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PhD. Thesis

Microaeration for biogas desulfurization – experimental and simulation study of various reactor types

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Faculty of Bioscience Engineering Department of Biosystems Engineering

Dizertace

Mikroaerace pro odstraňování sulfanu z bioplynu – experimentální a modelovací studie pro různé typy reaktorů

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Declaration

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"I hereby declare that I have worked out the thesis independently while noting all the resources employed as well as co-authors. I consent to the publication of the thesis under Act No. 111/1998, Coll., on universities, as amended by subsequent regulations."

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Word cloud made from the most used words of the microaeration review paper (Chapter 2).

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Summary

High hydrogen sulfide concentrations in biogas produced during anaerobic treatment of sulfate-rich substrates cause major problems such as inhibition of methanogenic populations, corrosion of concrete and steel, damage to cogeneration units, and toxicity to humans. Microaeration, i.e., the dosing of small amounts of air or oxygen into an anaerobic digester, is a highly efficient, simple and economically feasible technique to remove hydrogen sulfide from biogas. During microaeration, sulfide is oxidized by sulfide oxidizing bacteria to harmless elemental sulfur. However, microaeration has also potential drawbacks such as partial oxidation of organic substrate, aerobic hydrolysis, higher amounts of nitrogen in the biogas or clogging the walls and pipes with elemental sulfur.

In this thesis, the effectiveness of microaeration to remove large quantities of hydrogen sulfide from biogas (over 10 g H_2 S m⁻³) and to decrease the liquid phase sulfide concentration was demonstrated in lab-scale UASB and CSTR reactors, as well as in full-scale CSTR applications. Sulfide from both the gas phase and liquid phase was oxidized to solid elemental sulfur, ending up in the effluent and partly accumulating on the wall of the headspace compartment. Microaeration had no negative effect on the methanogenic activity nor on the efficiency of COD conversion. For suspended sludge, microaeration also improved its dewaterability. A model including sulfate reduction and sulfide oxidation processes was set up to describe microaeration in a UASB reactor. The model was validated using experimental data and showed a good fit in terms of gaseous H_2 S emissions and biogas flow rate.

Biomembranes were successfully introduced at lab-scale and pilot-scale as a novel method for hydrogen sulfide removal through microaeration. The biomembranes served as a support for biomass growth (biofilm) and provided a surface for elemental sulfur precipitation thus avoiding its accumulation in the pipelines. The transport, removal and permeation of hydrogen sulfide, nitrogen, oxygen, methane and carbon dioxide through the biomembranes were studied. Hydrogen sulfide was almost completely removed from biogas while the biomembrane prevented oxygen and nitrogen to contaminate biogas. Both chemical and biochemical sulfide oxidation were observed, the biochemical sulfide oxidation rate being faster.

The results of this thesis demonstrate that microaeration is an effective and promising method for sulfur removal, not only for use in small-scale applications, but also in the real wastewater treatment plant.

Souhrn

Vysoké koncentrace sulfanu v bioplynu jsou hlavním problémem spojeným s anaerobní čištěním substrátů bohatých na sírany. Sulfan způsobuje inhibici metanogenních populací, korozi betonu a oceli, ohrožuje funkci kogeneračních jednotek a je toxický pro člověka. Microaerace, tj. dávkování malého množství vzduchu nebo kyslíku přímo do anaerobního reaktoru, je vysoce účinná, jednoduchá a ekonomicky výhodná metoda odstraňování sulfanu z bioplynu. Během mikroerace se sulfid oxiduje na neškodnou elementární síru za pomoci sulfidy oxidujících bakterií. Mikroerace má ovšem i potenciální nevýhody, jako je částečná oxidace organického substrátu, aerobní hydrolýza, vyšší množství dusíku v bioplynu nebo zanesení stěn a potrubí reaktoru elementární sírou.

V této doktorské práci byla prokázána účinnost mikroaerace pro odstranění velkého množství sullfanu (více než deset tisíc mg m⁻³) z bioplynu a snížení koncentrace sulfidu v kapalné fázi jak v laboratorním (v UASB a CSTR reaktorech), tak v provozním měřítku. Sulfidy z plynné i kapalné fáze byly oxidovány na pevnou elementární síru, která částečně odcházela s odtokem a částečně se hromadila na stěnách plynového prostoru. Mikroerace neměla negativní vliv na metanogenní aktivitu či účinnost odstranění CHSK. U suspendovaného kalu zlepšila mikroerace odvodnitelnost. Dále byl vyvinut matematický model mikroaerace v UASB reaktoru, který zahrnoval procesy redukce síranů na sulfidy a oxidace sulfidů na elementární síru. Model byl validován pomocí experimentálních dat.

V laboratorním a v poloprovozním měřítku byla úspěšně otestována nová mikroaerční metoda pro odstraňování sulfanu z bioplynu za pomocí tzv. biomembrány. Biomembrány sloužila jako podpora růstu biomasy a poskytovala plochu pro srážení elementární síry, čímž se zabránilo její akumulaci na stěnách a potrubí reaktoru. Byl studován transport, odstranění a propustnost sulfanu, dusíku, kyslíku, metanu a oxidu uhličitého skrz biomembránu. Sulfan byl z bioplynu téměř zcela odstraněn, zatímco kontaminace bioplynu kyslíkem a dusíkem byla snížena. Byla pozorována jak chemická, tak biochemická oxidace sulfidů, přičemž rychlost biochemické oxidace byla vyšší.

Výsledky této práce ukazují, že mikroerace je vhodnou metodou pro odstraňování sulfanu z bioplynu a je vhodná nejen pro použití v laboratoři, ale i v reálném měřítku napřiklad na čistírnách odpadních vod.

Samenvatting

Hoge waterstofsulfideconcentraties in het geproduceerde biogas bij de anaerobe behandeling van sulfaatrijke substraten veroorzaken grote problemen, zoals inhibitie van methanogene populaties, corrosie van beton en staal, schade aan warmtekrachtkoppelingseenheden en toxiciteit voor de mens. Microaeratie, i.e. het toedienen van kleine hoeveelheden lucht of zuurstof aan een anaerobe digester, is een zeer efficiënte, eenvoudige en economisch haalbare techniek voor de verwijdering van waterstofsulfide uit biogas. Tijdens microaeratie wordt sulfide door sulfide-oxiderende bacteriën geoxideerd tot het onschadelijke elementaire zwavel. Microaeratie heeft echter ook mogelijke nadelen, zoals gedeeltelijke oxidatie van organisch substraat, aerobe hydrolyse, een verhoogde hoeveelheid stikstof in het biogas of de ophoping van elementaire zwavel op wanden en in buizen.

In dit proefschrift werd de effectiviteit van microaeratie voor de verwijdering van grote hoeveelheden waterstofsulfide uit biogas (meer dan 10 g $H_2S m^{-3}$) en voor de reductie van sulfideconcentraties in de vloeibare fase, aangetoond in laboratoriumschaal UASB en CSTR reactoren, evenals in volleschaal-CSTR-toepassingen. Sulfide uit zowel de gasfase als vloeibare fase werd geoxideerd naar vaste elementaire zwavel dat in het effluent terechtkwam en deels accumuleerde op de wand van het gasfasecompartiment. Microaeratie had geen negatief effect op methanogene activiteit of op de efficiëntie van COD-conversie. In het geval van gesuspendeerd slib verbeterde microaeratie ook de ontwaterbaarheid. Een model met inbegrip van sulfaatreductie- en sulfideoxidatieprocessen werd opgesteld voor de beschrijving van microaeratie in een UASB-reactor. Het model werd gevalideerd op bais van experimentele gegevens en gaf een goede overeenkomst in termen van H_2S -uitstoot en biogasdebiet.

Biomembranen werden succesvol geïntroduceerd op laboratoriumschaal en op pilootschaal als een nieuwe methode voor de verwijdering van waterstofsulfide door microaeratie. De biomembranen dienden als drager voor de groei van biomassa (biofilm) en leverden een oppervlak voor de afzetting van elementaire zwavel, waardoor de accumulatie ervan in de pijpleidingen vermeden werd. Het transport, de verwijdering en doorlating van waterstofsulfide, stikstof, zuurstof, methaan en koolstofdioxide via de biomembranen werden onderzocht. Waterstofsulfide werd bijna volledig verwijderd uit biogas, terwijl het biomembraan verhinderde dat zuurstof en stikstof het biogas vervuilden. Zowel chemische als biochemische sulfideoxidatie werden waargenomen; de biochemische sulfideoxidatie was sneller.

De resultaten uit dit proefschrift tonen aan dat microaeratie een effectieve en veelbelovende methode is voor zwavelverwijdering, niet alleen voor kleinschalige toepassingen maar ook op het niveau van de hele afvalwaterbehandelingsindustrie.

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It always seems impossible until it's done

Nelson Mandela

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List of Abbreviations

ABR	anaerobic baffled reactor
ADM1	anaerobic digestion model no. 1
AOX	adsorbable organic halogens
BMU	biomembrane unit
BOD₅	biochemical oxygen demand
BSA	bovine serum albumin
BTF	biotrickling filter
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
CST	capillary suction time
DAPI	4',6-diamidino-2-phenylindole
DI	deionized water
DO	dissolved oxygen
DWT	decentralized wastewater treatment
EBCT	empty bed contact time
EDTA	ethylenediamine-tetraacetic acid
EGSB	expanded granular sludge bed
ESMR	external silicone membrane reactor
FBR	fluidized bed reactor
G-L-S	gas liquid solid separator
HRT	hydraulic retention time
IC	internal circuit reactor
ICP-MS	inductively coupled plasma mass spectrometer
MBR	membrane bioreactor
MDU	microaerobic desulfurization unit
NWRWRF	northwest regional wastewater reclamation facility
OLR	organic loading rate
ORP	oxidation-reduction potential
ORP _{Ag}	oxidation-reduction potential with argent chloride electrode as reference
PID	proportional-integral-derivative
SCADA	supervisory control and data acquisition
SMA	specific methanogenic activity
SOB	sulfide-oxidizing bacteria

SOU	sulfide-oxidizing unit
SRB	sulfate-reducing bacteria
TN	total nitrogen
тос	total organic carbon
TSS	total suspended solids
T-SHAD	tire-sulfur hybrid adsorption denitrification process
UAF	up-flow anaerobic filter
UASB	up-flow anaerobic sludge blanket reactor
UMSB	up-flow microaerobic sludge blanket reactor
US EPA	United States Environmental Protection Agency
VFA	volatile fatty acid
VSS	volatile suspended solids

List of Symbols

Symbol	Description	Unit
Am	membrane surface area	m ²
Ar	reactor cross section area	m ²
с	concentration	mmoL L^{-1} or mg L^{-1}
С	carbon content	kmole C kg COD-1
D	diffusivity	$m^2 d^{-1}$
dt	time step	h
K _a	acidity constant without T-correction	Μ
k _{AB}	acid base constant	d ⁻¹
k _{chemox}	chemical oxidation rate	h ⁻¹
k _{dec}	decay rate	d ⁻¹ or h ⁻¹
k _{dis}	first order constant for composites disintegration	d ⁻¹
K _H	non-dimensional henry's law constant with T-correction (calculated from original KH in M.bar-1)	M _{liq} M _{gas} ⁻¹
\mathbf{k}_{hyd}	hydrolysis first order constant	d ⁻¹
Kı	inhibitory concentration	Μ
k _L a	volume-specific liquid-gas transfer coefficient	d ⁻¹
k _m	maximum uptake rate	kg COD S kg COD $X^{-1} d^{-1}$ or mmol S mg COD ⁻¹ h^{-1}
Ks	half saturation constant	kg COD m ⁻³ or mmol S L ⁻¹
Р	permeability of the gas through the membrane	mol m m ⁻² s ⁻¹ Pa ⁻¹
\mathbf{P}_{atm}	atmospheric pressure	bar
р	partial pressure	bar
p _i	partial pressure of the gas in the side at the beginning of a particular time step (<i>i</i>)	atm
P _{f,i-1}	partial pressure of the gas on the feed side in the previous time step (<i>i</i>)	Ра
$P_{p,i-1}$	partial pressure of the gas on the permeate side in the previous time step (<i>i</i>)	Ра
r ₁	transfer rate	$L m^{-2} h^{-1}$
R	gas law constant	bar M ⁻¹ K ⁻¹
r _{bioox}	biological oxidation rate	h ⁻¹
r _{chemox}	chemical oxidation rate	h ⁻¹
ρχ	biomass density	kg COD m ⁻³
r _p	granule radius	m
S	concentration	kg COD m ⁻³ or mmoL L ⁻¹ or mg L ⁻¹

Symbol	Description	Unit
t	duration of the time step	S
Т	temperature	°C or K
μ_{SOB}	maximum specific growth rate	h ⁻¹
V	volume	L or m ³
V ₀	volume of sludge at the beginning of measuring	L
V ₅	volume of foamy sludge after 5 min of bubbling	L
V _{Gs}	superficial gas velocity	$m d^{-1}$
V _{ST}	volume of foamy sludge 5 min after the gas flow is stopped	L
х	thickness of the membrane	m
X _{SOB}	SOB concentration	kg m⁻³
Y	biomass yield	kg COD kg COD ⁻¹ or g VSS $g^{-1} S^2$ or mg COD mmol S ⁻¹

Subscript

Symbol	Description	Symbol	Description
а	air side	O ₂	oxygen
аа	amino acids	li	lipids
ас	acetate	pr	protein
ac_ion	ionic acetate	pro	propionate
aSRB	acetate SRB	pro_ion	ionic propionate
b	biogas side	pSRB	propionate SRB
bSRB	butyrate SRB	S	elemental sulfur
biom	biomass	S ²⁻	sulfide
bu	butyrate	S ₂ O ₃ ²⁻	thiosulfate
bu_ion	ionic butyrate	SI	soluble inert
c4	valerate and butyrate	SO4 ²⁻	ionic sulfate
ch	carbohydrate	SOB	sulfide oxidizing bacteria
CH ₄	methane	su	monosacharides
CO ₂	carbon dioxide	va	valerate
fa	long chain fatty acids	Хаа	amino acid degraders
H ₂	hydrogen	Хас	acetate degraders
H ₂ O	water	XaSRB	acetate SRB
H_2S	hydrogen sulfide	XbSRB	butyrate SRB
Нас	acetic acid	Хс	complex particulate
Hbu	butyrate acid	Xc4	butyrate and valerate degraders
Hpro	propionate acid	Xfa	long chain fatty acid degraders
hSRB	hydrogen SRB	Xh2	hydrogen degraders
Hva	valerate acid	XhSRB	hydrogen SRB
IN	inorganic nitrogen	XI	particulate inert
ion	cations and anions	Xpro	propionate degraders
N ₂	nitrogen	XpSRB	propionate SRB
NH_3	ammonia	XSOB	SOB
NH ₄ _ion	ammonium	Xsu	monosaccharide degraders

Superscript

Symbol	Description	Symbol	Description	
α	reaction order	δ	reaction order	
в	reaction order	fin	final concentration	
γ	reaction order	init	initial concentration	

1. INTRODUCTION

1.1. Problem statement

Under anaerobic conditions, dissimilatory sulfate-reducing bacteria use sulfate as the terminal electron acceptor for the degradation of organic compounds while producing hydrogen sulfide. Hydrogen sulfide ends up in both the liquid effluent and biogas formed through the anaerobic digestion of organic material. High concentrations of hydrogen sulfide in biogas reduce its quality, since it causes corrosion of concrete and steel, compromises the functions of cogeneration units, produces emissions of unpleasant odors, is toxic to humans and generates emissions of sulfur dioxide during combustion. In addition, the presence of sulfide in the liquid phase causes corrosion of water transport systems and the accumulation of inert material in the sludge (e.g. metal sulfides). Moreover, sulfide is toxic to methanogens (already at concentrations above 50 mg L⁻¹) and may cause the inhibition of anaerobic processes. For all of these reasons, the production of sulfide is a major problem associated with the anaerobic treatment of sulfate-rich wastewater and organic wastes.

Available methods for sulfide removal from biogas can be classified into physico-chemical and biological methods. Operation at high temperature and pressure, as well as the need for additional equipment and chemicals, make physico-chemical methods energetically demanding and expensive. In contrast, biological methods based on the biochemical oxidation of sulfide to sulfate, thiosulfate and elemental sulfur involve lower operational costs with lower or no need for chemical addition. Biological removal of H₂S from biogas in closed anaerobic reactor (or digester) requires an electron acceptor. Therefore, a small amount of pure oxygen or air must be provided into the reactors for biological desulfurization.

