
Total sulfide	mg S·L ⁻¹	2.7	0.5		1.8	0.5			1.4 ± 1.1
ORP	mV	-330	-52		-117	-174	-266	-14	-167 ± 135
Primary thickened sludge									

Total sulfur	mg S·L ⁻¹	600		560			580
Soluble sulfur	mg S·L ⁻¹	37					37
Sulfate	mg S·L ⁻¹	4	5	11	15	12	9 ± 5
Total sulfide	mg S·L ⁻¹	44		9	19	9	20 ± 17
ORP	mV	-294	-280	-230	-235	-236	-255 ± 30
pH		5.7	5.9	5.3	5.3	5.5	5.5 ± 0.3

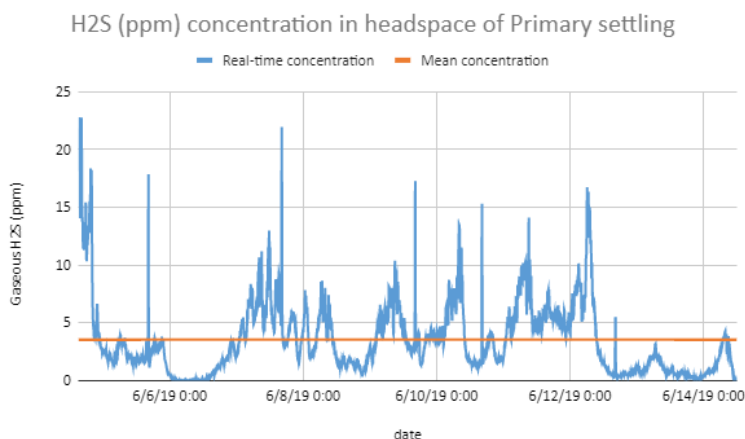
*standard deviation

The primary sludge characteristics in terms of sulfur considerably varied over time. This variation could be observed not only in the sulfate concentration but also in terms of other parameters such as ORP and pH (Table 4.3). For instance, the measured ORP ranged from -330 mV to -14 mV, indicating various oxidation and reduction conditions over time in primary sludge. Primary sludge seems to be influenced by the influent wastewater dynamics and rain events that influence the frequency of sludge discharge rate and sludge storage time in primary settling.

Despite these variations, a higher concentration of total sulfide was observed in the primary effluent (0.4 ± 0.2 mg S·L⁻¹, Table 4.3) and primary sludge (1.4 ± 1.1 mg S·L⁻¹) compared to the primary inlet (0.2 ± 0.1 mg S·L⁻¹), suggesting the formation of H₂S in the primary settling tank. Additionally, the increase in the concentration of sulfide in primary sludge is correlated with a lower concentration of sulfate in primary sludge (26 ± 10 mg S·L⁻¹, Table 4.3). Low sludge discharge leads to low oxidation-reduction potential conditions in the sludge blanket height and the creation of anaerobic conditions at the bottom of the primary settling tank (Carrera-Chapela et al., 2014; Vollertsen et al., 2008). A recent study (Bazemo et al., 2021) reported that 36% of H₂S emission from primary settling tank originated from H₂S formation in this unit.

Compared to primary settling, the operation of the primary thickener showed more stability. The long hydraulic retention time (2 days) in this unit enables the formation of reductive conditions, reflected in low ORP values (-255 ± 30 mV). These conditions led to a much lower concentration of sulfate in thickened primary sludge (9 ± 5 mg S·L⁻¹, Table 4.3). Additionally, an elevated concentration of sulfide (20 ± 17 mg S·L⁻¹) was observed in primary thickened sludge.

The concentration of H_2S (ppm) in the headspace of primary settling and thickening throughout the measurement campaign is plotted in Figure 4.3. The mean concentration of H_2S in the headspace of the primary settling tank was 3.6 ppm with a minimum and maximum concentration of 0 ppm and 23 ppm, respectively. Recent observations by Bazemo et al. (2021) suggest that H_2S emission in primary settling tanks was mainly due to the stripping of dissolved sulfide (64%) generated in the sewers. The emission of H_2S from the primary thickener was higher and varied between 11 ppm and 187 ppm with an average value of 68 ppm (Figure 4.3). The high emission from primary thickening is in agreement with literature that has selected primary thickeners as the main source for hydrogen sulfide production (Frechen, 2004).



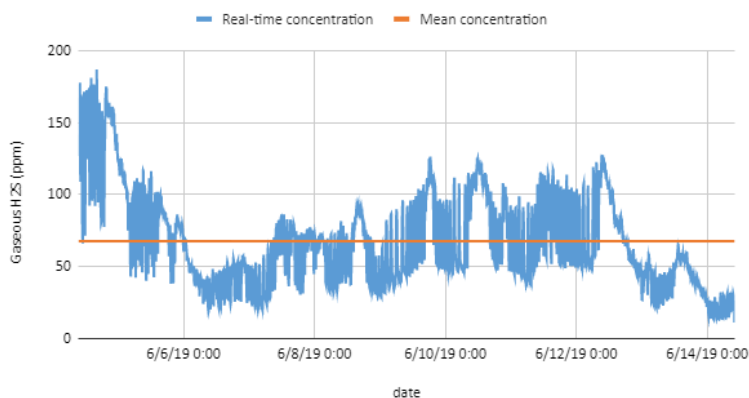
H₂S (ppm) concentration in headspace of Primary thickener

Figure 4.3 Concentration of H₂S in the headspace of primary settling and primary thickening.

Figure 4.4 presents the concentration of sulfate in the inlet of primary settling and gaseous H₂S concentration in the headspace of the primary settling tank over the 6th and 12th of June. There is a correlation between the increased concentration of sulfate with H₂S concentration in the headspace. For the data of 12th of June the decrease in the concentration of sulfate was correlated with the lower emission of H₂S from the primary settling tank to the gas phase.

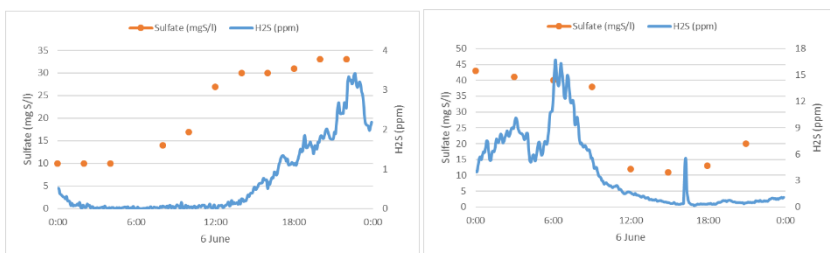


Figure 4.4 Correlation between sulfate concentration in the inlet of primary settling tank with H₂S concentration in the gas phase of this unit.

4.4.3 Secondary treatment

No significant change in the concentration of sulfate was detected in the pre-anoxic and anaerobic zone (Table 4.4) of the IFAS treatment unit. Information about the biological reduction of sulfate in the anaerobic zone of the activated sludge process is contradicting in literature. While some studies have detected

this reaction (Baetens et al., 2001; Ingvorsen et al., 2003; Lens et al., 1995; Wanner et al., 1987; Yamamoto-Ikemoto et al., 1994; Yamamoto et al., 1998, 1991), with a low rate of $0\text{--}3.1 \text{ mg SO}_4^{2-}\cdot(\text{g VSS}\cdot\text{h})^{-1}$ (Lens et al., 1995), others did not detect it in the anaerobic zone (De Beer et al., 1998; Schramm et al., 1999). The latter could be explained by the study of Ingvorsen et al. (2003) that showed sulfate reduction follows a linear pattern for the first 5 h with a low sulfate reduction rate, followed by an exponential phase. Given the much lower HRT of the anaerobic zone in the activated sludge process, the absence of sulfate reduction in our case is logical.

A slight increase in sulfate concentration could be seen in the following anoxic and aerobic zones (Table 4.4). The increase may be attributed to grab samples and measurement uncertainties rather than biological oxidation of sulfide to sulfate, because the concentrations of sulfide obtained in different stages of secondary treatment were negligible. The concentration of thiosulfate and sulfite were analysed on several samples, which were also below the detection range. In general, the result shows that sulfur species were barely affected during anaerobic, anoxic and aerobic conditions in the secondary treatment.

Similar to the influent wastewater and primary effluent, the majority of sulfur in the treated effluent was in the soluble fraction in the form of sulfate (Table 4.4). The value of total sulfur in the secondary sludge was $76 \text{ mg S}\cdot\text{L}^{-1}$ ($13 \text{ mg S}\cdot(\text{g dry solid})^{-1}$) and is consistent with the results of other studies which indicated a total sulfur content of secondary sludge in a range of 9.5 to $13.4 \text{ mg S}\cdot(\text{g dry solid})^{-1}$ (Dewil et al., 2006; Du and Parker, 2013; Fisher et al., 2017). The mass of total sulfur in the secondary sludge accounts for $4.5 \pm 1.1\%$ of total sulfur in influent wastewater (Section 3.4.1.3.2), indicating that a considerable amount of sulfur is directed towards the sludge treatment line. The fraction of sulfate in secondary sludge accounted for 44% of the total sulfur and the rest could be attributed to the sulfur assimilated in the biomass. The high sulfur content of the secondary sludge can have adverse effects on the treatment line, particularly in the anaerobic digestion of sludge, because of the formation of sulfide and subsequent release of H_2S (Dewil et al., 2008).

Table 4.4 The total sulfur, sulfate and sulfide measurements in the secondary treatment stage.

	unit	4/6	5/6	6/6	7/6	11/6	12/6	13/6	Mean ± Std*
Pre-anoxic									
Sulfate	mg S·L ⁻¹	45							
Total sulfide	mg S·L ⁻¹	<0.1							
Anaerobic									
Sulfate	mg S·L ⁻¹	47		21		32			
Total sulfide	mg S·L ⁻¹	<0.1				0.2			
Anoxic									
Sulfate	mg S·L ⁻¹	53				32			
Total sulfide	mg S·L ⁻¹	<0.1				0.1			
Aeration									
Sulfate	mg S·L ⁻¹	52							
Total sulfide	mg S·L ⁻¹	0.1							
Post anoxic									
Sulfate	mg S·L ⁻¹	50							
Total sulfide	mg S·L ⁻¹	<0.1							
Post aeration									
Sulfate	mg S·L ⁻¹	50				39			
Treated effluent									
Total sulfur	mg S·L ⁻¹	48				40	31		39.6
Soluble sulfur	mg S·L ⁻¹	43				39	29		36.9
Sulfate	mg S·L ⁻¹	43				35	27		35.1
Secondary sludge									
Total sulfur	mg S·L ⁻¹					76			
Sulfate	mg S·L ⁻¹	52		44	26	33	20		34.9 ± 13.0
Total sulfide	mg S·L ⁻¹	1.2	0.1			0.25			0.5
ORP	mV	6	-15	-163	-16	-15	49		-24 ± 66
pH		7.4	7.3	6.9	7	7.7	7.6		-7.3 ± 0.3

*standard deviation

The concentration of sulfide in the secondary sludge was 0.5 ± 0.6 mg S·L⁻¹ (Table 4.4), which is higher than those measured in anaerobic, anoxic and aerobic zones of secondary treatment. It could be attributed to a possible H₂S formation in the sludge blanket of the secondary settling tank. It has been reported that when anaerobic conditions develop in the bottom of the secondary settling tank, SRBs activity might recover leading to the biological reduction of sulfate to sulfide (Kjeldsen et al., 2004). On the other hand, a similar sulfate concentration was observed between secondary sludge and treated effluent, indicating an insignificant biological reduction of sulfate in the secondary settling tank. The secondary excess sludge was thickened in dynamic thickeners. Based on total sulfur mass flows presented in Forouzanmehr et al. (2021), 57% of the total sulfur entering this unit was directed towards the supernatant, thus lowering the sulfur content in thickened sludge.

4.5 Overview of sulfur transformation over the water treatment line

The data of water treatment lines are summarised in

Figure 4.5. Under anaerobic conditions, sulfate can be biologically reduced to sulfide. The anaerobic conditions in the water line occur in primary settling, anaerobic zone of biological treatment, the bottom of secondary settling when sludge is stored long enough and primary thickener. Sulfate measurements performed in all these locations showed that biological reduction of sulfate took place in the primary thickener and to a lesser extent, primary settling tank, while the formation of sulfide in the anaerobic zone and secondary settling was not observed. Furthermore, the biological reduction of sulfide in primary settling and thickening were accompanied by high emissions from these units.

Under anoxic and aerobic conditions, sulfide can be biologically oxidised to elemental sulfur and sulfate using nitrate and oxygen as electron donors, respectively. The low concentration of sulfide in the incoming wastewater and no sulfide formation during the anaerobic zone of secondary treatment lead to very low sulfide concentration in the secondary treatment. These observations mean that sulfate and sulfide reactions during secondary treatment are not significant in municipal WRRFs.

4.6 Conclusions

This study presents the quantification of sulfur species in the water treatment line of a municipal WRRF.

- Sulfate accounted for majority of total sulfur in influent wastewater, primary effluent and treated effluent. Throughout the water treatment line, sulfide was measured in low concentrations, while the semi-quantitative analysis did not detect thiosulfate and sulfite.
- The hourly measurements of sulfate and sulfide mass flows were found to highly vary and ranged between 94–310 kg S·h⁻¹ and 0.8–3.4 kg S·h⁻¹, respectively.
- The effect of IFAS-based biological treatment and secondary settling tank on the fate of sulfate was found negligible, since the concentration of sulfate remained relatively unchanged through the anaerobic, anoxic and aerobic reactors.
- The evaluation of total sulfur and sulfate in the primary sludge showed a high variation over time, which was attributed to the effect of influent dynamics and rain events on sludge storage time at the bottom of primary settling tank.
- A more stable operating condition and longer retention time during primary thickener promoted the biological reduction of sulfate to sulfide, leading to higher H₂S emission from this unit.

5

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

5.1 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.

5.2 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 (Figure 5.1). Degradable particulate organic sulfur would be converted to soluble organic sulfur in the 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 donor 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).

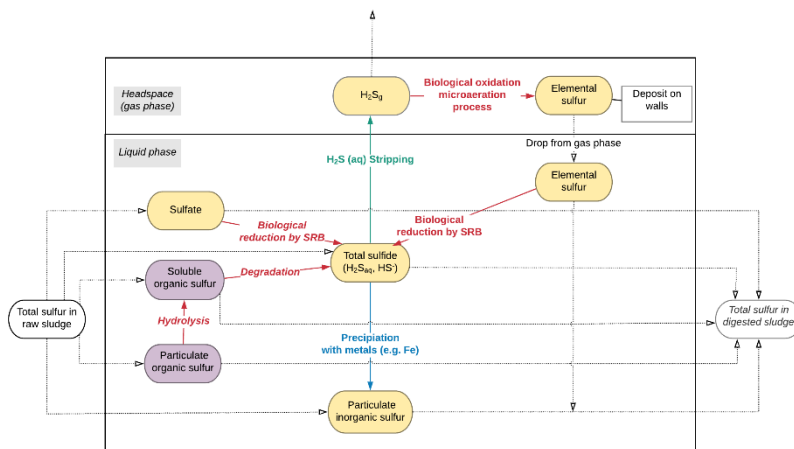


Figure 5.1 Sulfur species conversions during anaerobic digestion. Biological, chemical and physical reactions are indicated by red, green and blue colours. Organic and inorganic sulfur species are specified by yellow and purple colours. The dashed line shows the distribution of total sulfur in the raw 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 the 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). The direct effect of sludge thermal treatment on the sulfur cycle and formation of H_2S in the subsequent anaerobic digestion has not been investigated; however, the solubilisation and degradation of proteins during thermal hydrolysis (Donoso-Bravo et al., 2011; Shana et al., 2015; Wilson and Novak, 2009) could accelerate the formation of H_2S from organic sulfur in following anaerobic digestion.

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 H₂S production and methane yield for the two stages of sludge treatment.

5.3 Materials and methods

5.3.1 WRRF 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 m³) 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 is presented in Section 3.3.1.

5.3.2 Measurement campaign

5.3.2.1 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 H₂S production (Section 5.3.3). 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 Appendix D.

5.3.2.2 *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 H₂SO₄ in the presence of K₂Cr₂O₇ 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:

Organic sulfur fraction (OSF)

$$= (S_{\text{Total}} - S_{\text{Inorganic}})/S_{\text{Total}} \quad \text{Eq.5.1}$$

Particulate organic sulfur fraction (POSF)

$$= (S_{\text{Particulate}} - S_{\text{Particulate_Inorganic}})/S_{\text{Particulate}} \quad \text{Eq.5.2}$$

$$S_{\text{Particulate_Organic}} = \text{POSF} \times S_{\text{Particulate}} \quad \text{Eq.5.3}$$

$$S_{\text{Soluble_Organic}} = \text{OSF} \times S_{\text{Total}} - S_{\text{Particulate_Organic}} \quad \text{Eq.5.4}$$

$$S_{\text{Soluble_Inorganic}} = S_{\text{Soluble}} - S_{\text{Soluble_Organic}} \quad \text{Eq.5.5}$$

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

5.3.3 Batch tests

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

The tests were performed according to the biochemical methane potential (BMP) guidelines provided by a dedicated international working group (Holliger et al., 2016). Substrate to Inoculum ratio (S/I) was 0.5 on a VS basis. The substrates were collected from the inlet of the first stage digester, inlet and outlet of thermal hydrolysis unit, and outlet of second stage digester. Each reactor was flushed with nitrogen for at least 3 minutes to ensure anaerobic

conditions. For all samples, the test was performed in triplicates. Three blank tests containing only inoculum were incubated simultaneously to correct for the methane and H₂S produced by the inoculum. The digestion experiments were run for approximately 30 days. The biogas production was determined with the manometric method (Amodeo et al., 2020). The biogas composition was measured by gas chromatography using an Agilent 3000 micro gas chromatograph, equipped with a thermal conductivity detector (GC-TCD). Molsieve 5A (14 m length; pore size: 5 Å) and PoraPlotA (10 m length; 0.320mm ID) columns were used as stationary phases for GC-TCD, with Argon and Helium as carrier gases, respectively. The micro-GC was calibrated for H₂, H₂S, CO₂, CH₄, O₂ and N₂. Methane and hydrogen sulfide production were calculated in STP conditions (0 °C, 101325 Pa) after correction for moisture. At the end of each batch test, the digested sludge was analysed for total sulfur, soluble sulfur, and sulfate.

The contribution of biological sulfate reduction to formation of H₂S was calculated by the difference between initial and final sulfate concentrations. Produced sulfide in these experiments was the sum of H₂S 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.

5.4 Results

5.4.1 Long-term operation of anaerobic digesters

The two parallel first-stage digesters (D1a and D1b) were operated under similar conditions (Table 5.1): an HRT of 21 days and a VS load of 11011 and 11278 kg VS·d⁻¹ 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 CH₄·(g VS_{in})⁻¹, 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 $D1_{\text{feed}}$ 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 5.1 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	d	21 ± 2	21 ± 3	31 ± 6
Daily VS load	kg VS·d ⁻¹	11011 ± 1708	11278 ± 1774	9610 ± 1798
VS reduction (VSR) ¹	%	39 ± 5	41 ± 5	32 ± 5
Daily biogas production	Nm ³ ·d ⁻¹	5163 ± 1185	6012 ± 1302	6162 ± 1585
Methane yield	mL CH ₄ ·(g VS _{in}) ⁻¹	279 ± 54	316 ± 65	379 ± 66
Methane yield of BMP test ²	mL CH ₄ ·(g VS _{in}) ⁻¹	310 ± 6	-	226 ± 2
Total volatile solids reduction ³	%		55 ± 7	

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

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

³ $VSR_{\text{Total}} = (VSR_{\text{1st stage}} + VSR_{\text{2nd stage}}) / VS_{\text{in, 1st stage}}$

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 CH₄·(g VS_{in})⁻¹, which was higher than that obtained from BMP test 226 ± 2 mL CH₄·(g VS_{in})⁻¹. The latter is similar to the value of 236 mL CH₄·(g VS_{in})⁻¹ 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.

5.4.2 Total sulfur content and fractionation in and out anaerobic digesters

The total sulfur concentrations of anaerobic digesters (D1a and D2) are shown in Table 5.2. During the seven-week measurement campaign C1 (2018), the total sulfur concentrations in the inlet and outlet of D1a were $9.5 \pm 2.6 \text{ mg S} \cdot (\text{g dry solid})^{-1}$ and $11.6 \pm 2.7 \text{ mg S} \cdot (\text{g dry solid})^{-1}$, respectively. The increase of sulfur concentrations ($\text{mg S/kg dry solids}$) 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 5.2 Mean and standard deviation of total sulfur concentrations as $\text{mg S} \cdot (\text{g dry solid})^{-1}$ 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 dry solid) ⁻¹	D1a_{outlet} mg S·(g dry solid) ⁻¹	D2_{feed} mg S·(g dry solid) ⁻¹	D2_{outlet} mg S·(g dry solid) ⁻¹
C1 (2018)	9.5 ± 2.6 (n=22)	11.6 ± 2.7 (n=7)	12.3 ± 1.0 (n=6)	12.4 ± 1.5 (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)

The total sulfur mass flow decreased during both first stage (D1a) and second stage (D2) digestion (Table 5.3). In D1a, total sulfur flows decreased from $139 \pm 12 \text{ kg S} \cdot \text{d}^{-1}$ in the inlet to $117 \pm 13 \text{ kg S} \cdot \text{d}^{-1}$ in digested sludge. The total sulfur flow in the biogas (as H_2S) accounted for $6.3 \pm 1.2 \text{ kg S} \cdot \text{d}^{-1}$. 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 $\sim 11\%$ ($16 \text{ kg S} \cdot \text{d}^{-1}$), 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 $165 \pm 12 \text{ kg S}\cdot\text{d}^{-1}$ to $149 \text{ kg S}\cdot\text{d}^{-1}$ in digested sludge and $9.2 \pm 0.8 \text{ kg S}\cdot\text{d}^{-1} \text{ H}_2\text{S}$ in biogas, implying a 4% gap in sulfur mass flows.

Table 5.3 Average and standard deviation of total, particulate, and soluble sulfur mass flow as $\text{kg S}\cdot\text{d}^{-1}$ 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 ⁻¹	139 ± 12*	117 ± 13	6.3 ± 1.2	165 ± 12	149 ± 9	9.2 ± 0.8
	mg S·L ⁻¹	501 ± 42	422 ± 43	908 ± 107 (ppm)	1146 ± 58	1035 ± 33	1533 ± 68 (ppm)
Particulate sulfur (S _{Particulate})	kg S·d ⁻¹	119 ± 13	107 ± 13		110 ± 8	111 ± 10	
	mg S·L ⁻¹	431 ± 45	385 ± 44		762 ± 42	770 ± 56	
Soluble sulfur (S _{Soluble})	kg S·d ⁻¹	19 ± 2	10 ± 0.4		55 ± 4	38 ± 6	
	mg S·L ⁻¹	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 (n=6)	66 ± 5 (n=5)		60 ± 6 (n=7)	54 ± 6 (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 5.3). 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 Figure B in Appendix E). In addition to sulfur, elevated soluble fraction in thermally treated sludge was detected for COD (47% and 31% for C2 and C3, respectively, see Appendix E). 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 5.3,

the fate of soluble/particulate organic/inorganic sulfur in D1a and D2 can be deduced (Figure 5.2). From Figure 5.2, 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 $\text{kg S}\cdot\text{d}^{-1}$ and $33 \text{ kg S}\cdot\text{d}^{-1}$, respectively. In D1a, the uptake of particulate organic sulfur was significant ($21 \text{ kg S}\cdot\text{d}^{-1}$), while in D2 the uptake of soluble organic sulfur was more pronounced ($27 \text{ kg S}\cdot\text{d}^{-1}$).

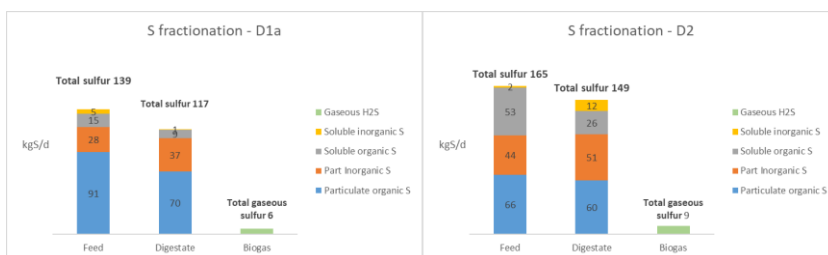


Figure 5.2 Fate of soluble/particulate organic/inorganic sulfur in D1a and D2, calculated from the fractionations given in Table 5.3.

Particulate inorganic sulfur increased after D1a and D2 with $+9$ and $+7 \text{ kg S}\cdot\text{d}^{-1}$, respectively. Soluble inorganic sulfur decreased in D1a ($-4 \text{ kg S}\cdot\text{d}^{-1}$) but increased in D2 ($+10 \text{ kg S}\cdot\text{d}^{-1}$). 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.