Among the biological desulfurization methods, microaeration has recently gained growing attention. Microaeration typically refers to the controlled dosing of a small amount of air or oxygen into the liquid or gaseous phase of anaerobic digesters. This method is reliable, simple and economically efficient. However, it has also some potential drawbacks such as partial oxidation of soluble substrate, clogging the walls and pipes with elemental sulfur and dilution of biogas with nitrogen in case air is used.

1.2. Objectives

The overall goal of this PhD thesis was to deal with H₂S emissions from the anaerobic treatment of wastewaters with high sulfate content by applying microaerobic conditions. This goal was reached through performing short-term and long-term lab-scale and pilot-scale experiments, combined with mathematical modeling and simulation.

1.3. Scope and organization of this thesis

The state of the art on microaeration has been **reviewed** in **Chapter 2**, summarizing important aspects of microaeration as well as indicating research gaps.

The experiments with microaeration comprise two types: **microaeration directly into the liquid phase of an anaerobic reactor (Chapter 3)**, more specifically a Continuous Stirred Tank Reactor or an

Up-flow Anaerobic Sludge Blanket reactor, and **microaeration into the gas phase of the reactor through biomembranes (Chapter 4)**, i.e. a membrane covered with a biofilm.

Concerning microaeration directly in the anaerobic reactor, **the kinetics of (bio) chemical sulfide oxidation** are studied in **Section 3.1**. Chemical and biochemical batch experiments were conducted at lab-scale to determine the sulfide transformations rate under microaerobic conditions and to set up the kinetic expressions to simulate the experimental data. **Section 3.2** deals with **microaeration in Continuous Stirred Tank Reactors (CSTR)**. The quality of biogas, digested sludge and sludge liquor were compared between anaerobic and microaerobic reactors. **Section 3.3** focuses on the **microaeration in Up-flow Anaerobic Sludge Blanket (UASB) reactor**. Detailed sulfur balances were set up for a strictly anaerobic and a microaerobic UASB. **A model** describing microaeration, **termed ADM1-S/O**, was set up in **Section 3.4** and validated against the experimental data from the UASB reactors of section 3.3. The lack of **full-scale experience with microaeration** in wastewater treatment plants has been overcome in **Section 3.5**, where seven microaerobic digesters in central Europe have been evaluated taking H₂S removal efficiency, operational remarks, and the quality change of digested sludge and sludge liquor into account.

Concerning microaeration through biomembranes, exploratory **lab-scale experiments** are presented in **Section 4.1**. A silicon tube covered with a microaerobic biofilm was studied with respect to the transport, removal and permeation of hydrogen sulfide, nitrogen, oxygen, methane and carbon dioxide. **Microaerobic pilot-scale experiences** are the topic of **Section 4.2**, where a 250 L CSTR was operated with a biomembrane placed in the gas phase. Particular attention was paid to the air dosing, in order to completely remove H_2S from biogas and simultaneously minimize oxygen and nitrogen leftovers in the biogas.

Chapter 5 focuses on **the possible usage of recovered elemental sulfur,** which is the ideal end-product of microaeration. Elemental sulfur has been used in the Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process for the removal of nitrogen through combined adsorption and autotrophic denitrification.

The overall conclusions and research perspectives are summarized in Chapter 6.

1.4. Schematic overview of this thesis



2. MICROAERATION FOR HYDROGEN SULFIDE REMOVAL DURING ANAEROBIC TREATMENT: A REVIEW (Article 1)

Krayzelova, L., Bartacek, J., Díaz, I., Jeison, D., Volcke, E.I.P., Jenicek, P. (2015). "Microaeration for hydrogen sulfide removal during anaerobic treatment: a review." Reviews in Environmental Science and Bio/Technology 14(4): 703-725.

Abstract

High sulfide concentrations in biogas are a major problem associated with the anaerobic treatment of sulfate-rich substrates. It causes the corrosion of concrete and steel, compromises the functions of cogeneration units, produces the emissions of unpleasant odors, and is toxic to humans. Microaeration, i.e. the dosing of small amounts of air (oxygen) into an anaerobic digester, is a highly efficient, simple and economically feasible technique for hydrogen sulfide removal from biogas. Due to microaeration, sulfide is oxidized to elemental sulfur by the action of sulfide oxidizing bacteria. This process takes place directly in the digester. This paper reviews the most important aspects and recent developments of microaeration technology. It describes the basic principles (microbiology, chemistry) of microaeration and the key technological factors influencing microaeration. Other aspects such as process economy, mathematical modelling and control strategies are discussed as well. Besides its advantages, the limitations of microaeration such as partial oxidation of soluble substrate, clogging the walls and pipes with elemental sulfur or toxicity to methanogens are pointed out as well. An integrated mathematical model describing microaeration has not been developed so far and remains an important research gap.

Keywords

Anaerobic digestion, biogas, elemental sulfur, hydrogen sulfide removal, microaeration, sulfide oxidizing bacteria

2.1. Introduction

Under anaerobic conditions, dissimilatory sulfate-reducing bacteria (SRB) use sulfate as the terminal electron acceptor for the degradation of organic compounds while producing hydrogen sulfide (H_2S). H2S ends up in both the liquid effluent and biogas formed through the anaerobic digestion of organic material. High concentrations of hydrogen sulfide in biogas reduce its quality, since it causes corrosion of concrete and steel, compromises the functions of cogeneration units, produces emissions of unpleasant odors, is toxic to humans and generates emissions of sulfur dioxide during combustion. In addition, the presence of sulfide in the liquid phase causes corrosion of water transport systems and the accumulation of inert material in the sludge (e.g. metal sulfides). Moreover, sulfide is toxic to methanogens (already at concentrations above 50 mg L⁻¹) and may cause the inhibition of anaerobic processes (Buisman et al., 1990a; Hao et al., 1996; Hulshoff Pol et al., 1998; Khanal & Huang, 2003b; Stucki et al., 1993; Zhou et al., 2007). For all of these reasons, the production of sulfide is a major problem associated with the anaerobic treatment of sulfate-rich wastewater and organic wastes.

Available methods for sulfide removal from biogas can be classified into physico-chemical and biological methods, as summarized in **Table 2.1**. Many commercial technologies are available on the market, such as SulfaTreat[®] (solid scavenger, iron sponge technology), SOXSIA[®] (sulfur oxidation and siloxane adsorption), THIOPAQ[®] (physical-chemical absorption with biological regeneration), DMT Sulfurex[®] (water scrubber), Sulfur-rite[®] (iron sponge technology), and Media-G2[®] (iron sponge technology).

Operation at high temperature and pressure, as well as the need for additional equipment and chemicals, make physico-chemical methods energetically demanding and expensive (Appels et al., 2008). In contrast, biological methods based on the biochemical oxidation of sulfide to sulfate, thiosulfate and elemental sulfur involve lower operational costs with lower or no need for chemical addition (Buisman et al., 1989; Syed et al., 2006). Biological removal of H₂S from biogas in closed anaerobic reactor (or digester) requires an electron acceptor. Therefore, a small amount of pure oxygen or air must be provided into the reactors for biological desulfurization.

Among the biological desulfurization methods, microaeration has recently gained growing attention. With microaeration, most authors refer to controlled dosing of small amount of air/oxygen into the liquid or gaseous phase of anaerobic digesters (**Figure 2.1**). This method is reliable, simple and economically efficient. However, it has also some potential drawbacks such as partial oxidation of soluble substrate or clogging the walls and pipes with elemental sulfur which are discussed later in this manuscript. This contribution reviews the important aspects of biological removal of sulfide during anaerobic treatment. Particular attention is paid both to the basic principles of sulfide oxidation (microbiology, chemistry) and the technological factors influencing this process. The need for further developments of microaeration, such as mathematical modeling, is discussed as well. Furthermore, the challenges and advantages of biological oxidation of sulfide are described, including economic considerations.

	Physico-chemical methods	Reagent	Parameters	Situation	Additional comments	Reference
	Precipitation	Iron chloride solution		small scale anaerobic digester	for liquid sulfide	Kapdi et al. (2005) Petersson and Wellinger (2009)
	Scrubbing	Sodium hydroxide	high pressure drop (high contact surface), long residence times	lab-scale two-stage co- current contactor (scrubber)	for gaseous H ₂ S large volume contactors	Couvert et al. (2008)
	Physical absorption	Water	pressurizing of biogas	counter-current packed column	high water consumpion for simultaneous removal of H ₂ S and CO ₂	Kapdi et al. (2005) Wellinger and Lindberg (1999)
	Chemical	Iron-chelated solutions	room temperature low gas pressure 1.2-2.2 bar	lab-scale counter- current gas-liquid contactor	for gaseous H_2S	Horikawa et al. (2004)
	absorption	Sodium hydroxide			for gaseous H ₂ S for very large gas volumes or high H ₂ S concentrations	Petersson and Wellinger (2009)
Chen adso		Iron oxides, iron sponge ption	temperature 25°C pressure less than 2 kPa	lab-scale upward or downward flow gas- solid contactors (semi- batch)	for gaseous H ₂ S limited regeneration (1×-2×)	Kohl and Nielsen (1997) McKinsey Zicari (2003)
	Chemical "dry" adsorption		temperature 40°C atmospheric pressure	usually two reaction beds	capacity 1000 Nm ³ gas h ⁻¹ limited regeneration	Petersson and Wellinger (2009) Wellinger and Lindberg (1999)
		Activated carbon (AC)	temperature 50-70°C pressure 7-8 bar 300 mg H ₂ S per 1 g of AC	usually two vessels for continuous system	for gaseous H ₂ S limited regeneration impregnation of AC needed	Bandosz (2002) Wellinger and Lindberg (1999)

 Table 2.1: The summary of physico-chemical and biological desulfurization methods others than microaeration

Biological methods	Electron acceptor	Dominant microorganisms	Situation	Additional comments	Reference
		SOB such as Thiobacillus sp., Sulfolobus sp.	digester	for gaseous and liquid $\rm H_2S$	Petersson and Wellinger (2009)
	Oxygen (pure O ₂	SOB such as Thiobacillus sp., Sulfolobus sp.	trickling filter with packing material	for gaseous H_2S	Petersson and Wellinger (2009)
Biochemical oxidation	or air)	Thiobacillus sp.	biological filter (combination of water scrubbing and biological oxidation)	for gaseous H ₂ S	Wellinger and Lindberg (1999)
		Thiobacillus sp.	lab-scale fixed-film bioreactors	for gaseous and liquid H_2S	Gadre (1989) Jensen and Webb (1995)
	Nitrite		lab-scale batch bioreactor	for liquid sulfide	Mahmood et al. (2007)
		Chemolitotrophic enrichment culture	lab-scale batch bioreactor	for liquid sulfide	Cardoso et al. (2006)
	Nitrate	Pure culture of Thiomicrospira sp. CVO	lab-scale batch and continuous bioreactor	for liquid sulfide	Gadekar et al. (2006)



Figure 2.1: The scheme of possible application of microaeration in anaerobic digesters with biogas and sludge recirculation: A - dosage in the liquid phase, B - dosage in the gas phase, C - dosage in the biogas recirculation.

2.2. Terminology

The action of dosing small quantities of air into the bioreactor is referred to by different terms in literature, such as "microaeration" (Duangmanee et al., 2007; Jenicek et al., 2013a; Jenicek et al., 2014; Jenicek et al., 2008; Jenicek et al., 2010; Krayzelova et al., 2014a; Tang et al., 2004; Tartakovsky et al., 2011), "limited aeration" (Zhou et al., 2007; Zitomer & Shrout, 2000), "aeration" (Bekmezci et al., 2011; Ikbal et al., 2003; Lohwacharin & Annachhatre, 2010), "microoxygenation" (Díaz & Fdz-Polanco, 2012; Díaz et al., 2011a; Díaz et al., 2011b; Fdz-Polanco et al., 2009; Ramos et al., 2012; Ramos & Fdz-Polanco, 2013; Ramos et al., 2014c; Ramos et al., 2013; Ramos et al., 2014d), "oxygenation" (Khanal & Huang, 2003a; Khanal & Huang, 2003b; Khanal & Huang, 2006; Khanal et al., 2003) or "moderate oxygenation" (van der Zee et al., 2007).

The terms "microaeration" or "microoxygenation" reflect (in most cases) the gas used. I.e. when air is dosed into the anaerobic reactor, the process has been called "microaeration", and when pure oxygen is used, the term "microoxygenation" has been applied. However, this has not been a strict rule and not all authors follow it.

Besides, it should be noted that the terms "microaerobic" (Díaz & Fdz-Polanco, 2012; Díaz et al., 2011a; Díaz et al., 2011b; Ramos et al., 2012; Ramos & Fdz-Polanco, 2014a; Ramos & Fdz-Polanco, 2013; Ramos et al., 2014c; Ramos et al., 2014d) or "microaerophilic" (Fdz-Polanco et al., 2009; Chu et al., 2005) are also applied to denote the reactor conditions (bulk liquid oxygen concentrations) as such, and at the same time referring to the act of oxygen dosage as "microoxygenation"

When referring to microaeration, the amount of oxygen is crucial. Several terms have been used when referring to the action of dosing oxygen to a culture. Authors were using the term "aeration/oxygenation" if the dose of oxygen was as high as 102-218 L O_2 L⁻¹ feed (Bekmezci et al., 2011). For the amount of oxygen between 2.6-6.4 L O_2 L⁻¹ feed (Lohwacharin & Annachhatre, 2010)

or 5.1 (Zhou et al., 2007), the authors used prefix "limited". Prefix "micro" was used when the amount of oxygen was 0.03-1.27 L O_2 L⁻¹ feed (Díaz & Fdz-Polanco, 2012; Díaz et al., 2011a; Díaz et al., 2010; Díaz et al., 2011b; Fdz-Polanco et al., 2009; Jenicek et al., 2014; Krayzelova et al., 2014a; Rodriguez et al., 2012). However, van der Zee et al. (2007) used the prefix "moderate" for 0.74-0.94 L O_2 L⁻¹ feed.

In this paper, the process of biological oxidation of sulfide is called "microaeration" if air was used for the oxidation of sulfide and "microoxygenation" if pure oxygen was used instead. As for the amount of air/oxygen dosed, we follow the criteria shown in **Figure 2.2**. The term "microaerophilic" is used only to refer to microorganisms.



Figure 2.2: The terminology for air/oxygen dosing based on the amount of oxygen dosed.

The concentration of dissolved oxygen (DO) is not a good control parameter for the microaeration process since the formation of elemental sulfur or sulfate proceeds at DO concentrations below 0.1 mg L⁻¹, which is the lowest detection limit of commonly available oxygen electrodes (Janssen et al., 1995). The oxidation-reduction potential (ORP) could make up a better control parameter to characterize microaerobic systems. However, a wide range of ORP values have been reported during microaeration: lower than -460 mV (Duangmanee et al., 2007); -320 to -270 mV (Nghiem et al., 2014); -265 mV (Khanal & Huang, 2003b; Khanal & Huang, 2006; Khanal et al., 2003); -230 to -180 mV (Khanal & Huang, 2003a); 0 to -200 mV (Kobayashi et al., 2012); and higher than -150 mV (Xu et al., 2012). This large variation is probably caused by the uniqueness of each system and its operational conditions. Moreover, it is often not clear whether the results are expressed as ORP_H (with hydrogen electrode as reference) or as ORP_{Ag} (with argent chloride electrode as reference).