5.4.3 Contribution of biological sulfate reduction to H₂S 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 5.4. 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 5.4: 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 ($\text{mg S}\cdot\text{L}^{-1}$)	12.9	1.4		12.5	6.0	
Soluble Fe ($\text{mg S}\cdot\text{L}^{-1}$)	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 (Figure 5.3), sulfide produced from the biological reduction of sulfate accounted for 56% ($420 \mu\text{g S/g VS}_{\text{in}} / 756 \mu\text{g S/g VS}_{\text{in}}$) and 28% ($256 \mu\text{g S/g VS}_{\text{in}} / 918 \mu\text{g S/g VS}_{\text{in}}$) 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.

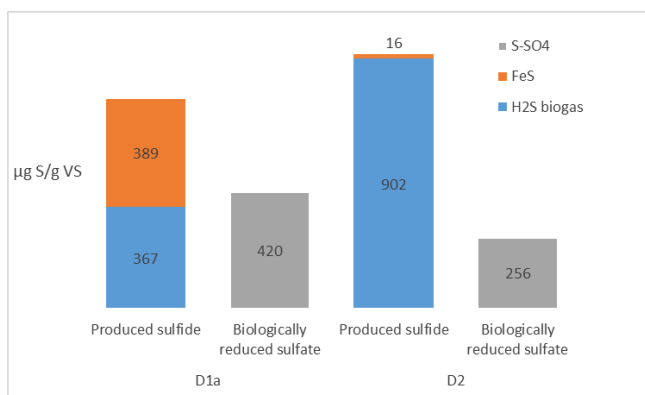


Figure 5.3 Comparison between sulfide production (either as H₂S in biogas or precipitated FeS) and biological sulfate reduction, for both first stage (D1a) and second-stage (D2) digestion. Values obtained from batch tests.

Based on these results, it was then assessed whether the degradation of sulfur-containing amino acids (cysteine and methionine) could explain the remaining difference between sulfide production and biologically reduced sulfate. Because methionine and cysteine were not analysed in this study, their concentrations in raw sludge and degradation rates in anaerobic digestion that were reported by Chen et al. (2019) were used (See Appendix F). Indeed, these authors have reported the content of hydrolytic cysteine and methionine in raw sludge as $0.46 \pm 0.01 \text{ mg} \cdot (\text{g dry solid})^{-1}$ and $3.60 \pm 0.01 \text{ mg} \cdot (\text{g dry solid})^{-1}$, respectively. In addition, the reported removal rate of cysteine and methionine in lab-scale anaerobic digestion was $34.78 \pm 7.87\%$ and $48.06 \pm 0.77\%$, respectively. With these values, the contribution of cysteine and methionine to the formation of sulfide were calculated, as $62 \mu\text{g S} \cdot (\text{g VS}_{\text{in}})^{-1}$ and $542 \mu\text{g S} \cdot (\text{g VS}_{\text{in}})^{-1}$, respectively, leading to a total potential sulfide formation of $1023 \mu\text{g S} \cdot (\text{g VS}_{\text{in}})^{-1}$. Although the calculated potential sulfide formation is higher than the measured sulfide ($756 \mu\text{g S} \cdot (\text{g VS}_{\text{in}})^{-1}$), these values are in the same order of magnitude. The difference could be explained by the fact that we did not measure all sulfide (remaining soluble sulfide and sulfide precipitated with other metals). Nevertheless, this result supports our previous statement that the degradation of organic sulfur is a major mechanism for the formation of H₂S in anaerobic digestion.

5.4.4 Profile of methane yield and H₂S in biogas of batch anaerobic digestion

H₂S formation and methane yield during anaerobic digestion batch experiments of samples taken from various stages are given in Figure 5.4. The yield of H₂S dramatically increased from 153 $\mu\text{g S}\cdot(\text{g VS}_{\text{in}})^{-1}$ to 921 $\mu\text{g S}\cdot(\text{g VS}_{\text{in}})^{-1}$ because of thermal hydrolysis. The increase is partially caused by the sulfate content (20-40 $\text{mg S}\cdot\text{L}^{-1}$) of the treated effluent which was added to the thermally treated sludge for dilution and cooling. Interestingly, the H₂S yield of digested sludge (D2_{outlet}) remained noticeable (352 $\mu\text{g S}\cdot(\text{g VS}_{\text{in}})^{-1}$). The methane yield also increased considerably from 53 $\text{mL CH}_4\cdot(\text{g VS}_{\text{in}})^{-1}$ in the inlet to 226 $\text{mL CH}_4\cdot(\text{g VS}_{\text{in}})^{-1}$ in thermally treated sludge.

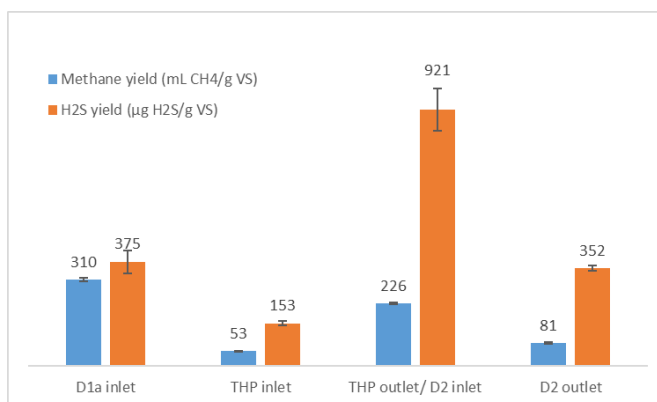


Figure 5.4 Profile of gaseous methane yield $\text{mL CH}_4\cdot(\text{g VS})^{-1}$ and hydrogen sulfide yield $\mu\text{g H}_2\text{S}\cdot(\text{g VS})^{-1}$ in the different stages.

5.5 Discussion

5.5.1 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 $15 \pm 7\%$ 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 $379 \pm 66 \text{ mL CH}_4 \cdot (\text{g VS}_{\text{in}})^{-1}$ to $314 \pm 58 \text{ mL CH}_4 \cdot (\text{g VS}_{\text{in}})^{-1}$, and VS reduction increased from $32 \pm 5\%$ to $43 \pm 6\%$. Further research is needed to assess the emission of volatile organic compounds in the off-gas stream of the thermal hydrolysis process.

5.5.2 The effect of intermediate thermal hydrolysis on organic matter solubilisation, methane production, and H₂S 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 $\text{CH}_4 \cdot (\text{g VS}_{\text{in}})^{-1}$, Figure 5.4) 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 5.3. 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.

5.5.3 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 5.3). In the first digestion stage the uptake of organic sulfur was 25% (Table 5.3, 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 (Figure 5.3). 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 5.3). 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).

5.5.4 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.

5.6 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

Conclusions & perspectives

6.1 Conclusions

6.1.1 Overview

Despite advances in processes and technologies, especially regarding resource recovery, water resource recovery facilities (WRRFs) still face substantial challenges associated with the sulfur cycle. Impacted aspects of WRRFs with sulfur cycle include design, operation, performance, economy, safety, the public image of these facilities. Nevertheless, limited attention has been given to sulfur in the research community compared to enormous efforts dedicated to treating organic compounds, nitrogen, and phosphorus. This research aimed to provide insights into the fate of sulfur species within municipal WRRFs based on full-scale data collection. As highlighted in Chapter 2, understanding of sulfur transformations is rarely investigated and verified based on full-scale data collection. Ultimately, understanding sulfur transformations will improve modelling based design, operation, and control of process treatment to alleviate the adverse impacts of sulfur.

This work improved our understanding of sulfur in WRRFs in four steps. Initially, a comprehensive literature view was performed to identify possible sulfur transformations in process treatment units and discussed the knowledge gaps and limitations within the existing literature. Then, a plant-wide distribution of total sulfur mass flows was obtained by considering water, sludge and gaseous streams of WRRFs (Chapter 3). Overall the results enabled identifying key sulfur streams, important spots for phase changing of sulfur mass flows (e.g. from sludge to gas phase). In the next step (Chapter 4), the transformation of sulfur species within the water treatment line of a municipal WRRF was investigated to identify the evolution of sulfur species under different redox environments. Finally, the transformation of sulfur species was investigated during the anaerobic digestion of raw sludge and thermally treated sludge (Chapter 5). This dissertation research gives new insights into the transformation of organic and inorganic sulfur, considering the impacts of emerging technologies such as thermal hydrolysis, and highlights future areas of research needed to minimise the negative impacts of the sulfur cycle.

6.1.2 Literature review

Sulfur can be present in various organic and inorganic forms and are reactive in different redox environments in wastewater treatment processes. Chapter 2 reviewed the literature on the transformation of various sulfur forms within process treatment units of a typical WRRF and highlighted limitations and knowledge gaps of existing studies. Sulfur transformation potentially impacts many stages of treatment, mainly through the generation and emission of volatile sulfur compounds, particularly hydrogen sulfide. The biological reduction of sulfate to sulfide during the anaerobic digestion process has been widely investigated in literature because of the adverse effects of sulfide on the performance of the anaerobic digestion process. However, these studies were primarily performed on sulfate-rich synthetic or industrial wastewater in lab-scale experiments, which may not necessarily be comparable to the full-scale municipal anaerobic digestion process due to the sulfate-limiting nature of municipal sludge. In addition, as highlighted in Chapter 2, organic sulfur contribution to H₂S formation are less explored, and only recently possible correlation between organic sulfur sources with H₂S concentration in biogas was suggested. This trend was also seen in anaerobic digestion models that mainly covers inorganic sulfur conversions, while organic sulfur aspects are mostly absent. It was also found that the existing modelling studies suffer from a lack of calibration and validation with full-scale anaerobic digestion processes.

The literature review also showed that the generation and emission of volatile sulfur compounds in emissive units such as primary thickener had not attracted much attention. In addition, the need for more investigation to understand the impact of emerging treatment techniques such as thermal hydrolysis on the fate of sulfur was highlighted in Chapter 2. Despite the impacts of sulfur on various aspects of WRRFs, sulfur studies at plant-wide levels lack in the literature compared to other elements such as organic matter, nitrogen and phosphorus. The review showed that sulfur management requires a plant-wide approach, meaning that sulfur transformation and interactions should be evaluated locally in every unit process and globally. A given example was multiple interactions

of S, P, Fe under different redox environments that provide an opportunity to reduce chemical requirements for phosphorus removal in activated sludge system and H₂S removal during the anaerobic digestion process.

6.1.3 Experimental design and data reconciliation of total sulfur mass flows

The effectiveness of combining mass balance-based experimental design and data reconciliation procedures was demonstrated for a reliable quantification of total sulfur flows in a relatively complex WRRF (Chapter 3). The experimental design procedure provided Pareto-optimal measurement layouts and proved to be efficient for the identification of key variables. In addition, this procedure allowed comparison and selection of optimum measurement layouts in terms of measurement costs and accuracy of the key variables. Measurements from full-scale WRRFs bear uncertainties for various reasons such as influent dynamics, sampling method and measurement errors. Data reconciliation and gross error detection provided better estimates for raw values of total sulfur mass flows in the water line and the sludge line, fitting the total flow and total sulfur mass balances and characterised by relatively high accuracy. The outcome of data reconciliation showed that gaseous sulfur loads were non-redundant in practice, meaning that their values cannot be accurately determined from measured variables and mass balances through data reconciliation. It was concluded that gaseous H₂S emissions, which represent a relatively low mass compared to water and sludge streams, need to be measured directly for obtaining reliable data.

6.1.4 Towards plant-wide sulfur management

Chapter 3 provided the result of a thorough investigation of total sulfur flow distribution to identify key sulfur flows over individual unit processes in a plant-wide context and pointing out their relative importance. The results revealed that the large majority of entering sulfur mass flow ($96 \pm 23\%$) left the plant through the treated effluent, indicating that the water treatment line hardly affected the incoming sulfur flows. However, the direction of sulfur towards the sludge treatment line through primary and secondary sludge,

which amounted to about 8% of the sulfur in the influent wastewater, caused high H₂S emissions in the primary thickener and both stages of anaerobic digestion. The sulfur in dewatered sludge accounted for $3.3 \pm 0.9\%$, while gaseous emissions amounted to $0.7 \pm 0.1\%$. Although gaseous sulfur accounted for a small portion of total sulfur, these gaseous streams are highly undesirable, leading to corrosion, odour emission and lower biogas quality. The result of Chapter 3 showed that secondary (drum) thickener was very effective for limiting sulfur flows towards the subsequent sludge treatment by directing the majority of entering sulfur flows towards the centrate. Sludge treatment through two-stage digestion configuration with intermediate thermal hydrolysis and pre and post dewatering considerably reduced (by 36%) the total amount of sulfur remaining in dewatered sludge. Detailed analysis of gaseous streams showed that most (gaseous) H₂S emissions, namely 42% of total H₂S emission, were produced in the second-stage anaerobic digester, ending up in the biogas. The higher H₂S production and emission from the second-stage digester suggested that the impact of thermal treatment on the production of H₂S was significant.

6.1.5 Transformation of sulfur in process treatment units

The concentration and form of sulfur species in WRRFs are affected by influent characteristics. Essential processes that can influence influent sulfur include seawater intrusion, industrial discharge, chemical dosage in sewer networks. In addition, a portion of the sulfate present in the sewer system can be converted to hydrogen sulfide by sulfate-reducing bacteria depending on the characteristics of the collection system. The sulfur speciation in the studied plant showed that incoming sulfur was mostly soluble as sulfate (Chapter 4). Sulfur speciation in the presettled wastewater and treated effluent remained similar to incoming wastewater. The sulfide concentration in the water treatment line was low, while the semi-quantitative analysis did not detect thiosulfate and sulfite. A closer look at different redox zones of IFAS-based secondary treatment showed that sulfate reduction to sulfide under anaerobic zone was negligible. Consequently, very low sulfide concentration meant that

the interaction of nitrogen and sulfide cycle in the process, autotrophic denitrification, could not occur.

Two processes with expected biological sulfate reduction were primary settling and primary thickeners. The results showed that sulfate significantly decreased during the primary thickening process, accompanied by high H₂S emission from this unit. In comparison, the condition of primary settling was more affected by influent wastewater, such as dynamics and rain events that affected the retention time in settling tanks, leading to highly variable characteristics of primary sludge such as sulfate concentration, ORP and solids contents. However, despite the high variation, a slight decrease in sulfate concentration and increase in sulfide concentration was seen in primary sludge.

6.1.6 Overall performance of the DLD process

The overall performance of two-stage digestion with intermediate thermal hydrolysis as a novel configuration was evaluated in Chapter 5 using long-term full-scale measurements. As expected, the performance of the first stage digestion calculated from long-term methane yield was similar to typical one-stage anaerobic digestion in literature. However, the literature on following thermal hydrolysis and second stage digestion were limited. The long-term evaluation revealed that the calculated methane yield was overestimated. Two reasons were identified 1) underestimation of VS measurement due to evaporation of volatile organic compounds at drying temperature (105°C) and 2) overestimation of biogas flow rate was attributed to deposits of elemental sulfur on the flowrate sensors generated from microaeration, estimated by operators in the range of ~20%. The methane yield of batch experiments was closer to the few values reported in the literature.

Intermediate thermal hydrolysis aimed at solubilising a part of the organic fraction undegraded during the first stage digestion to make it degradable in the second stage digester. The result of Chapter 5 showed that intermediate thermal hydrolysis effectively enhanced the solubilisation of organic matter and increased biodegradability of digested sludge, resulting in a significant

increase in methane yield and H₂S production during anaerobic digestion of thermally treated sludge.

6.1.7 Sulfur transformation in the DLD process

An important result of this investigation is that organic sulfur impacts the generation of H₂S during anaerobic digestion (Chapter 5). While the biological reduction of sulfate has been well established in the literature, few studies have discussed the role of organic sulfur compounds on the formation of H₂S during the anaerobic digestion process (Chapter 2). Chapter 5 shows that organic sulfur decreases during the anaerobic digestion process, obtained from a seven-week full-scale evaluation of anaerobic digesters. This decrease was mainly observed for particulate organic sulfur during anaerobic digestion of raw sludge, whereas soluble organic sulfur experienced a higher reduction during anaerobic digestion of thermally treated sludge. Additionally, the decrease of organic sulfur was higher than particulate inorganic sulfur (metal sulfide) production during both stages of anaerobic digestion, indicating the formation of other reduced sulfur forms (e.g. H₂S). Therefore, the full-scale investigation highlighted that the decrease of organic sulfur during the anaerobic digestion process had an important contribution to sulfide production.

Anaerobic digestion batch experiments on samples taken from each stage of two-stage anaerobic digestion with intermediate thermal hydrolysis further provided insights on the contribution of sulfate reduction to the formation of H₂S and the influence of THP on H₂S formation. The results showed that sulfate reduction could not explain all sulfide produced during experiments. This contribution was estimated to be 56%, and 28% of total sulfide (H₂S in biogas and precipitated FeS) produced in the first and second stages of digestion, respectively. These results provided insights into the sources of H₂S formation during anaerobic digestion and disputed the general view that considers sulfate as the single contributor to H₂S formation during anaerobic digestion. Indeed, H₂S formation from the organic sulfur conversion is significant, especially for thermally treated sludge. The potential impact of organic sulfur conversion for the production of H₂S during anaerobic digestion

of (thermally treated) sludge is an outcome of this dissertation that has substantial academic and engineering significance.

6.2 Perspectives

6.2.1 Application of experimental design and data reconciliation

There is an increasing interest in plant-wide modelling to answer existing questions in WRRFs, such as process optimisation and resource recovery. Despite advances in the development of mathematical models, data availability and quality for calibration and validation of models with full-scale data are staying behind. Therefore, there are numerous benefits of applying experimental design and data reconciliation for future studies, which aim at performing plant-wide investigations and modelling. Plant-wide studies deal with many sampling points, different measurements, and interactions between process treatment units, making the design of measurement campaigns difficult. In this regard, applying experimental design prior to conducting measurement campaigns enables optimum and economical measurement campaigns. Once collected, raw measurements often bear errors and uncertainty that adversely influence the data exploitation. For instance, using erroneous data for model calibration and validation would lead to laborious and unjustified model calibrations of kinetics and stoichiometric parameters. In this regard, it is essential to reconcile the raw measurements to verify (gross) errors and improve their accuracy before being implemented.

6.2.2 Plant-wide sulfur modelling

Future modelling studies should pay attention to the generation and emission of VSCs from primary thickener and primary settling tanks, knowing that these processes are often treated as point separators in the context of plant-wide modelling. These units were shown to be important emissive units in which generation and emission of H₂S occurred. Developing mathematical models that include both biochemical reactions for the generation of H₂S and emission mass transfer may provide better tools to estimate atmospheric emission from these units accurately.

At present, the control of H₂S in biogas is achieved by chemical dosing, microaeration process or biogas treatment processes to clean the biogas generated in anaerobic digestion. Besides investment requirements, there is a risk of failure to remove H₂S to an acceptable level required by gas turbines and engines. If H₂S generation in anaerobic digestion can be better understood and quantified by modelling, the concentration of H₂S in biogas could be predicted, and therefore control of H₂S releases could be optimally achieved. This research showed the importance of organic sulfur transformations in the generation of H₂S during anaerobic digestion. A critical challenge in applying organic sulfur transformations is that organic sulfur speciation and generation pathways are not well understood. Given that developed models mainly overlook organic sulfur reactions, future research should focus on incorporations of these reactions into anaerobic digestion models to improve the prediction of H₂S formation during the anaerobic digestion process. For this purpose, there is a need for future studies to perform speciation of organic sulfur, obtain kinetic information of H₂S formation and conversion so that the prediction of H₂S formation during anaerobic digestion models became more accurate. In addition, the interactions of sulfur cycle with other elements such as iron and other metals are also needed for achieving representative sulfur models.

This study showed that the thermal hydrolysis process increased the generation of H₂S during the anaerobic digestion process. These influences attributed to solubilisation and increased degradation of organic sulfur are not considered in the available THP models, mainly because organic sulfur is rarely included in modelling practises. The increased utilisation of THP in WRRFs makes it necessary to improve the THP models to reflect better its influence on sludge characteristics that lead to H₂S formation during anaerobic digestion.

The analytical methods of sulfur measurements, especially the organic fraction in the soluble phase and both organic and inorganic fraction in the particulate phase, deserve more detailed investigations in the future. Quantifying different organic sulfur species in the sludge samples and monitoring their conversions

during thermal hydrolysis and anaerobic digestion process will provide necessary knowledge for their incorporation into models.

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Appendix

Appendix A. List and short description of studies about sulfur transformations in wastewater treatment

	Reference	Short description
(1)	Chen and Szostak, 2013	A case study was performed at a lift station of a WRRF to systematically investigate H ₂ S emission using in situ monitoring techniques. The field data from both air and wastewater quality monitoring demonstrated that more H ₂ S (1 ppmv) was formed at a more negative redox potential, lower pH, and a higher temperature of wastewater.
(2)	Haaning Nielsen et al., 2004	The kinetics and stoichiometry of chemical sulfide oxidation in wastewater as a function of pH and temperature were investigated. A general rate equation and stoichiometric coefficient enabling chemical sulfide was proposed based on a series of experiments. <u>Sample were taken from sewer network and inlet of a WRRF.</u>
(3)	Baawain et al., 2019	This study (1) investigated the potential sources and concentration levels of H ₂ S emissions from a WRRF; (2) evaluated the efficiency of the odor control unit or Chemical wet scrubber; (3) compared the actual receptor data with those of the Gaussian plume dispersion model; and (4) identified the dispersion behavior of the emitted H ₂ S using data acquisition receptor. <u>Samplings were done in preliminary treatment units</u>
(4)	Prathna and Srivastava, 2021	This study investigated the feasibility of using ferric chloride as an agent for H ₂ S emission control in a WRRF. Influent sulfide concentrations at 11 WRRFs in Delhi were measured (ranged between 1.1 and 14.8 mg·L ⁻¹) and jar test studies performed on raw wastewaters. The results indicated that dosing 40 mg·FeCl ₃ ·L ⁻¹ led to significant proportional removal of sulfide, in addition to removal of TSS, BOD, phosphate and COD.
(5)	Wilmot et al., 1988	This study investigated the order of the oxidation reaction with respect to both sulfide and oxygen and determined the relative magnitude of the chemical and biological components of the oxidation reaction. <u>Samples in this study were taken from primary effluent.</u>
(6)	Fisher et al., 2018	This study investigated the emission of VSCs throughout biosolids processing at 6 WRRFs. The primary sludge and biosolids after dewatering and during storage were key stages producing nuisance odours as judged by the determination of OAVs. Emissions from the primary sludge were dominated by H ₂ S and appeared to be affected by the presence of organic matter, pH and Fe content
(7)	Catalan et al., 2009	This study investigated the emissions of VOSCs from the primary and secondary clarifiers at a Kraft mill. In the primary clarifier, dimethyl disulfide (DMDS) had the highest mean flux (0.83 µg s ⁻¹ m ⁻²). At the secondary clarifier, dimethyl sulfide (DMS) had the highest mean flux (0.024 µg s ⁻¹ m ⁻²).
(8)	Gutierrez et al., 2010	This paper investigated the impact of iron salt dosage to sewers for sulfide control on P removal during aerobic wastewater treatment. The work was conducted by integrating lab-scale experiments with mathematical modelling. The hydraulic retention time of iron sulfide in sewers was found to have a crucial impact on the settling of iron sulfide precipitates during primary settling, with a shorter HRT resulting in a higher concentration of iron sulfide in the primary effluent and thus enabling higher P removal. A model was proposed for FeS oxidation the subsequent iron phosphate precipitation.
(9)	Jeon et al., 2009	This study investigated flux profiles of the six odorous compounds emitted from primary settling, aerated tank and secondary settling of a WRRF using a Dynamic Flux Chamber (DFC). During summer, relatively higher amounts of the selected odorous compounds were emitted compared to winter. The dominant odor intensity contribution was caused by dimethyl disulfide (69.1%).
(10)	Bazemo et al., 2021	This study quantified VSC emission from primary settling tanks and investigated the impact of sewers, seasonality, and wastewater characteristics. Hydrogen sulfide and methyl mercaptan concentrations in the off-gas were dominant among the VSCs analyze. H ₂ S emission from primary settling mainly originated from the stripping of the dissolved sulfide formed in the sewers, whereas methyl mercaptan emission was more dependent on the conditions in the primary clarifiers (only 16% stripping);
(11)	Wanner et al., 1987	This study investigated the effect of anaerobic conditions on the occurrence of filamentous microorganisms in the biocenosis of activated sludge in laboratory systems. Synthetic wastewater was used incorporating glucose and ethanol as a carbon source Sulfate reduction may occur during anaerobic condition, leading to the collapse of the biological phosphorus removal mechanism. The effect of dissimilative sulfate reduction resulted in the excessive growth of <i>Thiothrix</i> and in deterioration of the settling properties of the activated sludge from the system.
(12)	Buisman et al., 1990	The non-catalysed chemical sulfide oxidation is considerably slower (75 times) than the biological sulfide oxidation at sulfide concentrations not exceeding 10 mg/l and about a factor 6 at sulfide concentrations up to 600mg·L ⁻¹ .
(13)	Yamamoto et al., 1991	This study investigated sulfate reduction in laboratory-scale anaerobic-aerobic activated sludge processes using the artificial wastewater made up of glucose and peptone. Sulfate reduction occurred in the anaerobic period of both sequential batch reactor system and the continuous anaerobic-oxic systems. When sulfate reduction ratio increased, Type 021N or <i>Beggiatoa sp.</i> grew in the sludge and settling characteristics became poor. The