2.3. Principles of microaeration

To understand the effect of oxygen dosage, it is necessary to understand the nature of both biological and chemical oxidation of sulfide. The most important bioconversions involved in aerobic sulfide removal are (Buisman et al., 1990b; Chen & Morris, 1972; Janssen et al., 1995; Kuenen, 1975):

$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$	$\Delta G^{\circ} = -169.35 \text{ KJ mol}^{-1}$	(Eq. 2.1)
$2HS^- + 4O_2 \to 2SO_4^{2-} + 2H^+$	ΔG° = - 732.58 KJ mol ⁻¹	(Eq. 2.2)
$2HS^- + 2O_2 \to S_2O_3^{2-} + H_2O$	ΔG° = - 387.35 KJ mol ⁻¹	(Eq. 2.3)

The biological removal of hydrogen sulfide (H_2S) is based on the biochemical oxidation of sulfide to elemental sulfur (S^0) or/and sulfate ($SO_4^{2^-}$). Some authors (Díaz et al., 2011b; van den Ende & van Gemerden, 1993) have also reported the production of thiosulfate ($S_2O_3^{2^-}$). Sulfide serves as the electron donor while oxygen serves as the terminal electron acceptor. Under oxygen limiting (microaerobic) conditions, at oxygen concentrations below 0.1 mg L⁻¹, sulfur is the major end-product of the sulfide oxidation (Eq. 2.1), with a partial oxidation to thiosulfate (van den Ende & van Gemerden, 1993). Sulfate is formed under sulfide limiting conditions and implies higher oxygen consumption per mole of sulfide (Eq. 2.2). Chemical oxidation of sulfide, resulting in the formation of mainly thiosulfate (Eq. 2.3) (Janssen et al., 1995) becomes important when biological activity of sulfide oxidizing bacteria is limited. This is the case especially in bioreactors highly loaded with sulfide. In such cases when oxygen is not consumed fast enough by sulfide oxidizing bacteria, the chemical oxidation of sulfide to thiosulfate becomes significant. From the economical point of view, sulfur formation is preferred, since it can potentially be recovered. Besides, the lower amount of oxygen needed for the oxidation to sulfur compared to sulfate implies lower energy consumption.

The formation of sulfur and sulfate can be controlled by the amount of oxygen supplied (Janssen et al., 1995). Theoretically, 0.5 mol $O_2/mol S^{2-}$ is necessary for the oxidation of sulfide to elemental sulfur (Eq. 2.1). According to Janssen et al. (1995) a maximal sulfur production of 73 ± 10% occurred at an O_2/S^{2-} consumption ratio in the range of 0.6 – 1.0 (mol L⁻¹ h⁻¹)/(mol L⁻¹ h⁻¹) with 0.7 as the optimum. According to Alcántara et al. (2004), sulfur-producing steady states were achieved at O_2/S^{2-} ratio ranging from 0.5 to 1.5. The maximum elemental sulfur formation (85% of the total influent sulfur) occurred at the ratio of 0.5. When the ratio was increased up to 2, sulfide was completely oxidized to sulfate. At O_2/S^{2-} as low as 0.15 mol/mol, the activity of sulfide-oxidizing severely decreased. According to the authors, it was probably related to an oxygen limitation in the culture which promoted sulfide accumulation in the reactor (Alcántara et al., 2004). At the ratios between 0.25 and 0.35 thiosulfate was detected in the culture. On the other hand, Díaz et al. (2011a) observed an increase in S₂O₃²⁻ concentration when increasing oxygen rate from 9.3 L d⁻¹ to 14.1 L d⁻¹. This indicated a slight overdose of oxygen.

Munz et al. (2009) observed that in some cases, there is less than 0.5 mol $O_2/mol S^2$ necessary for successful oxidation of sulfide to elemental sulfur. Authors observed 91%, 87%, and 85% of sulfide being converted to elemental sulfur at O_2/S^2 ratio of 0.015, 0.005, and 0.03 mol/mol, respectively. Also, they observed a strong effect of pH on the sulfide oxidation. The maximum elemental sulfur production decreased with increasing pH (from 85–91% to 53–59% at pH = 8 and 9, respectively).

According to Klok et al. (2013) biological oxidation of sulfide significantly depends on the concentration of sulfide. Sulfide oxidizing activity increased at sulfide concentrations from 0 to 0.15 mmoL L^{-1} . At concentrations from 0.3 to 1.0 mmoL L^{-1} , biological activity gradually decreased and increased again at sulfide concentrations from 1.0 to 5.0 mmoL L^{-1} . This was most likely the result of bacteria adaptation to high sulfide concentrations. (Buisman et al., 1990a) observed that the contribution of chemical oxidation of sulfide was larger when sulfur loading rate increased.

2.4. Microorganisms involved in microaeration

Sulfide-oxidizing bacteria (SOB) are the main group involved in sulfide oxidation under microaerobic conditions. In general, SOB are photoautotrophs or chemolithotrophs. Photoautotrophs use CO₂ as

the terminal electron acceptor while chemolithotrophs use oxygen (aerobic species) or nitrate and nitrite (anaerobic species). As microaeration always takes place in dark anaerobic fermenters, photoautotrophs cannot be involved in the process. Also, present paper focus on the dosing of limited amount of air or oxygen into an anaerobic reactor, therefore, chemolithotrophs using nitrite or nitrate as an electron acceptor will not be discussed.

In terms of energy and carbon sources, SOB can be classified into four groups: (1) obligate chemolithotrophs, (2) facultative chemolithotrophs, (3) chemolithoheterotrophs, and (4) chemoorganoheterotrophs (Tang et al., 2009). Obligate chemolithotrophs need CO₂ as carbon source and an inorganic energy source. All known *Thiomicrospira sp.*, many *Thiobacillus sp.*, and at least one *Sulfolobus sp.* belong to this category (Kuenen & Veldkamp, 1973; Matin, 1978). Facultative chemolithotrophs can grow either chemolithoautotrophically with an inorganic energy source and CO₂ as carbon source, or heterotrophically with organic compounds as carbon and energy source. Some *Thiobacilli sp.*, certain *Beggiatoa, Thiosphaera pantotropha*, and *Paracoccus denitrificans* are typical examples of facultative chemolithotrophic SOB (Friedrich & Mitrenga, 1981; Nelson & Jannasch, 1983). Chemolithoheterotrophs such as a few *Thiobacillus sp.* and some *Beggiatoa* strains generate energy from oxidation of reduced sulfur compounds. Chemoorganoheterotrophs can oxidize reduced sulfur compounds without deriving energy from them. *Thiobacterium, Thiothrix*, and some *Beggiatoa sp.* belong to this last group (Larkin & Strohl, 1983).

As far as pH and temperature are concerned, the requirements of various SOB species are diverse. Growth at pH values in the range 1 - 9 and temperatures ranging from 4 to 90 °C have been reported (Tang et al., 2009). The majority of known chemolithotrophic SOB are mesophilic, *Thiobacillus* being the only genera encompassing both mesophilic and thermophilic environments. Other important thermophilic genera are *Sulfolobus* and *Thermothrix*.

The most cited species of SOB found for the oxidation of sulfide was *Thiobacillus* sp. (Alcántara et al., 2004; Annachhatre & Suktrakoolvait, 2001; Maestre et al., 2010; Ravichandra P. et al., 2006) of *Hydrogenophilaceae* family (Luo et al., 2011), specifically *Thiobacillus denitrificans* (Krishnakumar et al., 2005; Lee & Sublette, 1993; Ma et al., 2006; Ongcharit et al., 1990), *Thiobacillus nivellus* (Myung Cha et al., 1999), *Thiobacillus baregensis* (Vannini et al., 2008), *Thiobacillus thiooxidans* (Takano et al., 1997) and *Thiobacillus thioparus* (Vlasceanu et al., 1997). SOB of *Halothiobacillaceae* family were observed by Vannini et al. (2008) (*Halothiobacillus neapolitanus*) and Luo et al. (2011). Other SOB found to participate on the oxidation of sulfide were of genus *Thiomicrospira* (Gadekar et al., 2006), *Thioonas* (Ng et al., 2004), *Thiothrix* (Cytryn et al., 2005; Maestre et al., 2010) with the specific species of *Thiothrix nivea* (Prescott et al., 2010), *Sulfurimonas* with the specific species of *Sulfurimonas* (Maestre et al., 2010), and *Acidithiobacillus* with the specific species of *Acidithiobacillus thiooxidans* (Lee et al., 2006).

2.4.1. SOB found in anaerobic reactors subjected to microaeration

Most of SOB found in microaerobic reactors for biogas production belong to phylum *Proteobacteria* or, exceptionally to phylum *Actinobacteria*. *Halothiobacillus sp., Acidithiobacillus sp.,* and *Sulfuricurvum sp.* were the most frequently cited species (**Table 2.2**). SOB were found almost exclusively in the headspace of the reactors or in the gas-liquid interphase suggesting that sulfide oxidation took place there.
Tang et al. (2004) observed a shift in the archaea population as the consequence of the introduction of microaeration. The size of *Methanosarcina sp.* population was reduced, while the size of *Methanoculleus sp.* population increased. In contrast, Ramos et al. (2014d) did not observe any particular impact on any of the archaeal populations while changing from anaerobic to microaerobic environment.

Genus	Phylum	Location	Aeration gas	Reference
Acidithiobacillus thiooxidans	Proteobacteria	Bottom of biotrickling filter	air	de Arespacochag a et al. (2014)
Arcobacter, Sulfuricurvum Acidithiobacillus,	ε -Proteobacteria γ -Proteobacteria	Headspace, liquid interphase	02	Ramos et al. (2014c)
Acinetobacter, Rhodococcus	γ -Proteobacteria Actinobacteria	Headspace	O ₂	Ramos et al. (2014c)
Acinetobacter, Arcobacter, Sulfuricurvum	Proteobacteria	Microaerobic desulfurization unit	O ₂	Ramos et al. (2013)
Halothiobacillus neapolitanus, Sulfurimonas denitrificans	Proteobacteria	Headspace	air	Kobayashi et al. (2012)
Halothiobacillus, Thiofaba	γ -Proteobacteria	Headspace	O ₂	Rodriguez et al. (2012)
Acidithiobacillus thiooxidans, Arcobacter mytili, Halothiobacillus neapolitanus, Thiomonas, Thiobacillus, Sulfuricurvum kujiense	Proteobacteria	Headspace (reactor with sludge recirculation)	O ₂	Díaz et al. (2011b)
Halothiobacillus kellyi, Arcobacter mytili,	Proteobacteria	Headspace (reactor with biogas recirculation)	0 ₂	Díaz et al. (2011b)

Table 2.2: Sulfide oxidizing bacteria found in anaerobic reactors subjected to microaeration

2.5. Technological and physical factors influencing microaeration

2.5.1. Oxygen dosing point and mixing method

• <u>Air dosing point</u>

Number of authors compared the efficiency of microaeration when air is dosed into the headspace or into the liquid phase of anaerobic digesters (**Figure 2.1**). When dosed into the headspace, oxygen can directly react with gaseous hydrogen sulfide and, therefore, the amount of air needed per given

amount of hydrogen sulfide is minimized (Díaz et al., 2011b; Ramos et al., 2012). This is important, because dosing lower amount of air induce lower contamination of biogas by nitrogen. On the other hand, when air is overdosed in order to assure complete H_2S removal, the excess oxygen will contaminate biogas (Díaz et al., 2010; Díaz et al., 2011b).

When air is dosed into the sludge, the intense contact between oxygen and the liquid phase will facilitates non-specific oxidation of degradable organic compounds, i.e. some losses of oxygen. This will increase the necessary air dosage and, hence, the contamination of biogas by nitrogen. Potentially, certain part of organic load can be oxidized along with sulfide, but the decrease of methane yield due to this oxidation is usually negligible (Krayzelova et al., 2014a).

Dosing air into the liquid phase also causes the decrease of sulfide concentration in the liquid phase (Díaz et al., 2011b; Krayzelova et al., 2014a; van der Zee et al., 2007; Zhou et al., 2007). However, this decrease is usually only about 20 to 30% (Krayzelova et al., 2014a) and cannot explain the large decrease in H₂S concentration in biogas. This implies that majority of H₂S oxidation takes place in the head space even if air is dosed into the liquid phase. Besides H₂S removal from biogas, the decrease of sulfide concentration in the liquid has the additional positive effect of decreasing sulfide toxicity towards methanogens.

<u>Mixing method</u>

The contact between oxygen and liquid phase is also intensified in digesters mixed by biogas recirculation. Analogically to dosing air into the liquid phase, this will increase the consumption of oxygen due to the reaction with organic compounds. Again, sulfide concentration in the liquid phase is decreased due to the intensified contact between oxygen and the liquid phase (Díaz et al., 2011a; Díaz et al., 2011b; Fdz-Polanco et al., 2009).

2.5.2. The location of sulfide oxidation and sulfur accumulation

For a proper design of microaeration, it is important to find out where the oxidation of sulfide occurs, i.e. whether it takes place in the biofilm covering the wall of the gas phase or in the liquid phase. Results from numerous microbial analyses (Table 2.2) revealed that SOB populations grow mainly on the walls of the headspace (Díaz et al., 2011b; Kobayashi et al., 2012; Ramos et al., 2014c; Rodriguez et al., 2012) or on the gas-liquid interphase Ramos et al. (2014c) suggesting that biological oxidation of sulfide takes place there. The intensity of microaerobic processes strongly depended on the available surface area in the headspace. Ramos et al. (2014a) operated a pilot reactor with variable size of headspace to investigate where the process of biogas desulfurization predominantly took place. In this study, oxygen was injected into the liquid phase. Hydrogen sulfide was entirely removed from the biogas when the digester had 25 L headspace and little or no H₂S removal was observed when the size of headspace was minimized to almost 0 L. Moreover, the deposition of elemental sulfur in the headspace could represent a clear indication that the oxidation takes place there (Ramos et al., 2012). Kobayashi et al. (2012) observed the accumulation of microbial mats, containing elemental sulfur as the dominant component, on the inner walls of a reactor headspace including ceiling, wall, net, and catwalk. Also Ramos et al. (2014c) and Rodriguez et al. (2012) observed the elemental sulfur accumulation all over the walls of the headspace. This indicates that the headspace of a bioreactor may act as a "biofilter", where SOB can grow on all available surfaces. The sulfur mats also serve as additional support material where new microbial mats develop. Furthermore, scanning electron microscopy revealed that these sulfur mats were formed mostly by upward filaments (perpendicular to the gas-liquid interphase) creating a support with large specific surface. This may help SOB in the competition for oxygen (Kobayashi et al., 2012).

In contrast, Díaz et al. (2011b) observed only partial accumulation of elemental sulfur in the top of headspace and on the walls while Díaz et al. (2011b) and Ramos et al. (2014d) did not observe any accumulation of elemental sulfur in the headspace. These authors suggested that the elemental sulfur formed in their reactors has most probably fallen into the liquid effluent. However, this suggestion could not be proved and it remains unclear why sulfur deposition on headspace walls was not observed in these cases.

According to Krayzelova et al. (2014a), only 10% of the produced elemental sulfur remained in the headspace of a UASB reactor, while 33% left the reactor with the liquid effluent. In this case, the small headspace of UASB-type reactors was probably responsible for the modest depositions of sulfur in the headspace. Large range of elemental sulfur concentrations detected in the effluent samples was also observed by van der Zee et al. (2007).

Additionally, sulfur deposition in the headspace was not reported when oxygen was sparged in fine bubbles into the bioreactors (Khanal & Huang, 2003a; Khanal & Huang, 2006; Zitomer & Shrout, 1998; Zitomer & Shrout, 2000), thus increasing oxygen transfer to the bulk liquid phase. Under such condition, sulfide oxidation seemed to take place only in the liquid phase. Under this condition a significant consumption of oxygen for aerobic oxidation of organic matter was observed and SOB were found in the sulfur mats formed in headspace walls. This may indicate that oxidation of organic matter out-competed the development of SOB in the liquid phase (Khanal & Huang, 2006; Zitomer & Shrout, 2000). The problems associated with elemental sulfur deposition on reactor walls and pipes will be discussed further.

2.5.3. Oxygen flow rate and biogas residence time in headspace

In general, bioreactors treating materials with low COD/S ratios, such as wastewater from brewery, sugar or paper industries (**Table 2.3**), produce large amounts of hydrogen sulfide. As a result of low COD/S ratios, these wastewater streams have been shown to require higher amounts of oxygen per volume of biogas (Zhou et al., 2007), in comparison to sewage sludge, agricultural wastes or manure. Normally, oxygen dosage (or equivalent air) between 0.3% and 3% of produced biogas in the bioreactor is enough to achieve efficient biogas desulfurization (**Table 2.3**). However, oxygen rate of up to 12% may be necessary if both gaseous and dissolved sulfide must be removed.

The residence time of biogas in the headspace is a key factor affecting sulfide removal efficiency, when providing oxygen/air injection into the headspace. Typically, removal efficiencies over 97% were obtained with residence times over 5h (**Table 2.3**). Schneider et al. (2002) found 88% removal efficiency with a residence time of 2.5h while it was lower than 40% under 1.25h. When the headspace was suppressed totally, the concentration of hydrogen sulfide in biogas produced with microaerobic treatment was similar to that found in unaerated digesters (Ramos et al., 2014a).