		authors suggested that sulfate reduction was a cause of growth of the filamentous bacteria which could utilize sulfide for energy source.
(14)	Yamamoto-Ikemoto et al., 1994	In anoxic-oxic conditions, denitrification bacteria grow predominantly, and the growth of sulfate reducing bacteria was suppressed. The growth of filamentous bacteria was also suppressed due to suppression of sulfate reduction. In anaerobic-oxic conditions (in absence of Nitrate), SRB grew predominantly and filamentous bulking due to Type 021 occurred. Sulfate reduction was the main trigger of filamentous bulking of the sulfur bacteria.
(15)	Yamamoto et al., 1998	Phosphate release and sulfate reduction occurred simultaneously under anaerobic conditions. When the sulfate reduction rates were high, there was a tendency for the maximum release of phosphate also to be high. This was explained by the fact that PAO utilized the acetate produced by SRB. When the sulfate reduction rate was high, the sulfide oxidizing rate was also high and filamentous bulking occurred. The results showed that sulfate reduction was a cause of filamentous bulking due to Type 021 N that could utilize reduced sulfur.
(16)	Lens et al., 1995	This study investigated a selection of aerobic biofilm reactors and activated sludge plants for the presence of SRB, sulfate reduction rate and sulfide oxidations SRB were present in a broad range of aerobic biofilm and activated sludge reactors, except in an activated sludge reactor aerated with pure oxygen. This presence was independent of the reactor configuration, the organic loading and the influent sulfate concentration. Sulfate reduction rate in activated sludge system was lower than that of biofilm systems.
(17)	Manz et al., 1998	This study investigated the relative abundance and spatial organisation of SRB in activated sludge flocs. Samples were taken from anaerobic, anoxic, aerobic and secondary settling of a WRRF. Members of the <i>Desulfobacteriaceae</i> and <i>Desulfovibrionaceae</i> were present in activated sludge. Individual sulfate-reducing cells could be visualized and the number of cells ranged from 0.5 to 8% of the total cell counts within all stages of the activated sludge process and the final clarifier.
(18)	Schramm et al., 1999	A combination of different methods was applied to investigate the occurrence of anaerobic processes in aerated activated sludge. Sulfate reduction could not be detected in any sludge in either the microsensor or the batch investigation, not even under short-term anoxic conditions. In contrast, the presence of sulfate-reducing bacteria was shown by fluorescence in situ hybridization with 16S rRNA-targeted oligonucleotide probes and by PCR-based detection of genes coding for the dissimilatory sulfite reductase.
(19)	Baetens et al., 2001	This study investigated deterioration of EBPR activity due to induced anaerobic sulfate reduction activity. Simultaneous occurrence of anaerobic sulfate 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 <i>Beggiatoa</i> spp. as dominant organism, was always observed concurrent with sulfate reduction, even at low levels.
(20)	Ingvorsen et al., 2003	This study investigated the kinetics of sulfate reduction in a modern WRRF during short- and long-term exposure to anoxic conditions. Under anoxic conditions, the sulfide production in the activated sludge followed a biphasic pattern, being linear for approximately 5 h, followed by an exponential phase with doubling times of sulfide production of 4.2-12.6 h. The number of SRB was relatively constant over the year, ranging from 2.1×10^5 to 1.1×10^6 cells ml ⁻¹ .
(21)	Kjeldsen et al., 2004	This study investigated the oxygen tolerance of SRB present in activated sludge by addressing the effect of aeration on the sulfate reduction activity and the survival of SRB in the sludge. SRB had the potential for quick proliferation during anaerobic storage of activated sludge. SRB only constitute a minor fraction of the total bacterial community in activated sludge. Oxygen exposure resulted in temporary metabolic inactivation of SRB but did not cause cell death.
(22)	Van Den Brand et al., 2015	This study investigated population diversity analyses of SRB for nine activated sludge WRRFs in the Netherlands and the effect of long-term (months) oxygen exposures on the SRB activity. All WRRFs have a similar SRB population. There was no seasonal impact on the SRB population. <i>Desulfobacter postgatei</i> , <i>Desulfovibrio desulfuricans</i> and <i>Desulfovibrio intestinalis</i> were the most common and dominant SRB species observed in these samples.
(23)	Guo, 2016	A Modeling study to investigate the interaction of sulfur, iron, and phosphorus in WRRF. Model calibration was not performed.
(24)	Rubio-Rincón et al., 2017	This study investigated how different anoxic and oxic contact times affect the inhibition and activity recovery of SRB under anaerobic conditions. The results of this study suggested that the proliferation of SRB is most likely to occur in BNR plants with an anaerobic fraction higher than 15% and operating at sludge retention times higher than 20 days (at a temperature of 20 °C).
(25)	Solon et al., 2017	Plant-wide modeling taking into accounts the interaction of P, S and Fe. Extending ASM2d in waterline and ADM1 for anaerobic digestion process. The model was not calibrated.
(26)	Hauduc et al., 2018 Hauduc et al., 2017	Incorporating sulfur transformations in a plant-wide model.
(27)	Salehin et al., 2019	This study investigated the feasibility of multiple reuse of iron salts dosed as a sulfide control method in a large full-scale sewer network for removal of phosphate and sulfide control during anaerobic digestion in the downstream WRRF through full-scale field

		studies. FeCl ₂ dosing for sewer corrosion control in the upstream sewer network was beneficially reused for P removal in the activated sludge tanks and subsequently for control of hydrogen sulfide during anaerobic digestion process at the downstream WRRF.
(28)	Rebosura et al., 2018	This study investigated the downstream effects of iron salt-dosing in sewers; on sulfide control in sewers, on phosphorus removal during wastewater treatment, and on H ₂ S control in anaerobic digester biogas production. Addition of ferric salts to sewers contributes to phosphorus removal from wastewater in the downstream WRRF. In-sewer dosing of FeCl ₃ reduces dissolved sulfide concentration in the downstream anaerobic sludge digesters, and consequently decreases the H ₂ S content in biogas.
(29)	Sekyiamah et al., 2008	This study investigated the most important factors affecting the formation and release of VSCs from primary settling and secondary settling. The sampling was performed in a municipal WRRF. The results show that DMS and DMDS were formed in the sludge blanket at the bottom of the secondary settling tank. The VSCs formed in the sludge blanket are returned by RAS pumps to the secondary aeration basin where the intense interaction with compressed air strips the VSCs from the wastewater.
(30)	Einarsen et al., 2000	This study investigated controlled dosing of nitrate to eliminate H ₂ S emission from primary thickener. This approach resulted in considerably better working environment and eliminated complaints from neighbours.
(31)	Flores-Alsina et al., 2020	A plant-wide model was extended and calibrated to mathematically describe the wastewater treatment works performance. Part of sulfur conversion were included in this study.
(32)	Novak and Park, 2010	This study investigated the impact of iron and aluminium addition on digestion and odor-causing compounds by using batch digestion of combined primary and waste activated sludge. The result of this study suggested that that iron and aluminium addition will benefit odor control.
(33)	Park and Novak, 2013	This study investigated the role of iron addition to control H ₂ S on volatile solid removal and odor causing compounds, specifically VOSC reduction. Iron(III) addition to digesters resulted in reduced H ₂ S and VOSCs in headspace gas of dewatered biosolids.
(34)	Roussel and Carliell-Marquet, 2016	In this study iron compounds were identified from full-scale digesters at WRRF to determine the main iron species according to their thermodynamic probability of formation under the specific environmental conditions experienced in each anaerobic digester. Results show that speciation of iron in the sampled anaerobic digesters was controlled by the solid phase through a primary reaction (sulfide precipitation to form pyrite and ferrous sulfide) and secondary reaction (phosphate precipitation to form vivianite).
(35)	Erdirencelebi and Kucukhemek, 2018	This study investigated dosing of iron (III) chloride on full-scale parallel ADs at different levels and patterns to determine the degree of H ₂ S reduction in the biogas, the time for AD response to the dosing level and the effect of additional alkali dosing. Dosing 24-105 mg FeCl ₃ ·L ⁻¹ significantly reduced the H ₂ S in biogas. The results indicated significant role of solid loading rate of primary sludge in H ₂ S production
(36)	Krayzelova et al., 2014	This study investigated the effect of microaeration in UASB reactor treating synthetic brewery wastewater and compared with a non-aerated UASB reactor. Microaeration has the potential to remove large quantities of hydrogen sulfide from biogas formed by the anaerobic treatment of sulfur rich wastewater in microaerobic UASB reactor (UMSB) reactor with granular sludge. Sulfur removed from the biogas and liquid in UMSB reactor was present as inorganic suspended solids in the effluent and partly accumulated on the wall of head space.
(37)	Jenicek et al., 2008	This study investigated the use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes. The application of the microaerobic conditions is an efficient method of the hydrogen sulfide removal from the biogas. The efficiency of the hydrogen sulfide removal from biogas of about 99% is a realistic value at a high initial concentration (4,000–8,000 mg·m ⁻³).
(38)	van der Zee et al., 2007	This study investigated the reaction stoichiometry and kinetics of the micro-aerobic oxidation of sulfide at low concentration levels in oxygen-exposed 'anaerobic' granular sludge. Air was added to the liquid phase. Introduction of a low airflow (0.7–0.9 m ³ ·m ⁻³ ·d ⁻¹ , corresponding to an O ₂ /S molar ratio of 8–10) to a fluidized bed reactor fed with low-sulfate vinasse was sufficient to reduce the biogas H ₂ S-content to an undetectable level. Sulfide was initially oxidized to elemental sulfur, thiosulfate and – most probably – polysulfide. Significant sulfate production did not occur.
(39)	Díaz and Fdz-Polanco, 2012	This study investigated the stability and robustness of the microaerobic removal when intrinsic variations to real digesters operation happen. Lab-scale experiment. The process recovered from oxygen supply withdrawal within hours when the original conditions were restored. The decrease of the sulfur load did not affect the concentration of hydrogen sulfide in the biogas in the short term and the reduction of the oxygen flow to adjust to the lower hydrogen sulfide production resulted in effective removal of the pollutant.
(40)	Nghiem et al., 2014	This study investigates the use of oxidation reduction potential (ORP) to regulate the injection of a small amount of oxygen into an anaerobic digester for reducing H ₂ S concentration in biogas. Six days after the ORP was increased to the range between -320 and -270 mV (from the natural baseline value of -485 mV), H ₂ S in biogas decreased from over 6000 ppm to as low as 30 ppm. H ₂ S concentration from the reference digester (without micro-oxygen injection) was over 4000 ppm. No discernible impacts of micro-oxygen injection on VS removal, COD removal, pH and alkalinity of the digestate, biogas production as well as biogas composition were observed.
(41)	Pokorna-Krayzelova et al., 2017	In this study, the ADM1 model was extended with sulfate reduction and sulfide oxidation to elemental sulfur. The main purpose was to study the effect of oxygen under microaerobic conditions. The model was tested with experimental data obtained from lab-scale UASBs.
(42)	van Leerdam et al., 2008	The goal of the present research is to assess the anaerobic degradation of MT at pH values of at least 8.0 and at 0.5 M sodium. The MT-degrading microbial community in the reactor