Reactor	OLR	Feed (COD:S ratio)	Reactive (dosing point)	Reactive Flow Rate	O₂:biogas ratio	O ₂ :H ₂ S _(g) ratio	Gas residence time in headspace	H ₂ S _(g) conc. without microaeration	H ₂ S _(g) removal efficiency	H ₂ S _(d) +HS ⁻ _(d) removal efficiency	Residual O2 in biogas	Reference
(volume in L)	$[g_{COD} L^{-1} d^{-1}]$				[%]	[mol mol ⁻¹]	[h]	[ppmv]	[%]	[%]	[%]	
Fully-mixed digester (10)	2	sludge (40)	Air (liquid)	1.6 Ld ⁻¹	1.7-9.2	1.3-7.4	n.a.	13,000	≥99	68	n.a.	Jenicek et al. (2014)
UASB (3)	8	synthetic brewery ww. (95)	Air (liquid)	1 Ld ⁻¹	2.5	3.9	n.a.	67,000	73	15	<0.1	Krayzelova et al. (2014a)
Fully-mixed digester (70)	2.3	sludge (72)	O ₂ (liquid)	ORP controlled (-320270 mV)	n.a.	n.a.	n.a.	6,000	≥99	n.a.	1-1.8	Nghiem et al. (2014)
Fully-mixed digester (7,000)	1.5-2.2 $g_{VS} L^{-1} d^{-1}$	sludge (-)	92-98% O₂ (headspace or liquid)	5-34 Lm ⁻³ d ⁻¹	1	0.9-2	10	2,500-4,900	99	≈0	<0.1	Ramos et al. (2014d)
Fully-mixed digester (250)	1-1.9 $g_{VS} L^{-1} d^{-1}$	sludge (-)	O ₂ (headspace or sludge rec.)	1.8-19 L_{biogas} m ⁻³	0.33-0.5	1	8	3,300-5,000	99	n.a.	<0.1	Ramos and Fdz- Polanco (2014a)
Fully-mixed digester (250)	1.4-2.9 $g_{vs}L^{-1}d^{-1}$	sludge (-)	O ₂ (sludge rec.)	4.4-6.2 Lm ⁻³ d ⁻¹	0.44-0.62	1.9-2.8	6	3,400	90	≈0	<0.03	Ramos and Fdz- Polanco (2013)
Fully-mixed digester (338,000)	$40-66 g_{manure} L^{-1} d^{-1}$	cow manure (-)	Air (headspace)	1% of biogas rate	~1	1.8-4.4	1.4	2,000-4,000	68	n.a.	n.a.	Kobayashi et al. (2012)
Fully-mixed digester (265)	n.a.	sludge (-)	O ₂ (liquid)	$0.16-0.46 \ LL_{feed}^{-1}$	0.9-2.5	2,5-7	7.6-0.2	3,500	0-99	n.a.	1-2	Ramos et al. (2012)
EGSB (4)	0.5-3.1	synthetic vinasse (12)	O ₂ (liquid)	0.37 Ld ⁻¹	4.7	1.7	2.4	25,000	72	40	4.1	Rodriguez et al. (2012), Lopes (2010)
Fully-mixed digester (250)	1.8-3.4	sludge (48-93)	O ₂ (headspace)	0.97 Ld ⁻¹	0.6-12	2-3.4	7.1-8.6	3,300-34,000	≥97	67-96	0.2-1	Díaz et al. (2011a)

Table 2.3: The overview of anaerobic reactors where the use of microaeration has been reported

Fully-mixed digester (250)	2.4-4.7	sludge (96-188)	O ₂ (headspace or sludge rec.)	0.25 LL _{feed} ⁻¹	1.4	1	6.3	13,000	≥ 98	88 (biogas recirculation)	0.6	Díaz et al. (2011b)
Fully-mixed digester (250)	1.9-4	sludge (143-310)	O ₂ (sludge rec.)	0.25 LL feed ⁻¹	1.2-1.5	1-1.4	6.6	12,000	97.5	≈ 0	1-1.4	Díaz et al. (2010)
Fully-mixed digester (250)	1.9-4	sludge (137-296)	Air (sludge rec.)	$1.27~\text{LL}_{\text{feed}}^{-1}$	1.2-1.5	1-1.4	5.3	10,000	> 99	≈ 0	1-1.4	Díaz et al. (2010)
Fully-mixed digester (2x 1,500,000)	3.5	sludge (-)	Air (sludge rec.)	n.a.	1.1	3.7	n.a.	3,300	99	n.a.	n.a.	Jenicek et al. (2010)
Fully-mixed digester (2,100,000)	3.5	sludge (-)	Air (sludge rec.)	n.a.	2.9	5.5	n.a.	5,600	99	n.a.	n.a.	Jenicek et al. (2010)
Fully-mixed digester (250)	1.9-4.5	sludge (152-369)	O ₂ (headspace or sludge rec.)	2.6-4.8 Ld ⁻¹	1.3-2.4	0.7-1.3	5-8	9,000-10,000	> 99	≈ 0 (sludge recirculation)	0.3-4.8	Fdz-Polanco et al. (2009)
Fully-mixed digester (11)	3.5	sludge (-)	Air (sludge rec.)	1.1 Ld ⁻¹	2.1	n.a.	n.a.	34	92	n.a.	n.a.	Jenicek et al. (2008)
CSTR + SOU (92+1)	1.2	sludge (690)	O ₂ (liquid)	7.2 Ld ⁻¹	3	10-14	n.a.	1,800-2,600	> 99	94	0.4-0.7	Duangmanee et al. (2007)
UASB (11)	2.8-12	sulfite pulp mill ww. (45-60)	Air (liquid)	45-90L/d	n.a.	n.a.	n.a.	5,000-23,000	-	20-30	n.a.	Zhou et al. (2007)
FBR (1.7)	3.5	synthetic vinasse (144)	Air (liquid)	1.2-1.5 Ld ⁻¹	n.a.	440-560	n.a.	0.71 mg-S d ⁻¹	>82	>52	n.a.	van der Zee et al. (2007)
UAF + SOU (4.5+2)	0.53-2.3 g _{тос} L ⁻¹ d ⁻¹	synthetic ww. (9)	O ₂ (liquid)	ORP controlled (-275265 mV)	n.a.	n.a.	n.a.	78,000	> 99	99	n.a.	Khanal and Huang (2006)
Fully-mixed digester (5)	1-8 g _{TS} L ⁻¹ d ⁻¹	synthetic waste (69)	Air	7.5% of evolved gas	1-2.1	n.a.	n.a.	680	99	n.a.	n.a.	lkbal et al. (2003)
Fully-mixed digester	n.a.	agricultural waste (-)	Air (headspace)	n.a.	0,3-0,4	1.3-1.7	2.5	2,500	88	n.a.	n.a.	Schneider et al. (2002)

UASB (up-flow anaerobic sludge blanket), EGSB (expanded granular sludge blanket), CSTR (continuous stirred tank reactor), FBR (fluidized bed reactor), SOU (sulfide oxidizing unit), UAF (up-flow anaerobic filter)

n.a. – not available

2.5.4. Removal of gaseous and dissolved sulfide and influence of pH

At pH around 7, at which anaerobic digestion typically occurs, $HS_{(d)}^{-}$ and $H_2S_{(d)}$ are the predominant sulfide species in the liquid phase (pK_{a1}=6.9, Migdisov et al. (2002)). The concentration of $H_2S_{(d)}$ increases when pH declines. Simultaneously, H_2S distributes between gas and liquid phases (dimensionless Henry's constant H = $c_G/c_L = 0.5$). Then, the value of pH influences sulfide distribution between liquid and gas phases and it is of particular importance when only $H_2S_{(g)}$ is removed by microaeration (i.e. by aerating the headspace). Assuming a constant amount of sulfur reduced by sulfidogenesis within the bioreactor, a lower pH results in a higher proportion of $H_2S_{(d)}$, a higher amount of $H_2S_{(g)}$ in the biogas to maintain the Henry's equilibrium and, consequently, requires a larger oxygen/air rate for efficient H_2S removal.

In those processes where sulfide removal occurs in the headspace, dissolved sulfide can be removed by increasing the contact between gas and liquid phases or by decreasing pH (to promote H₂S stripping). However, the required oxygen rate to remove both gaseous and dissolved sulfide species depends on the pH and the Q_{biogas}/Q_{effluent} ratio (m³ of biogas per m³ of liquid effluent) in the bioreactor as shown in Figure 2.3. Hence, at pH 7, the rate of oxygen needed to remove both gaseous and dissolved sulfide in digestion processes is lower than 1.3 times the rate necessary to remove exclusively gaseous sulfide with Q_{biogas}/Q_{effluent} ratios larger than 15. This was confirmed by switching from sludge to biogas recirculation (Díaz et al., 2011a; Díaz et al., 2011b; Fdz-Polanco et al., 2009) at pH close to 7 and $Q_{\text{biogas}}/Q_{\text{effluent}}$ =18. By contrast, processes with $Q_{\text{biogas}}/Q_{\text{effluent}}$ ratios below 5, such as industrial wastewater treatment (Krayzelova et al., 2014a; Rodriguez et al., 2012), would require a much higher rate of oxygen to remove dissolved sulfide than it is needed for biogas desulfurization only, and this effect is larger when pH increases. Consequently, at high pH or low Q_{biogas}/Q_{effluent}, removing dissolved sulfide may affect the profitability whether by raising the costs of pure oxygen supply or by excessive biogas dilution by nitrogen if air is used. This negative effect on the costs can be partially neutralized if severe inhibition on digestion is prevented under microaerobic conditions, because a large increase in methane productivity was observed (Khanal & Huang, 2006; Zitomer & Shrout, 1998) in this case.



Figure 2.3: Theoretical oxygen rate requirements for the microaerobic removal according to Eq. 2.1 assuming sulfide distribution obeys Henry's equilibrium. Oxygen rate to remove gaseous sulfide only is 1.

2.5.5. Reactor configurations

Over the years, microaeration has been tested in several different reactor configurations (**Table 2.3**). Reported configurations can be divided within two categories; a first one where oxygen/air is directly supplied into the reactor where the whole anaerobic digestion takes place, and, secondly, those configurations which comprise a chamber or separate unit where microaeration is performed.

• Microaeration directly inside anaerobic digesters

Within the first category, microaerobic H_2S removal has been traditionally used in digesters treating agricultural wastes in Germany because of the simplicity of its application and the convenience for biogas exploitation (Schneider et al., 2002). However, the most reported and successful application, including full-scale operation, is the digestion of sludge from WWTP under microaerobic conditions. In fully-mixed sludge digesters (10L – 2,100m³), microaeration can remove H_2S from biogas (2,500-34,000 ppm_v) with efficiency higher than 97% (Díaz et al., 2010; Fdz-Polanco et al., 2009; Jenicek et al., 2014; Jenicek et al., 2008; Jenicek et al., 2010; Ramos & Fdz-Polanco, 2014a). The lower efficiency found on full-scale microaerobic CSTR treating agricultural wastes, between 68% and 88% (Kobayashi et al., 2012; Schneider et al., 2002), is probably the consequence of the low biogas residence time in the headspace in comparison to sludge digesters (see section 5.3).

Recent research has broadened the usage of direct supply of oxygen to up-flow anaerobic sludge blanket (UASB) reactors, expanded granular sludge bed (EGSB) reactors, fluidized bed reactors (FBR) for the treatment of industrial wastewaters; particularly those from the brewery, sugar and paper industries that commonly present elevated sulfur load. The unaerated treatment of the wastewater of such industries resulted in a biogas with concentrations of H₂S higher than 20,000 ppm_v and up to 67,000 ppm_v, which was removed with efficiencies between 70% and 82% under microaerobic conditions (Krayzelova et al., 2014a; Rodriguez et al., 2012; van der Zee et al., 2007; Zhou et al., 2007). Furthermore, microaeration can increase the performance of the organic matter removal as a result of the reduction of sulfide inhibition to methanogens (Rodriguez et al., 2012; Zhou et al., 2007). An innovative approach of microaeration is the application of water electrolysis within UASB reactors so that O₂ is produced directly in the reactor; H₂S can be removed and the production of H₂ and the electrical current significantly enhanced anaerobic digestion (Tartakovsky et al., 2011).

A novel, recently reported, configuration is the application of membranes as a tool to provide required microaeration for sulfur oxidation. Membranes were already conceived many years ago as a way to provide bubble-less aeration in fermentation processes (Cote et al., 1988). However, only scarce reports are available where membranes are used as a way to provide aeration with the objective of sulfide oxidation. In principle, membranes could be used to transfer oxygen to the headspace or to the liquid phase of an anaerobic reactor. This would be accomplished by providing the flow of oxygen or air on one side of the membrane, and exposing the other side to the biogas in the headspace or the liquid phase of the reactor. Alvarez (2014) studied the use of silicon tubing as a way to provide microaeration to the headspace of an anaerobic reactor. Mass transfer coefficients for the different gases involved were determined (CH₄, CO₂, H₂S, O₂, N₂). The formation of a biofilm over the membrane surface was observed on the biogas side, similar to that formed on the surfaces of the headspace of anaerobic reactors subjected to microaeration. On the other hand, Camiloti et al. (2013) and Camiloti et al. (2014) reported the application of silicone tubes for the microaeration of the liquid phase of anaerobic reactors for wastewater treatment. In this case, a biofilm containing SOB was also formed, which was identified as responsible for a large part of the sulfur oxidation. The

application of membranes with selective permeability for oxygen represents a great opportunity, since they may partially reduce the dilution of the biogas with nitrogen, when air is used as oxygen source. Moreover, membranes preventing methane permeation would be required to avoid emissions of this gas to the atmosphere.

<u>Microaeration in separate compartments</u>

In the second category, a microaerobic unit (or compartment) is added to the process, thus maintaining the core anaerobic digestion unaerated. This allows the utilization of higher O_2 rates and avoids the accumulation of elemental sulfur in the headspace of the anaerobic digester. Hence, anaerobic baffled reactors (ABR) can be designed with a final compartment where microaeration is performed to remove the H₂S produced in the initial chambers under anaerobic conditions (Bekmezci et al., 2011; Fox & Venkatasubbiah, 1996). In a similar way, the sulfide-rich liquor and biogas, or the biogas alone, produced during anaerobic digestion can be treated in a sulfide oxidation unit (SOU) where microaeration is performed. When liquid and biogas were introduced into the SOU, increasing the ORP to around -265 from the natural anaerobic level of -290, H₂S was removed with efficiency higher than 99% (Khanal & Huang, 2006). Alternatively, the raw biogas produced in the digester can be treated in a SOU, inoculated with anaerobic sludge, which simulates the microaerobic conditions within the headspace of digesters. In this way S⁰ can be easily removed without affecting the digester (Ramos et al., 2013).

2.5.6. Microaeration process control

A variable oxygen rate is necessary in most reactors, as the consequence of feed composition/rate variations resulting in the varying production of sulfide. Besides, residual oxygen in the biogas must meet the requirements of the biogas utilization technology that will be employed afterwards. Oxygen content below 1% is required for fuel cells and below 3-0.5% (after carbon dioxide removal) for vehicle fuels or injection of upgraded biogas into the natural gas grid (Petersson & Wellinger, 2009). Optimal process control is the key to the successful microaeration in such cases. Oxygen supply can be controlled to cope with the changes of H₂S concentration and biogas flow (Ramos & Fdz-Polanco, 2014a). Proportional-integral-derivative (PID) controller was used to control the oxygen flow rate according to the H₂S concentration in biogas (Ramos & Fdz-Polanco, 2014a). Oxygen flow rate was set according to the difference (e) between the measurement and target H_2S concentration. H_2S concentration in biogas dropped below the set-point (0.01%) in a time range from 4.0-5.5 h, subsequently stabilizing at zero, while oxygen content remained around 0.05%. The microoxygenation level was optimal since it kept the removal efficiency above 99% with a minimum oxygen concentration in biogas. The flow of biogas was another parameter used for the control of H₂S concentration in biogas and for the control of oxygen supply in this paper. Approximately 3.5 and 5.0 L of O₂ per 1 m³ of biogas was needed to successfully remove 0.33% and 0.5% of H₂S from biogas, respectively. The average H₂S removal efficiency was 99% with 0.08% of oxygen in biogas. Ramos and Fdz-Polanco (2014a) suggested that biogas production could be an efficient regulating parameter under variable organic loading rate and steady sulfur load, while under non-steady sulfur load, H₂S concentration should be used as a regulating parameter instead.

When using biogas production as a control parameter, there is a danger that overdosing by air would increase apparent biogas production which would induce the increase of air dosage. Therefore this

strategy would only work in the case when the changes in biogas flow are considerably greater than the potential overdose by air. This was the case of the study by Ramos and Fdz-Polanco (2014a).

ORP has also been used for the control of oxygen dosing, in a chemostat (Khanal & Huang, 2003a) and a UAF system (Khanal & Huang, 2003b; Khanal & Huang, 2006; Khanal et al., 2003). In general, oxygen injection was automatically turned on whenever the reactor ORP was 10 mV below the target value. Pure oxygen was injected to the reactor until ORP was raised to 10 mV above the target level. During the operation of the chemostat, a target ORP value of -230 mV (50 mV above the anaerobic ORP level of -280 mV) almost completely removed the dissolved and gaseous sulfide (Khanal & Huang, 2003a). In the UAF, the target ORP value of -265 mV (25 mV above the ORP level of -290 mV) was set, which provided a dissolved sulfide removal over 98.5%,by converting it mainly to elemental sulfur with a production of small amount of thiosulfate (Khanal & Huang, 2003b; Khanal & Huang, 2006; Khanal et al., 2003). ORP as a tool for controlling microoxygenation was also used by Nghiem et al. (2014). In their case, an ORP probe was connected to a supervisory control and data acquisition (SCADA) system to control the digester. SCADA system was set to control valve dosing oxygen to maintain ORP level between -310 and -290 mV (the natural ORP level was -485 mV). Under such conditions, H_2S concentration decreased from over 6,000 mg L⁻¹ to just 30 mg L⁻¹.

No study was published that would use sulfide concentration in the liquid phase as the control parameter for the dose of air into the microaerobic reactor. This is most probably because the relation between H₂S concentration in biogas and in the liquid phase is not straightforward and large variations in H₂S concentrations in biogas often correspond to small or negligible variations in the liquid phase. This would largely depend on the oxygen dosing point (see section 2.5.1). However, even if air is dosed directly into the liquid phase, the changes in H₂S concentrations are relatively small compare to the changes in H₂S concentrations in biogas.

2.6. Mathematical modelling of sulfide oxidation

Mathematical modelling is an important tool which can provide valuable information that can help to understand the behavior of complex systems. There are many papers describing the kinetics of chemical oxidation of sulfide. The basic relation for the kinetic model can be expressed as follows (O'Brien & Birkner, 1977):

$$R_{chem.ox.} = k_m \cdot \left(S_{H_2S}\right)^{\alpha} \cdot \left(S_{O_2}\right)^{\beta}$$
(Eq. 2.6)

where $R_{chem.ox.}$ is the sulfide oxidation rate [mmoL L⁻¹min⁻¹], k_m is the rate constant [min⁻¹], S_{H_2S} is the H₂S concentration [mmoL L⁻¹], S_{O_2} is the O₂ concentration [mmoL L⁻¹], α is the reaction order with respect to the sulfide concentration [-], and β is the reaction order with respect to the oxygen concentration [-].

The summary of available kinetic parameters and the tested range of sulfide and oxygen concentrations are shown in **Table 2.4**. The parameters vary significantly across the literature. Different researchers used different analytical methods to determine sulfide and sulfide oxidation rate, and used different buffer solutions. Reported experiments were also conducted at different sulfide and oxygen concentrations ranging from 0 to 9.38 mmoL L⁻¹ and 0 to 1.10 mmoL L⁻¹, respectively. The reaction order of oxygen very likely depends on sulfide concentration (Buisman et

al., 1990a). Due to the uniqueness of each system, it is very hard to summarize the results and to make a unified conclusion.

k [min ⁻¹]	α	β	c(S ²⁻) [mmoL L ⁻¹]	c(O ₂) [mmoL L ⁻¹]	Reference
17.46	1.02	0.80	0-5.00	0.15	Klok et al. (2013)*
0.1165	1.00	1.00	0.04-0.10	saturated (25°C)	Luther et al. (2011)
0.57	0.41	0.39	0.16-9.38	0.003-0.266	Buisman et al. (1990a)
0.055	0.38	0.21	0.09-0.30	0.16-0.62	Wilmot et al. (1988)
67.6	1.15	0.69	0.05-0.20	0.60	Jolley and Forster (1985)
1.44	1.02	0.80	0.02-1.21	0.21-1.10	O'Brien and Birkner (1977)

Table 2.4: The kinetic parameters of chemical oxidation of sulfide described by the equation 6

* measured in the gas phase

Sharma et al. (2014) proposed the following kinetic expression for chemical oxidation of sulfide:

$$R_{chem.ox.} = k_m \cdot (S_{H_2S})^{\alpha} \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}}$$
(Eq. 2.7)

with k_m being 4.46 h⁻¹, α 0.56, and K_{O_2} 1.30 mg L⁻¹. H₂S oxidation rate was independent of the O₂ concentration above 5 mg L⁻¹, which they explained by Monod type equation.

Nielsen et al. (2004) included the effect of pH and temperature in their model of chemical oxidation of sulfide:

$$R_{chem.ox.} = \frac{k_0 + k_1 \cdot K_1 / S_{H^+}}{1 + K_1 / S_{H^+}} \cdot (S_{S^{2-}})^{\alpha} \cdot (S_{O_2})^{\beta} \cdot \theta^{T-20}$$
(Eq. 2.8)

where $S_{S^{2-}}$ is the concentration of total sulfide $[g m^{-3}]$, k_0 and k_1 are the rate constants for the oxidation of H₂S and HS⁻, respectively $[(g S m^{-3})^{1-\alpha} (g O_2 m^{-3})^{-\beta} h^{-1}]$, θ is the Arrhenius constant, T is the temperature [°C], and K_1 is the first dissociation constant for H₂S ($\approx 1.0 \cdot 10^{-7}$). The reaction order α and β were 0.9 and 0.2 respectively, θ was 1.06, and k_0 and k_1 fluctuated from 0.02 to 0.08 and from 0.25 to 1.00, respectively. The rate constants varied significantly and should be employed with caution. Moreover, the rate equation is valid within the pH and temperature intervals of 6-9 and 5-25°C, respectively (Nielsen et al., 2004).

For biochemical oxidation of sulfide, Monod-type equation for substrate utilization should be used as follows (Xu et al., 2013b):

$$\frac{dS_{S^{2-}}}{dt} = -\frac{\mu_{SOB}}{Y_{SOB}} \cdot \frac{S_{S^{2-}}}{K_{S,S^{2-}} + S_{S^{2-}}} \cdot \frac{S_{O_2}}{K_{S,O_2} + S_{O_2}} \cdot X_{SOB}$$
(Eq. 2.9)

where μ_{SOB} is the maximum specific growth rate [h⁻¹], Y_{SOB} is the yield coefficient for SOB [g VSS g⁻¹ S²⁻], $K_{s,S^{2-}}$ and K_{s,O_2} are sulfide and oxygen affinity constants [kg m⁻³], $S_{S^{2-}}$ and S_{O_2} are sulfide and oxygen concentrations [kg m⁻³], and X_{SOB} is the concentration of SOB [kg m⁻³].

Xu et al. (2013) presented an integrated model describing sulfur cycle processes of sulfate reduction, sulfide oxidation and sulfur bioreduction. They found out that the ratio of oxygen to sulfide is a key factor for controlling elemental sulfur formation.

Kinetic data for biological oxidation of sulfide found in the literature are summarized in **Table 2.5**. However, these kinetic studies were made in aerobic environments. It has been reported that the maximum specific activity for sulfide oxidation by SOB is different under aerobic and anaerobic conditions (McComas et al., 2001), i.e. 23.7 mg HS⁻g_{protein}⁻¹ min⁻¹ and 8.6 mg HS⁻g_{protein}⁻¹ min⁻¹, respectively. Yu et al. (2014) studied the microbial community structures in a biological desulfurization reactor under microaerobic conditions (0.02-0.33 mg L⁻¹). The results indicated that the microbial community functional compositions and structures were dramatically altered with elevated dissolved oxygen levels. Genes involved in sulfate reduction processes significantly decreased at relatively high dissolved oxygen concentration (0.33 mg L⁻¹), while genes involved in sulfur/sulfide oxidation processes significantly increased in low dissolved oxygen concentration conditions (0.09 mg L⁻¹) and then gradually decreased with continuously elevated DO levels. Therefore, the oxidation of sulfide under microaerobic (oxygen limited) conditions must be further studied.

b _{sob} [d⁻¹]	µ _{soв} [d⁻¹]	$K_{S,S^{2-}}$ [mg S ²⁻ L ⁻¹]	K_{S,O_2} [mg O ₂ L ⁻¹]	Υ _{SOB} [mg x mg ⁻¹ S ²⁻]	Dominant microorganisms	Reference
n.a.	0.67	11.00	0.0002	0.0900 (x = VSS)	SOB from activated sludge	Xu et al. (2013b)
0.130	n.a.	n.a.	n.a.	0.0380 (x = COD)	SOB of γ- Proteobacteria and Halothiobacillaceae class	Munz et al. (2009)
0.034	8.64	63.68	n.a.	0.0006 (x = ATP)	Thiomicrospira sp.	Gadekar et al. (2006)
n.a.	n.a.	8.96	n.a.	0.0891 (x = protein)	Thiobacilli sp.	Alcántara et al. (2004)
n.a.	7.20	0.32	n.a.	0.0969 (x = protein)	Pure culture of Thiobacillus thioparus	De Zwart et al. (1997)

Table 2.5: The kinetic parameters of biological oxidation of sulfide to elemental sulfu	ır
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n.a. – not available

Botheju et al. (2009) developed a model of oxygen effect in anaerobic digestion, however, the model focused on aerobic oxidation of soluble carbon and inhibition of strict anaerobic organisms, not on sulfide oxidation. Biomass dependent first order hydrolysis kinetics was used to relate increased hydrolysis rate to oxygen induced increase in biomass growth rate (Botheju et al., 2009; Botheju et al., 2010). An integrated model describing the effects of microaeration on biological and chemical oxidation of sulfide in anaerobic digestion has not been addressed yet. Therefore, mathematical modelling remains a research gap in microaeration.

2.7. Adverse effects of oxygen in anaerobic treatment

2.7.1. Oxygen toxicity to methanogens

Strict absence of oxygen has previously been considered as vital for anaerobic digestion, because of the toxicity of oxygen to methanogens (Zehnder, 1988). Later, methanogens were shown to be tolerant to certain oxygen concentrations or protected by facultative anaerobic bacteria in both granular (Guiot et al., 1992; Kato et al., 1993a; Kato et al., 1993b; Shen & Guiot, 1996) and suspended sludge (Estrada-Vazquez et al., 2003). Methanogens in granular sludge appear to be more tolerant to the presence of oxygen than methanogens in flocculent sludge. Based on the multilayer structure of anaerobic granular sludge, facultative anaerobes are predominant in the periphery of the granules, while oxygen-sensitive methanogens are located in the deeper layers, protected from the exposure to air (Guiot et al., 2011; Shen & Guiot, 1996). In most studies, no significant oxygen inhibition (Díaz et al., 2010; Díaz et al., 2011b; Fdz-Polanco et al., 2009; Jenicek et al., 2011a; Jenicek et al., 2014; Krayzelova et al., 2014a; Nghiem et al., 2014; Ramos & Fdz-Polanco, 2014a; Tang et al., 2004; Zhou et al., 2007) of methanogens was observed during microaeration. Only two studies (Jenicek et al., 2010; Zitomer & Shrout, 2000) reported slightly lower specific methanogenic activity in microaerobic reactor compared to anaerobic reactor.

2.7.2. Explosion risks of methane/oxygen mixtures

In general, mixing oxygen or air with biogas is undesirable because of the increased explosion risks of methane/oxygen mixture. However, the amount of oxygen dosed in microaerobic digestion is very small and it is quickly consumed. Therefore, it is far from the flammable range, which is typically 85-95% of air and 5-15% of methane by volume (Appels et al., 2008; Wase & Forster, 1984). The leakage of biogas in air should be considered as the higher threat compare to the mixing of a small amount of air/oxygen with biogas. During microaeration, the amount of oxygen or air in biogas should never reach these values. Most authors mentioned almost no or very limited amount of oxygen detected in biogas during microaeration (Krayzelova et al., 2014a; Ramos & Fdz-Polanco, 2014a; Ramos & Fdz-Polanco, 2013). Nonetheless, the explosion risk is always present when working with biogas and should not be underestimated.

2.7.3. Partial oxidation of organic substrate

When oxygen is present in anaerobic treatment methanogenic substrates or methane can be partially oxidized. However, the oxygen dosing rate typically applied during microaerobic removal of sulfide (0.001-0.01 kg m⁻³ d⁻¹) and organic loading rate (ORL) of digesters expressed in COD in the same oxygen units (1-10 kg m⁻³ d⁻¹) are three orders of magnitude different. Therefore, the amount of oxidized substrate cannot be significant. Some authors observed lower methane production in microaerobic reactors compare to anaerobic reactors caused probably by an aerobic degradation of organic matter (Khanal & Huang, 2003a; Kobayashi et al., 2012; Ramos & Fdz-Polanco, 2013; Rodriguez et al., 2012). However, most authors report no or negligible decrease of methane production due to microaeration (Díaz et al., 2011a; Díaz et al., 2010; Díaz et al., 2011b; Fdz-Polanco et al., 2009; Jenicek et al., 2010; Krayzelova et al., 2014a; Nghiem et al., 2014). In these cases the dose of oxygen was not controlled according to the sulfide content (or it was controlled very roughly by ORP). Therefore, oxygen was apparently overdosed or digesters were in unbalanced conditions which contributed to the decrease of methane production.

The partial oxidation of organic compounds in anaerobic digester can improve the efficiency of volatile suspended solids removal (VSS). The evaluation of side-effects of microaerobic sulfide removal during anaerobic digestion showed the decrease in VSS/TSS ratio of the digested sludge in all experiments with microaerobic conditions, due to its better VSS degradation (Jenicek et al., 2008).

2.7.4. Clogging the walls and pipes of microaerobic reactor with elemental sulfur

According to some authors, microaeration takes place solely or almost solely in reactor headspace (Díaz et al., 2011b; Kobayashi et al., 2012; Ramos et al., 2014c; Rodriguez et al., 2012). The whitish deposition of elemental sulfur on the walls and pipes can clog the system resulting in headspace overpressure and biogas leakage. de Arespacochaga et al. (2014) operated a biotrickling filter with a solid oxide fuel cell for on-site electricity and thermal energy production. Around 70% of H₂S removal was done by partial oxidation to elemental sulfur which increased the pressure drop over the column, reduced the availability of the treatment line, and eventually led to a fuel cell shutdown. A cleaning interval of less than 14 months is necessary to minimize microaeration costs (Ramos et al., 2014c). Ramos et al. (2014b) opened their microaerobic reactors, cleaned the surface of its headspace, removed the liquid interface, and restarted microaeration. Hydrogen sulfide removal was not affected, however, it was not clear which mechanism (biological or chemical oxidation) played the main role in this set-up. The collection of elemental sulfur is a remaining challenge in microaeration technology and requires further research, especially in full-scale applications.

2.7.5. Dilution of biogas by nitrogen from air

By using air for microaeration, nitrogen will remain and dilute biogas. This is especially challenging when biogas with low amount of methane (around 50%) is produced, e.g. from lignocellulose (Chandraa et al., 2012), because then, even small dilution of biogas may complicate its further use in cogeneration unit. Celis (2012) reported that when extremely high H_2S concentrations (around 12,000 ppm) must be removed, the concentration of N_2 to increased up to 20% in biogas. It caused a decrease of methane concentration below 50% and such concentration is too low for most cogeneration units. However, the replacement of air by oxygen solved the nitrogen dilution of biogas without affecting digestion and desulfurization efficiency.