		sludge was studied as well. At a sodium concentration of 0.5 M and a pH between 8 and 9 complete MT degradation to sulfide, methane and carbon dioxide was possible at a maximum loading rate of 22 mmol MT L ⁻¹ day ⁻¹ and a hydraulic retention time of 6 h.
(43)	Parker and Wu, 2006	This study developed a mathematical model that is based upon the ADM1 structure to describe the formation and emission of odorous compounds in anaerobic sludge digestion.
(44)	Du and Parker, 2012	This study investigated the kinetics of VOSC generation from methionine and their subsequent degradation in mesophilic and thermophilic anaerobic sludge digestion. In addition, a mathematical model was developed to describe the conversion. Methyl mercaptan was found to be the only VOSC directly generated from methionine degradation. Methyl mercaptan was methylated to form dimethyl sulfide and both methyl mercaptan and dimethyl sulfide were subsequently degraded to H ₂ S. Mixed-second order kinetics were found to best fit the VOSC generation and conversion processes.
(45)	Knobel and Lewis, 2002	This study developed a mathematical model to describe the dynamic and steady-state behaviour of an anaerobic digester, treating high sulfate waste waters.
(46)	Lizarralde et al., 2010	This study investigated a new mathematical model to describe the process of sulfate reduction and possible scaling that take place in a UASB reactor fed with high sulfate concentration wastewater. Model was calibrated with experimental data obtained from a lab-scale UASB. Simulated H ₂ S concentration in the biogas was higher than measured values.
(47)	Barrera et al., 2015	An extension of ADM1 with sulfate reduction was proposed, calibrated and validated for the description of the anaerobic digestion of cane-molasses vinasse (high strength and sulfate rich wastewater). Calibration and validation was performed using lab-scale data.
(48)	Poinapen and Ekama, 2010	This study developed a 2-phase (aqueous-gas) kinetic model for biological sulfate reduction using primary sewage sludge as carbon source.
(49)	Flores-Alsina et al., 2016	This study develops a mathematical model for phosphorus (P), sulfur (S) and iron (Fe) interactions for dynamic simulations of anaerobic digestion processes. The state variables of sulfur compounds were sulfate, sulfide and elemental sulfur.
(50)	Fedorovich et al., 2003	Extending ADM1 with sulfate reduction The model was tested using the available data in the literature
(51)	Batstone, 2006	This study reviewed current knowledge, and requirements for future modelling of anaerobic domestic wastewater treatment. Modeling of biological sulfate reduction was discussed in this study.
(52)	Carrera-Chapela et al., 2016	This study developed a mathematical model with focus on the H ₂ S generation with a reduce number of parameters of five stages was developed. The model and parameters were calibrated and validated with experimental data from two pilot scale anaerobic reactors treating sewage sludge.
(53)	Ahmed and Rodríguez, 2017	This study evaluated available sulfate reduction models for anaerobic digestion process in terms of their structure and complexities.
(54)	Dai et al., 2017	This study investigated alkaline fermentation method for the simultaneous enhancement of methane yield and decrease in H ₂ S production.
(55)	Du and Parker, 2013	The composition of sulfur in primary and waste-activated sludge (WAS) before and after lab-scale anaerobic digestion at 35 and 55°C was characterized.
(56)	Donoso-Bravo et al., 2018	This study developed and tested a mathematical model of the microaerobic digestion process based on the ADM1 model using experimental data from pilot-scale anaerobic digesters operated under microaerobic conditions. The model's prediction potential was largely enhanced by adding retention parameters that account for the activity of sulfide oxidizing bacteria retained inside the anaerobic digester, which have been consistently shown to be responsible for a large share of the H ₂ S removed.
(57)	Jeniček et al., 2017	This study reported the experiences obtained during long-term operation (in the period of years 2003-2015) of 7 microaerobic digesters in central Europe in terms of H ₂ S removal and changes in the quality of sludge and sludge liquor. The hydrogen sulfide removal efficiency of about 99% can be achieved at a high initial H ₂ S concentration (above 4000 mg m ⁻³). The achievement of hydrogen sulfide concentration in tens of mg m ⁻³ is realistic.
(58)	Mejia Likosova et al., 2013	This study investigated the optimal process conditions to achieve an effective solid-liquid separation and phosphate recovery from iron phosphate sludge via sulfide addition. In particular, the effects of pH, mixing time, sulfide dosing rate and settling time have been investigated in detail. Effective phosphorus recovery from iron phosphate sludge can be achieved by sulfide addition, reaching 70 ± 6% recovery at a S/Fe stoichiometric molar ratio of 1.5 and increasing up to 92 ± 6% as the S/Fe molar ratio increased to 2.5
(59)	Kazadi Mbamba et al., 2019	This study investigated mechanisms of iron and phosphorus in pilot-scale data to evaluate control and operational strategies. The main novelty of this contribution is to predict P and Fe transformations in both water and sludge lines. The interaction of sulfur with iron and phosphorus were included.
(60)	Chen et al., 2019	This study investigated the effects of THP at different temperatures on the metabolism of amino acids including methionine and cysteine in sewage sludge in high solid AD. 120 °C and 160 °C were set as the typical temperatures because of their different promoting effects on organic matter solubilization and high solid AD performance of dewatered sludge.
(61)	Liu et al., 2015	This study investigates the release of organic sulfur in sludge as sulfur-containing odors at 100–400 °C. It was observed that the formation of H ₂ S, the predominant odorant, significantly increases at temperatures above 150 °C.
(62)	Dhar et al., 2011a	This study investigated the impact of thermo-oxidative pretreatment on various volatile sulfur compounds removal in continuous flow anaerobic digestion. For the pretreated feed digester, the H ₂ S, DMS and MT concentrations in biogas decreased by 75%, 40%, and 10% compared to the control digester, respectively.

(63)	Dhar et al., 2011b	This study investigates the impact of pretreatment on WAS prior to anaerobic digestion using a laboratory scale system combining mechanical and chemical (H ₂ O ₂ and iron salt) pretreatments. Different chemical dosages have significantly decreased dissolved sulfide concentration before anaerobic digestion.
(64)	Li et al., 2020a	One of the objectives of this study was to identify the characteristics of VSC emissions during HSAD (including VSC emission rate and content) and explored the effects of THP on VSC emissions. VSCs are directly produced during THP. The concentration of soluble amino acids decreased, while the concentration of SO ₄ ²⁻ increased.
(65)	Li et al., 2020b	This study investigated the effect of solid content, initial pH, and thermal hydrolysis on the VSCs conversion in AD. It was postulated that THP may reduce the sludge particle size, increasing the contact of sulfur compounds with invertase, thus favoring VSCs conversion.
(66)	Abelleira-Pereira et al., 2015	This study showed that the hydrogen sulfide (H ₂ S) average content (%) in the biogas coming from BMP tests of the sludge pretreated at 170°C (0.110 ± 0.040%) was found to be almost double of that in the biogas produced in the BMP tests of raw sludge (0.060 ± 0.010%).
(67)	Higgins et al., 2011	The goal of this research was to investigate the mechanisms of biosolids odor production and pathogen indicator regrowth, and observe factors that contribute to the stimulation of microbial growth from the originally stable liquid biosolids.
(68)	Park et al., 2014	This study investigated anaerobic degradation of individual amino acids (including cysteine and methionine) in order to estimate the anaerobic digestion of the amino acids that are formed by the hydrolysis of sewage sludge.
(69)	Chen et al., 2011	This study investigated whether a preferential stimulation of microorganisms in anaerobically digested biosolids can occur after dewatering and if it can lead to pathogen indicator regrowth and odor generation upon storage. Laboratory incubation simulating biosolids storage indicates that both odorant generation, based on total volatile organic sulfur compound concentrations (VOSCs) and pathogen indicator regrowth, based on fecal coliform densities follow similar formation and reduction patterns.
(70)	Erdal et al., 2008	This study reported recent findings on biosolids cake odor reduction.
(71)	Chen et al., 2007	This study investigated the use of aluminum sulfate, which was added just prior to condition, for its potential on storage biosolids odor control by reducing accessibility of soluble proteins in biosolids.
(72)	Novak et al., 2006	This study investigated the management of odor-causing compounds from anaerobically digested biosolids and dewatered sludge cakes. The main odor-causing chemicals were volatile sulfur compounds, which included hydrogen sulfide, methanethiol, and dimethyl sulfide. Volatile sulfur compound production peaked in 3 to 8 days and then declined. The decline was a result of conversion of organic sulfur compounds to sulfide.
(73)	Gruchlik et al., 2017	This study investigated odorous compounds present in biosolids and sludge produced at WRRFs and determine if the best odor reduction strategy. Alum addition at the 4% Al dose was effective in reducing odor in anaerobically digested biosolids.
(74)	Higgins et al., 2006	This study investigated the mechanisms for production and degradation of volatile organic sulfur compounds (VOSCs), key odor causing compounds produced by biosolids.
(75)	Higgins et al., 2008	This study investigated the hypothesis that bioavailable protein and, more specifically, the sulfur-containing amino acids within the protein, can be degraded by proteolytic enzymes to produce odor-causing compounds—mainly volatile sulfur compounds (VSCs)—during biosolids storage.
(76)	Zerlottin et al., 2013	This study investigated the occurrence of spontaneous self-heating of sludge after drying, to understand its nature, course and remediation.
(77)	Poffet et al., 2008	This study investigated the characteristics of dried sewage sludge granules and the possible reasons for the observed self-heating phenomenon. It was found that a cascade of reactions and a series of circumstances are responsible for the thermal runaway, where each of them isolated would not lead to an event.
(78)	Bertani et al., 2016	This study investigated the onset of spontaneous heating in dried sludge originated from primary and biological treatment of both municipal and industrial wastewater. All the experimental evidences indicated that the most relevant processes involved the Fe/S/O system in a series of interconnected redox processes.

Appendix B. Experimental design

This section contains supplementary materials concerning the application of the experimental design procedure to the studied plant, described in Chapter 3.

Appendix B1: Experimental design procedure

Appendix B2: The simplified process flow diagram of the studied plant

Appendix B3: Key variables

Appendix B4: Mass balances

Appendix B5: Data inventory

Appendix B6: Pareto-optimal front

B1. Experimental design procedure

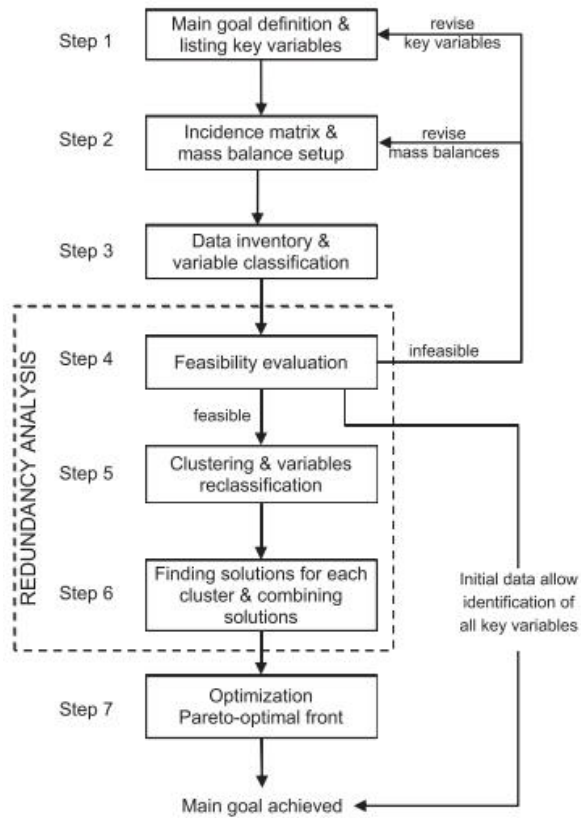


Figure B1. Experimental design procedure for the selection of sets of (additionally) measured variables that allow the identification of key variables (Le et al., 2018).

B2. Process flow diagram of the studied plant

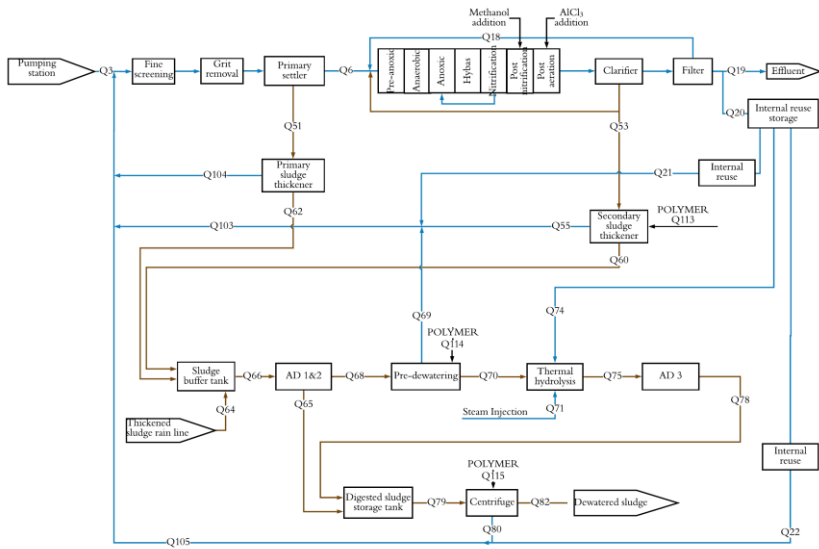


Figure B2. Process flow diagram of the studied plant.

B3. Key variables

The important water, sludge, and gas streams in regard to sulfur flows were selected as key variables. These streams were selected as key variables because higher accuracy for them were required.