2.8. Additional advantages of microaeration

2.8.1. Enhancement of hydrolysis

Since hydrolysis is often considered as the bottleneck of the anaerobic digestion of solid materials (Myint et al., 2007), improving this limiting step can improve the whole process (Botheju & Bakke, 2011). An adequate microaeration intensity can significantly enhance the hydrolysis of carbohydrate and protein in food waste by 21-27% and 38-64%, respectively (Xu et al., 2014). A sufficient microaeration strategy should be employed during the early period of digestion to enhance the hydrolysis of easily biodegradable organics, promote acidogenesis, and avoid the accumulation of lactic acid (Zhu et al., 2009). Johansen and Bakke (2006) studied the effects of microaeration on hydrolysis of primary sludge and observed 50-60% increase in the rate of the hydrolysis of carbohydrates and proteins. The extra hydrolyzed products were oxidized to carbon dioxide or incorporated into new biomass. The increase of soluble proteins due to microaeration was also observed by Diak et al. (2013) together with the increase of ammonia. Microaeration effectively

solubilized COD, and improved the subsequent degradation of COD. However, the increase of carbohydrates was not observed. On the other hand, Nguyen et al. (2007) reported no enhancement of hydrolysis by microaeration, but the applied amount of air per kilogram of total solids per day was 10x lower than in the study of Johansen and Bakke (2006).

Moreover, microaerobic assays presented shorter lag-phase than the anaerobic assays in the study conducted by Díaz et al. (2011c). This resulted in faster production of methane during the first steps of the cellulose degradation. The maximum methane production in the anaerobic assay was observed on day 19 while in the microaerobic assay it was observed before day 15.

2.8.2. Better recovery from shock loading or serious decrease of pH

Wang et al. (2014) described that microaeration was a promising strategy to handle shock loading in anaerobic treatment of coal gasification wastewater. The recovery time was shortened from 23 to 11 days under natural condition. Ramos and Fdz-Polanco (2013) subjected microaerobic digester to a hydraulic overload. Microaeration improved the biogas quality and oxygen seemed to contribute to a stable digestion system, which increased the ability to deal with overloads. Also Jenicek et al. (2010) observed faster methanogenic bacteria recovery after the inhibition caused by overloading. Aero-tolerant methanogenic culture was added to anaerobic digester to improve the recovery time after organic overload or toxicity upset (Tale et al., 2015). In contrast to the anaerobic enrichment, the aerated enrichments were more effective, resulting in faster recovery of methane and COD removal rates.

After a shock-load of sucrose, the pH in the complete-mix methanogenic reactors recovered more quickly under microaeration conditions (Zitomer & Shrout, 1998). Aeration may prevent pH decreases in other highly loaded systems since volatile acids were potentially oxidized and carbon dioxide and hydrogen were stripped out. O'Keefe et al. (2000) observed no adverse effect of aeration on the microbial activities in anaerobic digester.

2.8.3. Better sludge quality

Microaeration also appeared to improve the quality of the digested sludge in the way of lower foaming potential and better dewaterability (Jenicek et al., 2011a; Jenicek et al., 2011b; Jenicek et al., 2014). The extent of foaming problems was lower in microaerobic digester compare to anaerobic digester.

2.8.4. Production of elemental sulfur

As mentioned previously, there is a lack of technology available to recover elemental sulfur from bioreactors where microaeration is applied. However, if this technology were to be developed, the elemental sulfur could be used in bioleaching processes (Tichý et al., 1994) or for the autotrophic sulfur-oxidizing denitrification (Krayzelova et al., 2014b; Zhou et al., 2011). The biologically produced elemental sulfur has some distinctly different properties as compared to "normal" inorganic (orthorhombic) sulfur (Kleinjan et al., 2003). The density of biologically produced sulfur is lower and the particles have hydrophilic properties whereas orthorhombic sulfur is known to be hydrophobic with higher density. Due to this, the biologically produced sulfur could be more available and suitable for microorganisms compared to the chemically produced one. More information about biologically

produced elemental sulfur can be found in the papers by Janssen et al. (2009) and Kleinjan et al. (2003).

2.9. Economic considerations

When considering microaeration to remove sulfide, air is, at least initially, the most economical alternative; however, biogas dilution with nitrogen (1-8%) when air is employed may result in a lower performance of biogas combustion or higher costs during biogas upgrading to remove nitrogen. In fact, a recent economic evaluation revealed that the utilization of concentrated oxygen (92-98%) presented higher net present value (NPV5 and NPV20) than the utilization of pure oxygen or air to substitute the current addition of FeCl₃ to the anaerobic digesters of a full-scale WWTP producing 550 m³ h⁻¹ of biogas. This alternative presented the lowest operational costs per cubic meter of biogas treated (0.0019 EUR) compared to air, pure oxygen supply and the addition of FeCl₃ (0.0027 EUR, 0.0039 EUR and 0.0100 EUR, respectively) (Díaz et al., 2015).

2.10. Needs for further research

Microaeration as a method for biogas desulfurization has been gaining attention over the past years and it has been often used in full-scale digesters in agricultural applications (personal communications with plant operators and Schneider et al. (2002)). However, some theoretical and practical aspects of microaeration still remain unclear and need further research. This is important both for introduction of microaeration into new fields (high rate digesters for wastewater treatment) and for optimization of microaeration in current application (agricultural digesters).

2.10.1. Mechanism of sulfide oxidation

There is still discussion to what extend bacteria are responsible for the oxidation of sulfide under microaerobic condition. It is clear that both biotic and abiotic processes run in parallel (Buisman et al., 1990a), but the rates of these processes in microaerobic digesters are not well quantified yet.

Moreover, the exact metabolic pathway of sulfide oxidation under microaerobic condition is not well defined. It is not clear yet, what is the role of intermediate sulfur species such as sulfite, thiosulfate, polysulfide, and polythionates. It is also not clear, to what extend can be elemental sulfur repeatedly reduced to sulfide and how this process contributes to the overall oxygen consumption and reduction of methane yield.

2.10.2. Control of microaeration

To maximize the efficiency of microaeration, precise control of air dosing is needed. In the current applications, microaeration often cannot cope with sudden changes of sulfide concentration in biogas induced e.g. by the start of intermittent mixing (personal communication with plant operators). It can be expected that similar problems will take place in high-rate digesters should microaeration be introduced for them too.

The spatial control of microaeration, i.e. the spatial distribution of the formation of elemental sulfur is even more pressing problem. In current applications, most of sulfur forms on the walls of reactor's headspace (Kobayashi et al., 2012; Ramos et al., 2012; Ramos et al., 2014c; Rodriguez et al., 2012) and is expected to continually fall of into the liquid effluent (Ramos et al., 2014d). However, partial or

complete clogging of biogas piping has also been reported (de Arespacochaga et al., 2014). When introduced into high-rate digesters such as UASB, IC or EGSB, formation of sulfur will partially take place in the three-phase separators of these reactors (Krayzelova et al., 2014a) which may seriously impair the function of the digester. Therefore, new methods for controlled safe sulfur formation in dedicated compartments of the digesters should be developed. The application of biomembranes (biofilm grown on the surface of membrane modules) for air delivery is one of the promising options (Alvarez, 2014). This technique would facilitate sulfur formation directly on the surface of these membranes and thus preventing the clogging of three-phase separators.

2.10.3. Microbiology

There are several reports describing the microbiological composition of microaerobic biofilms, but there has been very little systematic work on this topic. Most of the knowledge on SOB microbiology is derived from studies with pure SOB cultures (De Zwart et al., 1997) or environments different from microaerobic digesters such as activated sludge biotrickling filters etc. (Alcántara et al., 2004; Munz et al., 2009; Xu et al., 2013b).

2.10.4. Mathematical modelling

Microaeration as a method for biogas desulfurization in anaerobic digestion has not been modelled yet and remains an important research gap. Although, there are a few papers describing sulfate reduction and sulfide oxidation (Xu et al., 2013b), the conditions of limited amount of oxygen are specific and require its own modelling approach.

2.11. Conclusions

Although the interest in microaeration for hydrogen sulfide removal from biogas in full-scale has been steadily growing, only over 40 papers on this topic have been published during the last decade. Interestingly, while microaeration has been widely applied in full-scale anaerobic digesters for solid substrates (biogas plants), microaeration in anaerobic reactors for wastewater treatment such as UASB reactor has been rarely studied or applied.

The following highlights were extracted from recent literature:

- The accumulation of elemental sulfur and the growth of SOB biofilm have been most often observed in the headspace (or on the gas-liquid interphase) of anaerobic bioreactors, as the result of microaeration taking place in the gas phase. However, there are reports showing that microaeration can take place also in the liquid phase.
- The residence time of biogas in the headspace and available surface area are the key factors affecting the efficiency of hydrogen sulfide removal through sulfur oxidation in the headspace.
- Intensified contact between oxygen and anaerobic biomass may improve the removal of dissolved sulfide, decrease the amount of oxygen in biogas and increase the rate of hydrolysis. This effect can be facilitated when the reactor is mixed by biogas or when air/oxygen is dosed into the liquid phase.
- An integrated mathematical model describing microaeration has not been developed so far. Such model would greatly improve the understanding of the process and research on this topic is of high priority.

3. MICROAERATION DIRECTLY IN ANAEROBIC REACTOR

3.1. KINETICS OF (BIO)-CHEMICAL SULFIDE AND THIOSULFATE OXIDATION UNDER MICROAEROBIC CONDITIONS (Article 2)

Pokorna-Krayzelova, L., Selan, L., Jenicek, P., Volcke^r E.I.P., Bartacek, J. Kinetics of (bio) chemical sulfide and thiosulfate oxidation under microaerobic conditions. Prepared for submission.

Abstract

Hydrogen sulfide is a toxic and highly undesirable product of the anaerobic treatment of wastewater containing sulfate. It can be removed through microaeration, a simple and cost-effective method involving the application of oxygen limiting conditions (i.e., DO below 0.1 mg L⁻¹). However, the exact behaviour and transformations of sulfide and thiosulfate in these conditions are still not very clear. Chemical and biochemical sulfide and thiosulfate oxidation under microaerobic conditions were studied in batch reactor. The biochemical experiments were conducted using a pure culture of *Sulfuricurvum kujiense*. Under microaerobic conditions, elemental sulfur was the major end-product of both chemical and biochemical sulfide oxidation. Thiosulfate was oxidized only chemically. During chemical experiments, elemental sulfur was formed in suspension, while flakes appeared during biochemical experiments. The experimental results were used to estimate a mathematical model including chemical and biochemical oxidation of sulfide and thiosulfate.

Keywords

Batch experiments; Mathematical modelling; Microaeration; Oxygen; Sulfide oxidation; Sulfuricurvum kujiense

3.1.1. Introduction

Anaerobic treatment of wastewater with high sulfur content leads to the reduction of these compounds to liquid and gaseous hydrogen sulfide (Ramos et al., 2013). Sulfide in the dissolved form can inhibit methanogenic and acetogenic organisms and may lead to the accumulation of inert material in the sludge (e.g. metal sulfides) and to the deterioration of aerobic post treatment systems (activated sludge bulking; excessive growth of phototrophs) (Sarti & Zaiat, 2011). Gaseous sulfide is toxic and flammable and its presence in biogas results in the emission of sulfur dioxide upon combustion (Tang et al., 2009).

Biochemical desulfurization processes are considered to be attractive alternatives to the physicalchemical techniques, because of their low energy and chemical dosage requirements, easy and automated operation, the long life expectancy of system elements, the potential for elemental sulfur recovery, the absence of a solid waste stream (Díaz et al., 2011a; Ramos et al., 2014a; Ramos et al., 2013; Ramos et al., 2014d; Tang et al., 2009).

Microaeration is a biochemical desulfurization method that is based on the introduction of small amount of oxygen into an anaerobic system. This simple sulfide removal technique has already been applied at full scale (Jeníček et al., 2017). Oxygen or air can be dosed directly into the reactor to oxidized sulfide to elemental sulfur, so no additional process units are required (Krayzelova et al., 2015; Krayzelova et al., 2014a; van der Zee et al., 2007).

The oxygen availability is the main factor determining the final sulfur products (Janssen et al., 1995). Under oxygen limiting (microaerobic) conditions, at oxygen concentration below 0.1 mg L⁻¹, sulfur is expected to be the main end product of biological sulfide oxidation (Roosta et al., 2011), with a partial biological oxidation to thiosulfate (van den Ende & van Gemerden, 1993). On the other hand, sulfate is the dominant end-product under higher oxygen availability (Roosta et al., 2011). Chemical oxidation gains importance especially in the systems with higher sulfide concentration (Janssen et al., 1995). Under those conditions, biochemical activity may be limited and sulfide is oxidized chemically, mainly to thiosulfate (Janssen et al., 1995; van der Zee et al., 2007).

Mathematical models are a helpful tool for process understanding and for simulating its performance. Pokorna-Krayzelova et al. (2017b) presented a model describing a model for microaeration in UASB reactor, including several pathways for sulfate reduction to sulfate and including biochemical oxidation of sulphide to elemental sulfur. Further oxidation of elemental sulfur to sulfate was neglected and so was chemical sulfide oxidation. Roosta et al. (2011) estimated kinetics for biochemical sulfide oxidation in a fed batch reactor; at a DO concentration of 0.5-6 mg L⁻¹. Xu et al. (2013b) described the kinetic of biochemical sulfide oxidation under the DO concentration from 0.03 to 0.3 mg L⁻¹ but in their case, chemical sulfide oxidation was neglected.

This study compared chemical and biochemical oxidation of sulfide and thiosulfate under microaerobic conditions (DO below 0.1 mg L^{-1}). Biochemical sulfide oxidation experiments were conducted with the pure culture of *Sulfuricurvum kujiense* which utilizes sulfide and thiosulfate as an electron donor and oxygen under microaerobic condition as an electron acceptor (Díaz et al., 2011a; Ramos et al., 2014a). A simple mathematical model of sulfide and thiosulfate oxidation in microaerobic conditions was described based on the experimental results.

3.1.2. Materials and Methods

Experimental set-up

A batch reactor of 2 L total volume was used for the experiments of chemical and biochemical sulfide and thiosulfate oxidation under microaerobic conditions (**Figure 3.1.1**). The reactor temperature was kept at 35 °C.



Figure 3.1.1: The scheme of experimental reactor: 1 - stirring plate, 2 - reactor, 3 - magnetic stirrer, 4 - DO probe, 5 - ORP probe, 6 - nitrogen reservoir, 7 - sampling point, 8. air pump.

The experiments were conducted in the cultivation medium MBM 1020 (DSMZ, for *Sulfuricurvum kujiense*). The MBM medium consists of (per 1000 mL): 0.2 g NaNO₃, 0.2 g KH₂PO₄, 0.2 g NH₄Cl, 0.4 g MgCl₂ $^{\circ}$ 6H₂O, 0.2 g KCl, 0.1 g CaCl₂ $^{\circ}$ 2H₂O, 2.5 g Na₂S₂O₃ $^{\circ}$ 5H₂O, 0.1 mg EDTA, 0.4 mg FeSO₄ $^{\circ}$ 7H₂O, 0.02 mg ZnSO₄ $^{\circ}$ 7H₂O, 0.006 mg MnCl₂ $^{\circ}$ 4H₂O, 0.06 mg H₃BO₃, 0.04 mg CoCl₂ $^{\circ}$ 6H₂O, 0.002 mg CuCl₂ $^{\circ}$ 2H₂O, 0.006 mg Na₂MoO₄ $^{\circ}$ 2H₂O.

Prior to each experiment, the medium was stripped with nitrogen gas to decrease the oxygen concentration below 0.01 mg L⁻¹. The headspace of reactor was flushed with nitrogen gas from the nitrogen reservoir to remove the oxygen leftovers. The reactor was sealed and blank samples for sulfide, sulfate and thiosulfate were taken. The chemical and biochemical sulfide oxidation experiments were initiated by injecting 10-15 mL of sulfide and thiosulfate stock solution to obtain the initial sulfide concentration around 8 to 10 mg L⁻¹. It turned out to be nearly impossible to prepare a sulfide stock solution without thiosulfate being present. The pH was kept at 7 ± 1 by using

2 M HCl and 4 mg L^{-1} NaOH solutions. The oxygen concentration was kept below 0.1 mg L^{-1} and the experiments were stopped when the concentration of oxygen reached that value.

Dissolved oxygen (DO), oxidation reduction potential (ORP), pH, and the concentration of sulfide, sulfate and thiosulfate were hourly measured. The sulfide and thiosulfate removal rates were determined as the change of initial and final concentration over the measured period of time. Elemental sulfur formation was calculated as the difference between initial and final concentrations of sulphide, thiosulfate and sulfate (Eq. 3.1.1).

$$m_{S^0} = -\left(c_{S^{2-}}^{fin} - c_{S^{2-}}^{init}\right) - \left(c_{S_2O_3^{2-}}^{fin} - c_{S_2O_3^{2-}}^{init}\right) - \left(c_{SO_4^{2-}}^{fin} - c_{SO_4^{2-}}^{init}\right)$$
Eq. 3.1.1

Biochemical sulfide oxidation

Biochemical sulfide oxidation experiments were conducted with pure culture of *Sulfuricurvum kujiense* (DSMZ 16994). This strain was obtained from the German Collection of Microorganisms and Cell Cultures laboratory. *Sulfuricurvum kujiense* belongs to facultative anaerobic, chemolitotropic, sulfur oxidizing bacteria and utilizes sulfide and thiosulfate as an electron donor and oxygen under microaerobic condition as an electron acceptor (Kodama & Watanabe, 2004). The experiments were conducted in triplicates.