Table B3. List of key variables in the water, sludge, and gas streams used for the first step of the experimental design.

<i>Description</i>	<i>Symbol</i>	<i>Description</i>	<i>Symbol</i>
<i>Water streams</i>			
Influent wastewater	MS3	Inlet of thermal hydrolysis	MS70
Pre-settled wastewater	MS6	Inlet of anaerobic digester 3	MS75
Treated Effluent	MS19	Outlet of anaerobic digester 3	MS78
<i>Sludge streams and centrates</i>		<i>Gas streams</i>	
Primary sludge	MS51	Biogas digestion 1&2	MS121
Secondary sludge	MS53	Biogas digestion 3	MS122
Thickened primary sludge	MS62	Emission flux primary settler	MS124
Inlet anaerobic digesters 1&2	MS66	Emission flux primary thickener	MS125
Outlet anaerobic digesters 1&2	MS68	Emission flux sludge buffer tank	MS126
Centrate of pre-dewatering	MS69		

B4. Mass balances

Table B4 presents the mass balances over the subsystems of the studied plant. Notations consist of a combination of the variable name and the flow number in the process flow diagram. Mass balances were set up in a linear form, implying the flow rate and total sulfur mass flows entering and leaving the boundaries which were drawn around individual and/or grouped unit processes. All unit processes were assumed completely mixed and the same densities were assumed for all streams. Gas streams which were expected to contain a significant amount of sulfur were taken up as key variables. The sulfur content in the remaining gas streams was assumed negligible – they were therefore not included in the mass balances.

Table B4. Mass balances of the studied plant.

#	Units	Mass balances	Group
1	Primary settling	$Q3 - Q6 - Q51 + Q103 + Q104 + Q105$	
2	Secondary treatment	$Q6 - Q19 - Q20 - Q53$	
3	Primary thickener	$Q51 - Q62 - Q104$	
4	Secondary thickener	$Q53 - Q55 - Q60$	
5	Sludge buffer tank	$Q60 + Q62 + Q64 - Q66$	
6	Digestion first stage	$Q66 - Q65 - Q68$	
7	Pre-dewatering	$Q68 - Q69 - Q70$	Flow (Q,
8	Thermal hydrolysis	$Q70 + Q71 + Q74 - Q75$	$m^3 \cdot day^{-1}$)
9	Digestion second stage	$Q75 - Q78$	
10	Storage of digested sludge	$Q65 + Q78 - Q79$	
11	Post-dewatering	$Q79 - Q80 - Q82$	
12	Reject water (103)	$Q55 + Q69 + Q21 - Q103$	
13	Reject water (105)	$Q80 + Q22 - Q105$	
14	Primary settling	$MS3 - MS6 - MS51 + MS103 + MS104 + MS105$ - MS124	
15	Secondary treatment	$MS6 - MS19 - MS20 - MS53$	
16	Primary thickener	$MS51 - MS62 - MS104 - MS125$	Total sulfur
17	Secondary thickener	$MS53 - MS55 - MS60$	(MS,
18	Sludge buffer tank	$MS60 + MS62 + MS64 - MS66 - MS126$	$kg \cdot day^{-1}$)
19	Digestion first stage	$MS66 - MS65 - MS68 - MS121$	
20	Pre-dewatering	$MS68 - MS69 - MS70$	

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#	Units	Mass balances	Group
21	Thermal hydrolysis	MS70 + MS71 + MS74 - MS75	
22	Digestion second stage	MS75 - MS78 - MS122	
23	Storage of digested sludge	MS65 + MS78 - MS79 - MS127	
24	Post-dewatering	MS79 - MS80 - MS82	
25	Reject water (103)	MS55 + MS69 + MS21 - MS103	
26	Reject water (105)	MS80 + MS22 - MS105	

B5. Data inventory

The results of data inventory are presented in Table B5. Based on the experimental design procedure, the initially measured, initially unmeasured, and unmeasurable variables were identified. The typical values and uncertainty were estimated for each variable, knowing that the relative magnitude of the estimation was sufficient. These estimations were made either using the values from available dataset or by expert knowledge, literature, or other WRRFs. The unmeasured flow rates were considered as unmeasurable, because they were only small fraction of all flow rates in the studied plant. Consequently, the additional measurements concerned only total sulfur concentration and the cost was therefore proportional to the number of additional sampling points for the measurement of total sulfur concentration.

Table B5. Data inventory in terms of flows (Q) and concentrations of total sulfur (S) in the studied plan.

Stream (PFD)	Variable Stream ↓ →	Q m ³ ·d ⁻¹			S gS·m ⁻³				
		m	c	a	b	m	c	a	b
3	Influent wastewater	1	140 000	5	20	50	10		
6	Pre-settled wastewater	1	150 000	5	20	50	10		
51	Primary sludge	1	1 800	5	20	100	15		
103	Reject water	1	4 667	5	20	50	15		
104	Filtrate of primary thickener	1	816	5	20	50	15		
105	Reject water	1	1 000	5	20	50	15		
18	Backwash water of filter	1	1 000	5	20	50	10		
19	Treated Effluent	1	140 000	5	20	50	10		
20	Internal reuse water	1	3 810	5	20	50	10		
53	Secondary sludge	1	3 621	5	20	100	15		
55	Filtrate of dynamic thickener	x	2 915	5	20	50	15		
60	Thickened dynamic sludge	1	242	5	20	500	15		
62	Thickened primary sludge	1	300	5	20	500	15		
64	Thickened rain sludge	1	30	5	20	500	15		
65	Bypass of thermal hydrolysis	1	30	5	20	500	15		
66	Inlet anaerobic digesters 1&2	1	671	5	20	500	15		
68	Outlet anaerobic digesters 1&2	1	671	5	20	500	15		
69	Filtrate of Pre-dewatering	x	600	5	20	50	15		
70	Inlet of thermal hydrolysis	1	101	5	20	2 000	15		
71	Steam injection to thermal hydrolysis	1	23	5	50		5		
74	Internal reuse water to thermal hydrolysis	1	80	5	20	50	10		

75	Inlet of anaerobic digester 3	1	200	5	20	1 000	15
78	Outlet of anaerobic digester 3	1	200	5	20	1 000	15
79	Inlet of post-dewatering	1	200	5	20	1 000	15
80	Filtrate of post-dewatering	x	150	5	20	100	15
82	Dewatered sludge	1	40	5	20	3 000	15
21	Internal reuse to reject water	1	1 200	5	20	50	10
22	Internal reuse to reject water	1	700	5	20	50	10
111	Methanol addition	1	3	5	1	0	
112	AlCl ₃ addition	1	2	5	1	0	
113	Polymer dynamic thickening	1	45	5	1	0	
114	Polymer addition pre-dewatering	1	45	5	1	0	
115	Polymer addition post-dewatering	1	84	5	1	0	
121	Biogas digestion 1&2	1	14 000	5	1	1	5
122	Biogas digestion 3	1	8 000	5	1	1	5
124	Emission flux primary settler	1	500	5	1	0.5	10
125	Emission flux primary thickener	1	500	5	1	0.5	10
126	Emission flux sludge buffer tank	1	500	5	1	0.5	10
127	Emission flux digested sludge storage	1	500	5	1	0.5	10

(*) Stream number in the process flow diagram (Figure A2), **m** = indicating whether this flow/concentration variable is initially measured (1), not measured (empty), unmeasurable (x), **c** = weighing factor represents the cost of a single measurement, **a** = typical value of the measurement (order of magnitude of the typical values is more important than the absolute values), **b** = expected uncertainty of the measurement. Typical values and expected uncertainties were determined based on the expert knowledge, literature, and data available from other projects.

B6. Pareto-Optimal front

Table B6 lists specifications of the Pareto-optimal solutions in terms of number and type of additionally measured variables, cost of additional measurement, average variance of new estimates of key variables results.

Table B6. Pareto-optimal solutions that allow the key variables to be identifiable

#	a	c	v	S3	S6	S51	S103	S104	S53	S60	S62	S64	S65	S66	S68	S70	S75	S78	S79	S82
1	11	220	7,80	1			1	1		1	1	1	1		1	1	1	1		1
2	12	240	5,01	1			1	1	1	1	1	1	1		1	1	1	1		1
3	13	260	4,66	1			1	1	1	1	1	1	1		1	1	1	1		1
4	14	280	4,56	1			1	1	1	1	1	1	1		1	1	1	1		1
5	15	300	4,48	1		1	1	1	1	1	1	1	1		1	1	1	1		1
6	16	320	4,44	1	1	1	1	1	1	1	1	1	1		1	1	1	1		1
7	17	340	4,41	1	1	1	1	1	1	1	1	1	1		1	1	1	1		1

= solution number

a = number of additionally measured variables (in terms of flow and concentration)

v = standard error of the key variables. The closer v is to unity, the higher the accuracy is.

c = total cost of additional measurements

(1) variables are selected to be measured additionally or (empty) not

Appendix C. Data reconciliation and gross error detection

This section contains supplementary materials concerning the data reconciliation discussed in Chapter 3.

Appendix C1: Data reconciliation procedure

Appendix C2: Overview of raw measurements- input of data reconciliation

Appendix C3: Results of data reconciliation for total sulfur mass flows

Appendix C4: Gross error detection

C1. Data reconciliation procedure

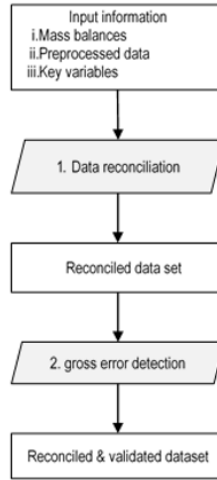


Figure C1. Data reconciliation procedure (Le 2019).

Three common test reported in literature were used in this study namely the global, measurement, and nodal tests (Table C1). Firstly, one can compare each of the new estimates with the original measurements, $y - x$ (Eq. C1). Secondly, one can substitute each of the measurements into the active constraints, $f(y)$. In these two cases the variances of the respective residuals can be derived easily. Thirdly, the sum-of-squares of the residuals of Eq. C1 has a known distribution and can therefore be used.

Table C1. Basic tests in gross error detection (Verheijen, 2010) applied in this study

Test	Description	Measure
Global test	Weighed sum of residuals squared gives an overall view	$y - x$
Nodal test	Each individual constraint misfit is considered	$f(y)$
Measurement test	Each individual measurement is considered	$\sum (y - x)^2$

C2. Overview of raw measurements- input of data reconciliation

Table C2 provides an overview of measured variables and their raw values, comprising flow rates and total sulfur concentrations in water and sludge streams as well as gaseous sulfur (H_2S) emissions. The flow rates were recorded from the SCADA system. The mass of gaseous sulfur was calculated by multiplying the concentration of hydrogen sulfide with the ventilation flow rate on each unit process. The sampling from waterline and sludge line were repeated 1 to 4 times during the measurement campaign. The uncertainty was calculated using the quadrature sum of the following uncertainties: the measurement errors, standard error and error of sampling method. Expert knowledge and available data from other campaigns were used to evaluate the obtained uncertainties.

Table C2. Overview of the raw measurements, i.e. flow rate (Q), the concentration of total sulfur (S) and total sulfur mass flow of the gas streams (MS), in terms of the number of analysis, mean values, and uncertainty of the measurements.

Water and sludge streams							
Variable	Measuring frequency	Mean value ($m^3 \cdot d^{-1}$)	Uncertainty (%)	Variable	Number of samples	Mean value ($g \cdot S \cdot m^{-3}$)	Uncertainty (%)
Q3	continuous	137151	9	S3	3	39	30
Q6	continuous	150693	9	S6	3	42	24
Q19	continuous	142771	11	S19	3	40	26
Q51	continuous	1802	9	S51	4	140	78
Q53	continuous	3985	10	S53	2	77	23
Q60	continuous	231	8	S60	3	480	23
Q62	continuous	375	7	S62	3	567	25
Q64	continuous	35	19	S64	1	343	25
Q65	continuous	27	6	S65	2	407	25
Q66	continuous	697	6	S66	3	440	24
Q68	continuous	666	6	S68	3	407	25
Q70	continuous	108	6	S70	4	2370	23
Q75	continuous	212	6	S75	4	1052	23
Q78	continuous	212	6	S78	4	890	23
Q79	continuous	220	17	S79	1	800	29
Q103	continuous	5672	8	S103	1	37	30
Q104	continuous	1436	10	S104	1	20	30
Gaseous H_2S emissions							
Variable	Measuring frequency	Mean value ($g \cdot S \cdot d^{-1}$)	Uncertainty (%)				

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MS121	continuous	15435	29
MS122	continuous	18309	15
MS124	continuous	2051	23
MS125	continuous	6301	23
MS126	continuous	1250	25
MS127	continuous	405	32

C3. Results of data reconciliation for total sulfur mass flows

Table C3. Results of data reconciliation for total sulfur mass flows(MS) data.

#	variable	Raw estimate	error (kg.day ⁻¹)	sD%	Reconciled	error (kg.day ⁻¹)	sD%*	Δx	k
1	Influent wastewater	5351	1670	31	5817	947	16	108	68
2	Pre-settled wastewater	6379	1633	26	5939	945	16	93	66
3	Backwash	41	11	28	41	8	19	100	52
4	Treated effluent	5741	1540	27	5595	943	17	98	63
5	Reuse water	156	41	26	151	40	27	97	3
6	Primary sludge	252	79	25	215	40	19	85	96
7	Secondary sludge	305	77	25	260	45	17	85	65
8	Filtrate secondary thickening	153	43	28	149	36	24	98	30
9	Thickened secondary sludge	111	27	25	111	25	23	100	15
10	Thickened primary sludge	212	55	26	181	37	20	85	55
11	Thickened rain sludge	12	4	31	12	4	31	102	0.2
12	Bypass thermal hydrolysis	3	3	25	11	3	25	100	1
13	Inlet 1st stage digestion	307	75	25	303	35	12	99	78
14	Outlet 1st stage digestion	271	69	25	277	35	13	102	74
15	Centrate pre-dewatering	15	92	628	50	40	79	344	81
16	Inlet thermal hydrolysis	256	61	24	227	29	13	89	78
17	Dilution water	4	1	26	3	1	26	98	4
18	Inlet 2nd stage digestion	222	52	24	230	26	11	104	75
19	Outlet 2nd stage digestion	188	44	23	213	26	12	113	65
20	Inlet post-dewatering	176	61	34	224	26	12	128	82
21	Centrate of post-dewatering	27	9	35	30	9	30	113	5
22	Dewatered sludge	149	69	47	194	40	20	130	67
23	Reject water	210	65	31	249	48	19	119	47
24	Filtrate of primary thickener	29	9	32	29	9	31	99	6
25	Reject water	57	13	23	58	13	23	103	0.1
26	Reuse water	48	27	57	50	27	54	103	4
27	Reuse water	30	9	30	28	8	30	93	11
28	Primary settling emission	1.97	0.45	23	1.97	0.45	23	100	0.0
29	Primary thickener emission	5.43	1.23	23	5.43	1.23	23	100	0.0
30	Sludge buffer tank emission	1.22	0.24	20	1.22	0.24	20	100	0.0
31	1st stage digestion emission	14.19	4.06	29	14.41	4.05	28	102	0.4
32	2nd stage digestion emission	16.87	2.53	15	16.97	2.53	15	101	00.3
33	Digested sludge emission	0.36	0.11	32	0.36	0.11	32	100	0.0

Raw estimate = calculated mass flows from raw values; error = standard error of the measurement; sD% = relative standard error of the measurement calculated as error/ raw estimate; reconciled = new estimate from data reconciliation; sD%* = standard error of the new estimate, Δ (%) = ratio reconciled variable to raw estimate
 $i_x = \frac{\text{precision improvement}}{\text{var}(x) - \text{var}(\hat{x})} \times 100$, where var(x) and var(\hat{x}) are the variance of raw estimate and reconciled, respectively
 I = identified variables, *Key variables are shown in bold

C4. Gross error detection

Concerning the global test, there was no evidence of gross error in the data set because the value of sum square error (21.53) was smaller than the critical level (27.59, derived from chi-square distribution with 17 degrees of freedom and significant level of 0.05).

In the case of measurement test, there was no evidence of gross error because the t-statistics of every measurement test was smaller than the critical t-statistic. The critical t-statistic was calculated from the significant level of 0.05 with 44 degrees of freedom (number of reconciled variables).

In the case of the nodal test, there was no evidence of gross error because the t-statistic of every mass balance test was smaller than the critical t-statistic. The critical t-statistic was calculated from the significant level of 0.05 with 464 degrees of freedom (Active constraints = mass balances which contain only measured variables).

Table C4. Results of gross error detection for flow rate, total sulfur concentration and total sulfur mass flow

a) Global test: No gross error detected
System degree of redundancy = 17, Sum square error = 21.53, xvalue = 27.59, pvalue = 0.20 (at $\alpha = 0.05$)
b) Measurement test: NO gross error detected (df = 44, critical t-statistic = 3.25, p-value min = 0.00)
c) Nodal test: NO gross error detected (df = 464, critical t-value = 3.87, p-value min = 0.00) number of active constraints = 463

Appendix D. Overview of measurement campaigns

The simplified process flow diagram of sludge treatment line is given in Figure D1. During the measurement campaigns grab samples were collected from specified sampling points.

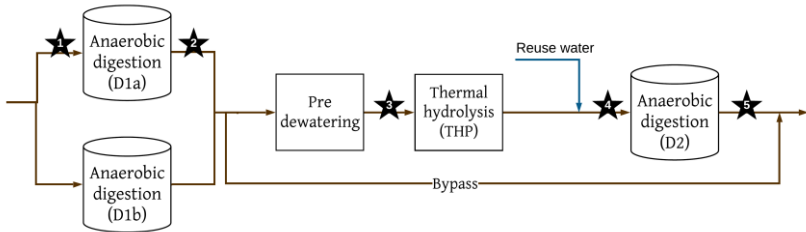


Figure D1. The configuration of two-stage anaerobic digestion with intermediate thermal hydrolysis. Sampling points are shown with ★

The details of measurement campaign are provided in Table S1, which included measurement period, type of measurements and number of analysis.

Table D1. The details of measurement campaigns.

Campaigns	Type of measurement	D1a _{feed} (1)*	D1a _{outlet} (2)	THP _{inlet} (3)	D2 _{feed} (4)	D2 _{outlet} (5)
C1 28/5/2018-18/7/2018	Total sulfur, dry solids, volatile solids	22	7		6	6
	Total particulate sulfur	10	7		6	6
	Total soluble sulfur	10	7		6	6
	Organic sulfur	22	6		6	5
	Particulate organic sulfur	6	5		7	6
C2 (4/6/2019-14/6/2019)	Total sulfur, dry solids	3	2		4	4
C3 (22/10/2020)	Total sulfur, sulfate, soluble iron, soluble and total COD, VFA	1	1	1	1	1

Appendix E. Effect of thermal hydrolysis on sludge solubilisation

The effect of full-scale intermediate thermal hydrolysis on the solubilisation of organic matter and total sulfur are given in Figure E1. The soluble COD accounted for 47% and 31% for the samples of C2 and C3, respectively (Fig. 3). The VFAs (acetic, butyric, and propionic acids) accounted for 4% of the total COD, indicating its formation through hydrolysis of organics. The soluble sulfur fraction remained relatively similar in C1 (2018) and C2 (2019) which accounted for 34% and 41% of total sulfur, respectively. Although the soluble fraction of COD and sulfur in the dewatered sludge entering thermal hydrolysis were not analysed, the soluble fraction in this stream is expected to be low given the high solids content (~20%) of dewatered sludge. Therefore, it is reasonable to attribute the measured soluble fraction of COD and sulfur of thermally treated sludge to the effect of thermal hydrolysis.

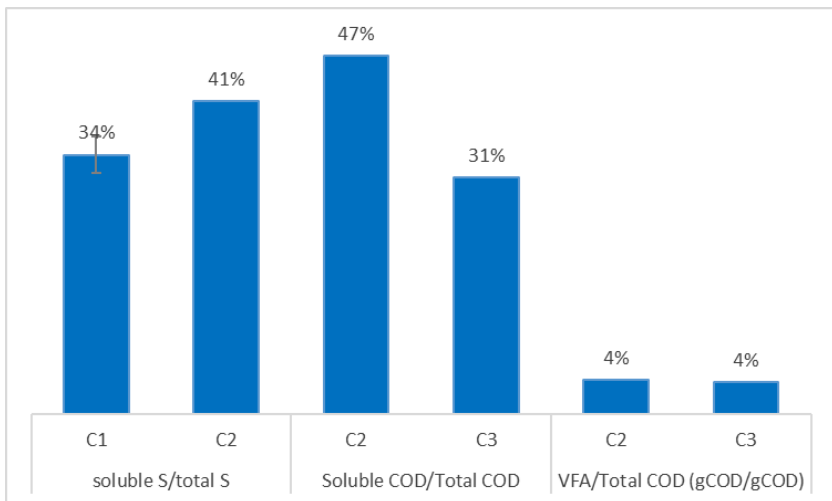


Figure E1. The soluble fraction of total sulfur, soluble fraction of total COD as well as VFA to COD ratio in thermally treated sludge, as an indication of solubilisation in thermal hydrolysis. C1, C2 and C3 refer to measurements performed in 2018, 2019 and 2020, respectively.

Appendix F. Estimating the contribution of cysteine and methionine to sulfide production

The concentration of cysteine and methionine was not directly analysed in this study. Instead, the reported concentrations and removal rates of cysteine and methionine in anaerobic digestion were taken from the study of Chen et al. (2019). They reported the concentration of cysteine and methionine in the raw sludge as 0.46 and 3.6 mg·(g dry solids)⁻¹. In addition, the removal rate of cysteine and methionine in the anaerobic digestion were 34.8% and 48.1%. By taking these values and considering the dry solids (4.4%) of raw sludge in our study, the mass of cysteine and methionine removed during batch anaerobic digestion were calculated to be 420 µg and 4545 µg, respectively. The degradation of cysteine under anaerobic conditions has been found to generate H₂S. The methionine degradation has been observed to lead to the formation of volatile organic sulfur compounds (VOSCs) (i.e. MT and DMS) (Du and Parker, 2012). VOSCs concentrations in digesters are reduced by methanogens that mediate the degradation of VOSCs to H₂S (Parker and Wu, 2006). We assume that sulfide is the final product of both cysteine and methionine degradation. Therefore, the degradation cysteine and methionine mass flows will lead to sulfide formation of 111 µg (62 µg S·(g VS_m)⁻¹) and 975 µg (542 µg S·(g VS_m)⁻¹), respectively.

Curriculum vitae

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Work experiences

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List of publications

Scientific Journals

Forouzanmehr F., Le Q.H., Solon K., Maisonnave V., Daniel O., Buffiere P., Gillot S., Volcke E.I.P., Plant-wide investigation of sulfur flows in a water resource recovery facility (WRRF). *Science of the Total Environment*, p.149530.

Conferences

Forouzanmehr F., Le Q.H., Solon K., Maisonnave V., Daniel O., Buffiere P., Gillot S., Volcke E.I.P., Plant-wide investigation of sulfur flows in a water resource recovery facility (WRRF). Virtual 7th IWA Water Resource and Recovery Modelling Seminar (WWRmod 2021). (Oral presentation).

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FOLIO ADMINISTRATIF

THESE DE L'UNIVERSITE DE LYON OPEREE AU SEIN DE L'INSA LYON

NOM : FOROUZANMEHR		DATE de SOUTENANCE : 10/12/2021
Prénoms : Farhang		
TITRE : Sulfur transformations in water resource recovery facilities (WRRFs) - plant-wide assessment with focus on anaerobic digestion		
NATURE : Doctorat	Numéro d'ordre 2021LYSEI109	
Ecole doctorale : Chimie de Lyon		
Spécialité : Chimie		
RESUME :		
<p>Le soufre est un élément clé, souvent négligé, dans les stations d'épurations des eaux usées (STEP). Il subit des conversions biologiques et physico-chimiques dans les procédés unitaires dominants, tandis que son émission sous forme de sulfure d'hydrogène (H₂S) peut provoquer des nuisances olfactives, de la corrosion et avoir des effets négatifs sur la production de biogaz. Malgré son importance, peu d'informations sont disponibles sur le devenir du soufre dans les STEPs, notamment en ce qui concerne la caractérisation à grande échelle et à l'échelle de l'usine. Ce travail de recherche doctorale présente les avancées dans notre compréhension de la caractérisation du flux de soufre total et de la transformation du soufre pendant les processus de traitement des eaux usées des STEP.</p>		
MOTS-CLÉS :Wastewater treatment, Sulfur transformation, anaerobic digestion, thermal hydrolysis		
Laboratoire de recherche : DEEP (Déchets Eaux Environnement Pollutions) et Biosystems control (BioCo) research group		
Directeur de thèse: Pierre BUFFIERE, Eveline VOLCKE et Sylvie GILLOT		
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Composition du jury :		
Rapporteur, Piet LENS, Professeur, UNESCO-IHE Delft		
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