Chemical sulfide oxidation

To prevent the biological activity during chemical sulfide oxidation, MDM medium solution was autoclaved prior to use and the batch reactor was washed with ethanol and distilled water. The experiments were conducted in triplicates.

Model set-up

Based on the results from biochemical and chemical experiments, four main sulfur conversion processes were modelled: biochemical oxidation of hydrogen sulfide to elemental sulfur by sulfide oxidizing bacteria (SOB) (Eq. 3.1.2), biochemical oxidation of elemental sulfur to sulfate by SOB (Eq. 3.1.3), chemical oxidation of hydrogen sulfide to thiosulfate (Eq. 3.1.4), and chemical oxidation of thiosulfate to sulfate (Eq. 3.1.5). The decay of SOB was also incorporated in the model. The hydrogen sulfide acid-base reaction was taken up in **Table 3.1.1** for reasons of completeness, even though pH was kept constant in the model. For each process, the stoichiometric coefficients were calculated by closing the COD and sulfur balances (**Table 3.1.1**). Monod-type kinetic equations were used to describe the biochemical oxidation rates.

$$H_2S + 0.5O_2 \xrightarrow{SOB} S^0 + H_2O$$
 Eq. 3.1.2

$$S^0 + 1.5O_2 + H_2O \xrightarrow{SOB} H_2SO_4$$
 Eq. 3.1.3

$$H_2S + O_2 \rightarrow 0.5S_2O_3^{2-} + 2H^+ + 0.5H_2O$$
 Eq. 3.1.4

$$S_2 O_3^{2-} + 0.5 O_2 \rightarrow S O_4^{2-} + S^0$$
 Eq. 3.1.5

The model was implemented in Aquasim 2.0 (Reichert, 1998).

	Components i →	1	2	3	4	5	6	7	8	
A _{ij}	Processes j ↓	S ₅₀₄ ²⁻	S _{HS} -	S_{H_2S}	$S_{S_2O_3^{2-}}$	S _S	S ₀₂	X _C	X _{SOB}	Process rate (ρ _i .g COD L ⁻¹ d ⁻¹)
1	Uptake of H_2S by X_{SOB}			-1		1	$-\frac{(16-Y_{SOB})}{32}$		Y _{SOB}	$\rho_1 = k_{m.H_2S.SOB} \cdot \frac{S_{H_2S}}{K_{S.H_2S.SOB} + S_{H_2S}} \cdot X_{SOB} \cdot \frac{S_{O_2}}{K_{S.O_2.SOB} + S_{O_2}}$
2	Uptake of S ⁰ by X _{SOB}	1				-1	$-\frac{(48-Y_{SOB})}{32}$		Y _{SOB}	$\rho_{2} = k_{m.S.SOB} \cdot \frac{S_{S}}{K_{S.S.SOB} + S_{S}} \cdot X_{SOB} \cdot \frac{S_{O_{2}}}{K_{S.O_{2}.SOB} + S_{O_{2}}}$
3	Chemical H ₂ S oxidation			-1	0.5		-1			$\rho_4 = k_{H_2S \ chem.ox} \cdot \left(S_{H_2S}\right)^{\alpha} \cdot \left(S_{O_2}\right)^{\beta}$
4	Chemical S ₂ O ₃ ²⁻ oxidation	1			-1	1	-0.5			$\rho_3 = k_{S_2 O_3^{2-} chem.ox} \cdot (S_{S_2 O_3^{2-}})^{\gamma} \cdot (S_{O_2})^{\delta}$
5	Decay of X _{SOB}							1	-1	$\rho_5 = k_{dec, X_{SOB}} \cdot X_{SOB}$
A1	H ₂ S acid-base reaction		-1	1						$\rho_{A1} = K_{AB,H_2S} \cdot \left(S_{HS^-} \cdot S_{H^+} - \frac{K_{a,H_2S}}{S_{H_2S}} \right)$
Com	position matrix				•					
g CC	D per unit	0	64	64	64	48	-32	1	1	
mole	e S per unit	1	1	1	2	1	0	0	0	
		sulfate (mole S L ⁻¹)	Aydrogen sulfide ion (mole S L^{1})	Hydrogen sulfide (mole S L ⁻¹)	rhiosulfate (mole S L ⁻¹)	Elemental sulfur (mole S L ⁻¹)	Dxygen (mole O ₂ L ⁻¹)	Composites (g COD L ⁻¹)	SOB degraders (g COD L ⁻¹)	

Table 3.1.1: Stoichiometric matrix A_{ij} and composition matrix for chemical and biochemical sulfide oxidation

Parameter estimation

Maximum H_2S uptake rate ($k_{m,H2S,SOB}$), Yield (Y_{SOB}) and decay rate (k_{dec}) were determined separately by experiments with the pure culture of SOB *Sulfuricurvum kujiense*. Maximum uptake rate was determined based on the maximum uptake of sulfide by SOB over period of time. Decay rate constant was calculated based on SOB concentration decrease over time. Yield was determined by the growth of SOB over the decrease of sulfide concentration during time.

All other kinetic parameters were estimated by model calibration by the least squares method, minimizing the sum of squared errors for all compounds simultaneously.

Analytical methods

The dissolved oxygen concentration (DO) and the oxidation reduction potential (ORP) were measured by LD0101 probe (Hach Lange Company, Germany); pH was measured with a SensoLyt probe (WTW s.r.o., Czech Republic). The concentration of sulfide, sulfate and thiosulfate were measured with spectrophotometer DR 3900 (Hach Lange Company, Germany) applying the following protocols: APHA (2012) for sulfide, sulfate according to Horáková (2007) and thiosulfate concentration as in Nor and Tabatabai (1975).

3.1.3. Results

Chemical sulfide oxidation

The average results of the chemical sulfide oxidation experiments are shown in **Figure 3.1.2**. The concentration of oxygen was below 0.1 mg L⁻¹ (with average ORP -227 mV). Sulfide and thiosulfate were oxidized (removed) at an average rate of 12.06 mg S L⁻¹ d⁻¹ and 6.83 mg S L⁻¹ d⁻¹ over the time period of 7 hours respectively. The concentration of sulfate was stable during the experiments. The rate of sulfate oxidation was only 0.03 mg S L⁻¹ d⁻¹. During the experiments, the colour of the medium changed from colourless to slightly yellowish.





Biochemical sulfide oxidation

The average results of biochemical sulfide oxidation experiments are shown in **Figure 3.1.3**. The concentration of oxygen was below 0.1 mg L⁻¹ (with average ORP -240 mV). Sulfide and thiosulfate were oxidized (removed) at a rate of 29.86 mg S L⁻¹ d⁻¹ and 5.25 mg S L⁻¹ d⁻¹, resp. The concentration of sulfate was stable during the experiments. The rate of sulfate oxidation was 0.56 mg S L⁻¹ d⁻¹. During the experiments, slightly yellowish flakes appeared in the medium.



Figure 3.1.3: Evolution of the sulfur species concentrations during biochemical oxidation experiments

Model calibration

The maximum H₂S uptake rate, decay rate, and the yield coefficient were determined by the experiments with pure culture of *Sulfuricurvum kujiense* (data in Supplementary material, Section 8.1). The maximum H₂S uptake rate, $k_{m,H2S,SOB}$, was 482 mmol S mg⁻¹ COD h⁻¹, the decay rate, k_{dec} , was 0.24 h⁻¹ and the yield coefficient, Y_{SOB}, was 10.37 mg COD mmol⁻¹ S.

The remaining parameters were estimated by fitting simulated data to the experimental results for biochemical oxidation (**Figure 3.1.3**). **Table 3.1.2** summarizes the obtained kinetic parameter values.

Parameter	Description	Unit	Value
α	Reaction order with respect to H ₂ S	-	1.1 ⁽²⁾
β	Reaction order with respect to O ₂	-	0.9 ⁽²⁾
γ	Reaction order with respect to $S_2O_3^{2-}$	-	0.5 ⁽²⁾
δ	Reaction order with respect to O ₂	-	0.5 ⁽²⁾
k _{dec.xSOB}	Decay rate	h ⁻¹	0.24 (1)
k _{m.H2S.SOB}	Maximum H ₂ S uptake rate	mmol S mg COD ⁻¹ h ⁻¹	482 ⁽¹⁾
k _{m.S.SOB}	Maximum S ⁰ uptake rate	mmol S mg COD ⁻¹ h ⁻¹	0.001 ⁽²⁾
K _{s.H2S}	Half saturation constant for H ₂ S	mmol S L ⁻¹	0.001 ⁽²⁾
K _{s.O2}	Half saturation constant for O ₂	mmol $O_2 L^{-1}$	0.1 (2)
K _{s.S}	Half saturation constant for S ⁰	mmol S L ⁻¹	0.1 (2)
$k_{H2S.chemox}$	Chemical H ₂ S oxidation rate	h ⁻¹	0.001 ⁽²⁾
k _{s2O3.chemox}	Chemical $S_2O_3^{2-}$ oxidation rate	h ⁻¹	10 ⁽²⁾
Y _{SOB}	Biomass yield	mg COD mmol S ⁻¹	10.37 ⁽¹⁾

Table 3.1.2: Model parameters and their values as calculated ⁽¹⁾ (Supplementary materials, Section 8.1) or obtained through model calibration ⁽²⁾ (**Figure 3.1.4**)

The simulated concentration of sulfide, thiosulfate and DO showed a good fit with the experimentally measured one (**Figure 3.1.4**), corresponding with a Root-Mean-Square Error of 0.065 for DO concentration, 0.192 for thiosulfate concentration, and 0.738 for sulfide concentration. The sulfate concentration was overestimated with the model, corresponding with a Root-Mean-Square Error of 2.481. The total sulfur concentration in model compare to experiment is slightly higher (21.33 mg S L⁻¹ for experiments compare to 22.83 mg S L⁻¹ for model at the end).



Figure 3.1.4: Model fit (simulation results) to the experimental results for biochemical oxidation

3.1.4. Discussion

Chemical versus biochemical sulfide oxidation

Under oxygen limiting (microaerobic) conditions, at oxygen concentrations below 0.1 mg L^{-1} , elemental sulfur is the major end product of the chemical and biochemical sulfide oxidation. The chemical and biochemical sulfide oxidation rates were determined as 12.06 and 29.86 mg S L⁻¹ d⁻¹), so biochemical sulfide oxidation is about two and a half times faster. Assuming that chemical sulfide oxidation approximately 60% is created by bacteria and 40% due to chemical oxidation of sulfide. According to Alcántara et al. (2004), the activity of SOB severely decreased at oxygen to sulfide ratios of 0.15 mmoL O₂ mmoL S²⁻ or less. In this study the SOB were active even at the O₂/S²⁻ ratio of 0.011 mmoL O₂ mmoL S²⁻.

During chemical sulfide oxidation 99.8% of elemental sulfur and 0.2% of sulfate was formed, while during biochemical sulfide oxidation 98.4% of elemental sulfur and 1.6% of sulfate was observed. Munz et al. (2009) observed 91% of elemental sulfur oxidation at similar molar ratio 0.015 mmoL O_2 mmoL S^{2^2} . Apparently, even the small difference of 0.004 mmoL O_2 mmoL S^{2^2} (0.011 in the present paper compared to 0.015 of Munz et al. (2009) paper) can make the difference of 7.8% of elemental sulfur (98.4% in the present paper compare to 91% of Munz et al. (2009) paper).

The thiosulfate oxidation rate was in biochemical as well as chemical conditions almost the same (i.e. 5.25 and 6.83 mg S L⁻¹ d⁻¹, resp.) showing that thiosulfate oxidation under the microaerobic conditions proceeds mainly chemically. According to Janssen et al. (1995) chemical oxidation of sulfide to mainly thiosulfate becomes important when biological activity of SOB is limited. In this study SOB were active and thiosulfate was still oxidized mainly chemically.

During the experiments, the colour of the medium changed from colourless to slightly yellowish indicating the formation of elemental sulfur (Chen & Morris, 1972). While during the chemical experiments, the sulfur was in the form of yellowish suspension, in biochemical experiments, yellow flakes appeared in the reactor. This is in accordance with the findings of Janssen et al. (2009) and Kleinjan et al. (2003), who observed the same difference in the properties of biologically produced sulfur compare to the chemically produced one.

Model calibration

Overall, presented model predictions correlated well with experimental data. The concentration of DO, sulfide and thiosulfate showed a good fit. The concentration of sulfate was overestimated in the simulations. Underestimation of sulfate concentration could be caused by sulfate reduction back to hydrogen sulfide which was not incorporated in the model. All thiosulfate was converted, probably to sulfate and elemental sulfur (see Eq. 3.1.5) however no sulfate appeared in the system during the experiments. The ORP was on average -240 mV during the experiment, which is too low for sulfate to be present in water. The oxidation of elemental sulfur to sulfate occurs in the system at the ORP of +352 mV while the reduction of elemental sulfur to sulfide takes place at ORP -225 mV (Vohlidal, 1985). In the present model, thermodynamics was not taking into account (only kinetics).

The kinetic parameter values of chemical sulfide oxidation obtained in this study were compared with the literature (**Table 3.1.3**). The chemical H_2S oxidation rate of 0.06 min⁻¹ were comparable with the results of Wilmot et al. (1988) (0.055); however, the reaction orders, α and β , were different. The

concentration of sulfide was comparable, but the concentration of oxygen was 53-207× lower in the present study. Comparably, the reaction orders, α and β , in this study (1.1 and 0.9, resp.) are similar to the results of O'Brien and Birkner (1977) (1.02 and 0.8, resp.); however, the chemical H₂S oxidation rate is different. Again, it could be caused by the different oxygen concentration (70-367× lower in this study). In general, the results available in the literature are hardly comparable with the results from this study. The experimental conditions vary paper from paper making the comparison nearly impossible.

k _{H2S.chemox} min ⁻¹	α	β	c (S ²⁻) mmol S ²⁻ L ⁻¹	c (O ₂) mmol O ₂ L ⁻¹	References
0.06	1.1	0.9	0.27	0.003	This paper
0.57	0.41	0.39	0.16-9.38	0.003-0.266	Buisman et al. (1990b)
0.055	0.38	0.21	0.09-0.30	0.16-0.62	Wilmot et al. (1988)
67.6	1.15	0.69	0.05-0.20	0.60	Jolley and Forster (1985)
1.44	1.02	0.80	0.02-1.21	0.21-1.10	O'Brien and Birkner (1977)
-	0.81-0.99	0.19-0.16	0-0.25	0-0.13	Nielsen et al. (2004)
1	1.34	0.56	0.05-0.20	0.16-0.08	Chen and Morris (1972)

Table 3.1.3: The kinetic parameters of chemical sulfide oxidation

 1 k_{H2S.chemox} depended on the pH value and varied from 11.8 to 16.38 M $^{-1}$ h $^{-1}$

Table 3.1.4 summarizes the results of kinetics of biochemical sulfide oxidation to elemental sulfur found in the literature and compares it with this study.

μ _{soв} [d ⁻¹]	K _{s,H2S} [mg S ²⁻ L ⁻¹]	K _{s,02} [mg O ₂ L ⁻¹]	Y _{sob} [mg x mg ⁻¹ S ²⁻]	Reference
204.9	0.032	3.2	0.32 (COD)	This study
0.67	11.00	0.0002	0.0900 (x = VSS)	Xu et al. (2013b)
8.64	63.68	n.a.	0.0006 (x = ATP)	Gadekar et al. (2006)
n.a.	8.96	n.a.	0.0891 (x = protein)	Alcántara et al. (2004)
7.20	0.32	n.a.	0.0969 (x = protein)	De Zwart et al. (1997)

Table 3.1.4: The kinetic parameters of biochemical oxidation of sulfide to elemental sulfur

n.a. – not available

Also in this case, the comparison is hardly possible. To the best of author's knowledge, there are no papers available describing kinetic parameters for chemical and separately biochemical sulfide oxidation in one (oxygen limited) system. Xu et al. (2013b) describes the kinetic of biological sulfide oxidation; however, they neglected chemical sulfide oxidation. Wilmot et al. (1988) found 12-56 % contribution of biological component oxidation; however its initial conditions were 10-20 mg L⁻¹ for oxygen and 6-9 mg L⁻¹ for sulfide. The results showed variation in contribution of biological oxidation with source of wastewater. They suggested that at low sulfide concentration, biological reaction is expected to be more significant.

3.1.5. Conclusions

Chemical and biochemical sulfide and thiosulfate oxidation was studied under microaerobic conditions.

- Under microaerobic conditions (DO below 0.1 mg L⁻¹) elemental sulfur is the major endproduct of both, chemical and biochemical, sulfide oxidation.
- During the biochemical sulfide oxidation approximately 60% of elemental sulfur is created by bacteria and 40% by chemistry.
- Biochemical sulfide oxidation was about two and a half times faster compared to chemical sulfide oxidation.
- Comparison of the chemical and biochemical oxidation rates confirmed that thiosulfate (formed by chemical oxidation of hydrogen sulfide) is in the microaerobic conditions oxidized only chemically.
- Suspended elemental sulfur was formed during chemical oxidation, while flakes appeared during biochemical experiments.
- Chemical and biochemical parameter values were estimated through model calibration.

3.2. IMPROVING PRODUCTS OF ANAEROBIC SLUDGE DIGESTION BY MICROAERATION (Article 3)

Jenicek, P., Celis, C.A., Krayzelova, L., Anferova, N., Pokorna, D. (2014). "Improving products of anaerobic sludge digestion by microaeration." Water Science and Technology 69(4): 803-809.

Abstract

Biogas, digested sludge and sludge liquor are the main products of anaerobic sludge digestion. Each of the products is influenced significantly by specific conditions of the digestion process. Therefore, any upgrade of the digestion technology must be considered with regard to quality changes in all products. Microaeration is one of the methods used for the improvement of biogas quality. Recently, microaeration has been proved to be a relatively simple and highly efficient biological method of sulfide removal in the anaerobic digestion of biosolids, but little attention has been paid to comparing the quality of digested sludge and sludge liquor in the anaerobic and microaerobic digestion and that is why this paper primarily deals with this area of research. The results of the long-term monitoring of digested sludge quality and sludge liquor quality in the anaerobic and microaerobic digesters suggest that products of both technologies are comparable. However, there are several parameters in which the "microaerobic" products have a significantly better quality such as: sulfide (68% lower) and soluble chemical oxygen demand (COD) (33% lower) concentrations in the sludge liquor and the lower foaming potential of the digested sludge.

Keywords

Anaerobic digestion; Biogas desulfurization; Microaerobic conditions; Microbial activity; Sludge liquor; Sludge quality

3.2.1. Introduction

Currently, anaerobic digestion is a key technological stage of sludge management in large municipal wastewater treatment plants, enabling the transformation of organic pollution into energy. Anaerobic digestion is the only process with a highly positive energy balance and the produced biogas can cover a substantial part of wastewater treatment energy requirements (Jenicek et al., 2012). Over the past few decades many intensification methods were proposed for focusing on an increase in biogas production, because the biodegradability of sludge organic matter is limited. In addition to biogas production maximization, another important aim of advanced sludge technologies lies in the quality improvement of all anaerobic digestion products: biogas, sludge and sludge liquor.

Biogas is utilized as a renewable energy source and its sufficient quality determines its energy valorisation. In particular, hydrogen sulfide (Lens et al., 1998) and siloxane (Dewil et al., 2006) content can cause many operational problems. Sludge disposal is usually a costly operation and so the reuse of sludge is strongly encouraged because of sustainability. In terms of sludge reuse routes, the content of specific pollutants, rheological properties, dewaterability and other sludge properties are of great importance.

Sludge liquor separated during digested sludge dewatering is rejected into the activated sludge system and has a considerable influence on wastewater treatment operational costs and the effluent quality. Thus, any improvement to sludge liquor quality can be beneficial for the wastewater treatment process.

One of the ways to improve anaerobic digestion products seems to lie in the application of microaerobic conditions. In this paper, microaerobic digestion means a digestion with limited (trace) oxygen consumption. With respect to the oxidation-reduction potential (ORP), microaerobic conditions can be characterized by a limited ORP increase caused by micro-consumption of oxygen in comparison with anaerobic conditions (Khanal & Huang, 2003b). The microaerobic conditions are obtained by dosing a limited amount of air or oxygen into the anaerobic digester. It has been proved that in a mixed culture, even strict anaerobes can survive without any inhibition, supposing that facultative microorganisms are able to consume the present oxygen quickly and completely (Jenicek et al., 2011a; Zitomer & Shrout, 1998).

Until now microaerobic conditions have been used for efficient hydrogen sulfide removal from biogas. (Fdz-Polanco et al., 2009; Khanal & Huang, 2003b; van der Zee et al., 2007). However, less attention has been paid to the comparison of digested sludge and sludge liquor quality after anaerobic and microaerobic digestion. This paper seeks to fill this gap in knowledge by evaluating the quality of all digestion products.

3.2.2. Materials and Methods

Digesters set-up

Our experiments were carried out in two continuously stirred, semi-continuously fed laboratory-scale digesters of 10 L working volume, identical to those used in our previous microaerobic experiments reported earlier (Jenicek et al., 2010). The total time of parallel operations for both digesters was 300 days. The start-up of both reactors was carried out in anaerobic conditions. After the start-up, the operation of the first reactor (R-1) was changed to microaerobic mode when air was dosed

continuously to the bottom of the digester with a peristaltic pump. The air flow rate was gradually increased from 0.3 to 1.6 L d⁻¹. The second reactor (R-2) remained anaerobic and served as a referential reactor. Thickened waste activated sludge from a municipal wastewater treatment plant was used as the substrate for the reactors; sulfur content was increased by sodium sulfate addition. The average sludge composition was as follows: total suspended solids (TSS) 69.2 \pm 6.5 g L⁻¹, volatile suspended solids (VSS) 49.4 \pm 3.6 g L⁻¹, pH 7.10 \pm 0.19. The reaction mixture was kept homogeneous by means of mechanical mixing and the operational temperature was kept at 40 \pm 1°C as it was the original operational temperature of the inoculum. Volumetric loading rates of the digesters were 2.0 g L⁻¹ and 0.15 g L⁻¹ d for chemical oxygen demand (COD) and SO₄²⁻ respectively during the evaluation period. Both of the reactors had equal hydraulic and solids retention times: approx. 30 days.

Biogas and sludge composition

Analytical procedures were carried out in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The biogas composition and volatile fatty acids concentrations were determined with a gas chromatograph GC 8000^{TOP} equipped with a heat conductivity detector HWD 800. The elemental composition of the sludge was assessed by X-ray fluorescence analysis, using the ARL 9400 XP sequential WD-XRF spectrometer. It is equipped with an Rh anode end-window X-ray tube type 4GN fitted with a 75 µm Be window. All peak intensity data were collected in vacuum by WinXRF software.

Foaming potential (FP) and foam stability

These parameters were assessed as additional characteristics of the digested sludge because of the increased presence of filamentous bacteria that cause sludge foaming. It has been reported that anaerobically treated excess activated sludge exhibits a tendency to cause digester foaming. In order to describe and compare foam quantity and quality a so called 'bubble test' has been developed (Zaplatilkova, 2004). The testing was based on the test procedure described by Pagilla et al. (1996) and modified with respect to the character of the anaerobic sludge. The final test was carried out by bubbling 1 litre of sludge using nitrogen with the flow rate of 1 L min-1 in 2 L volumetric cylinders. The level of foamy sludge was recorded after 5 minutes of bubbling and the FP was calculated from this value (Eq. 3.2.1). Then, 5 min after the gas flow was stopped the level of foamy sludge was recorded again to calculate the index of stability (IS), (Eq. 3.2.2). FP describes the capacity of sludge to create foam. IS provides information about the stability of the foam created.

$$FP = \frac{V_5}{V_0}$$
 (Eq. 3.2.1)

$$IS = \frac{(V_{ST} - V_0)}{(V_5 - V_0)} \times 100$$
 (Eq. 3.2.2)

where V_0 is the volume of sludge at the beginning of measuring (usually 1 L); V_5 is the volume of foamy sludge after 5 min of bubbling; V_{ST} is the volume of foamy sludge 5 min after the gas flow is stopped.

Microscopy

Microscopic analysis of the sludge samples was performed in accordance with Jenkins et al. (1993). The microscopic examination in wet mounts (Olympus BH2-RFCA – \times 125, \times 250 magnification and phase contrast \times 1250 magnification) was aimed at examining basic biomass characteristics (morphological properties, structure, presence and types of protozoa/metazoa and zoogloeal colonies). Gram and Neisser staining procedures were applied to determine the abundance of filamentous microorganisms and to identify them.

CST

Capillary suction time (CST) is a simple parameter and a method used for the characterization of sludge dewaterability. The CST is the time necessary to collect a unit volume of filtrate of the sludge undergoing filtration in a standard-sized CST funnel, when placed upon a standard grade of chromatography paper. The CST test was found to be accurate, if the product of solid concentration and specific resistance to filtration is of interest (Scholz, 2005). The original circular setup introduced by Baskerville and Gale (1968) and the Whatman-17 filter paper were used to conduct the measurement.

3.2.3. Results and discussion

Digestion performance

When comparing the performance of both microaerobic and anaerobic digesters it can be stated that, despite the air dosing, the production of methane is comparable in both digesters. Biogas production is higher in the microaerobic digester because the rest of the air supplied – i.e. nitrogen – remains in the biogas. However, methane production is similar in both digesters. Furthermore, the sludge digestion efficiency expressed by VSS degradation efficiency is not significantly different as shown in **Table 3.2.1**. Apparently lower VSS degradation could be a consequence of the potentially higher growth and biomass yield of facultative bacteria. A fundamental difference is in the hydrogen sulfide content in biogas; thanks to microaeration more than 99% of H₂S was removed from the biogas in the final period of digester operation. This confirms the desulfurization potential of the microaerobic technology as previously reported (Fdz-Polanco et al., 2009; Jenicek et al., 2008) being also effective for the specific composition of treated sludge such as high Fe and S content. Significant differences were confirmed (ORP_H value) in the ORP corresponding to the report by Khanal and Huang (2003b). The comparison of H₂S concentrations in biogas during all experimental periods is shown in **Figure 3.2.1**.

Parameter	Microaerobic digester R1	Anaerobic digester R2
Volumetric loading rate – COD (g L ⁻¹ d ⁻¹)	2.0	2.0
Biogas production (L d^{-1})	4.62 ± 0.46	3.67 ± 0.52
Air dose (L d ⁻¹)	1.6	-
CH_4 production (L d ⁻¹)	2.33 ± 0.24	2.41 ± 0.35
Specific CH ₄ production (L kg ⁻¹ VSS _{added})	188 ± 21	195 ± 28
VSS degradation efficiency (%)	58.1 ± 2.7	59.9 ± 3.2
H ₂ S removal efficiency (%)	99.7 ± 0.2	-
ORP _H (mV)	-507 ± 7	-547 ± 5

 Table 3.2.1: Comparison of digestion results in final period of experiment (average value and standard deviation)





Biogas composition

As mentioned earlier the H_2S concentration was the main difference in biogas quality. Even extremely high hydrogen sulfide concentrations were almost completely removed. Another difference was caused by the remaining nitrogen gas from the supplied air, which is inert and dilutes all the other components of biogas, especially methane, whose concentration decreased from 63% to 50%. If the decrease of methane concentration is not acceptable, then the dilution can be avoided by substituting the air with oxygen. A slightly lower ratio of CH_4/CO_2 in biogas from the microaerobic digester indicates that a small portion of oxygen is consumed during the oxidation of organic matter. The content of nitrogen was surprisingly high in anaerobic digester. Here, the nitrogen could originate from air getting into the digester with the treated sludge doses, from denitrification and/or from the decomposition of nitrogenous organic compounds. The average biogas composition in the final experimental period is illustrated in **Table 3.2.2**.

Parameter	Microaerobic digester R1	Anaerobic digester R2
CH ₄ (vol. %)	50.4 ± 1.3	63.0 ± 1.2
CO ₂ (vol. %)	24.8 ± 2.1	29.0 ± 0.9
N ₂ (vol. %)	25.0 ± 2.2	4.8 ± 0.2
H ₂ (vol. %)	< 0.1	< 0.1
H_2S (mg m ³)	51.7 ± 14.4	17600 ± 490

Table 3.2.2: Comparison of biogas quality in final period of experiment (average value and standard deviation)

Sludge liquor composition

The sludge liquor composition differed significantly in many parameters as shown in Table 3.2.3 and Figure 3.2.2, the most important difference being that of soluble COD during the whole duration of the experiment – it was about 3 g L⁻¹ lower in the microaerobic digester. The reason for such a result may lie in better degradability of some organic compounds when anaerobic and aerobic conditions are combined. Volatile fatty acids (VFA) concentration was found to be 3.5x higher in the anaerobic digester compared to the VFA concentration in the microaerobic digester. However, that only causes the COD difference in the range of tens of milligrams. Earlier, it had already been found to be true for adsorbable organic halogens (AOX) compounds for example. (Jenicek et al., 2008). Abnormally high concentration levels of N_{ammon} (sum N-NH₄⁺and N-NH₃) appeared in both reactors because highly thickened waste activated sludge was digested. However, the N_{ammon} concentration in the microaerobic digester was always slightly lower, about 4% on average. Microaerobic conditions also brought about an expected effect – a lower soluble sulfide concentration, which is in direct relation to highly efficient biogas desulfurization. On the other hand, thiosulfate and sulfate concentrations were higher in R1, but both concentrations can be classified as negligible in comparison with the total sulfur content in the digesters. The microaerobic digester had its lower maximum of the volatile fatty acid concentration when compared to the anaerobic digester during the period in which the digestion process was negatively affected in both digesters, 100 versus 200 mg L⁻¹.

Parameter	Microaerobic digester R1	Anaerobic digester R2
рН	8.5 ± 0.1	8.6 ± 0.1
Dissolved inorganic salts (g L^{-1})	8.7 ± 0.6	8.8 ± 0.5
Soluble COD (g L ⁻¹)	5.8 ± 0.4	8.7 ± 0.5
Volatile fatty acids (mg L^{-1})	16.6 ± 0.6	58.8 ± 1.9
Dissolved sulfide (mg L ⁻¹)	162 ± 31	506 ± 50
Thiosulfate (mg L ⁻¹)	3.5 ± 0.9	1.9 ± 0.2
Sulfate (mg L ⁻¹)	19 ± 2	37 ± 2
N _{ammon} (g L ⁻¹)	2.88 ± 0.10	3.00 ± 0.10
P-PO ₄ ³⁻ (mg L ⁻¹)	55 ± 9	65 ± 10

Table 3.2.3: Sludge liquor quality in final experimental stage (average value and standard deviation)



Figure 3.2.2: Difference in COD and N_{ammon} concentrations in both digesters.

Sludge composition

No significant differences were found in sludge composition except for sulfide and sulfur content as shown in **Tables 3.2.4** and **Table 3.2.5**. The sulfide content is lower in the microaerobic sludge due to its oxidation to elemental sulfur and for the same reason the elemental analysis of the sludge found the higher total sulfur content in the microaerobic sludge. Indications of the slightly higher VSS content in biomass of the microaerobic digester could be a consequence of the potentially higher growth and biomass yield of the facultative bacteria as mentioned in the comment on the performance of the digesters. Small differences were found in the elemental composition of the digested sludge from microaerobic and anaerobic reactors (**Table 3.2.5**). The expected increase of sulfur content due to the precipitation of elemental sulfur in the microaerobic sludge was confirmed. However, this increase was not particularly high because the sludge containing the accumulated sulfur was continuously removed from the digester. Even at extremely high H₂S concentrations, the maximum daily production of elemental sulfur was 35 mg. High Fe content is caused by the Fe³⁺ addition in the wastewater treatment plant, where the treated activated sludge originates from. The presence of iron also contributes to H₂S removal in both reactors due to the precipitation of FeS.

Parameter	Microaerobic digester R1	Anaerobic digester R2
TSS (g L^{-1})	31.98 ± 1.30	31.66 ± 1.11
VSS (g L^{-1})	17.96 ± 1.02	17.17 ± 1.24
VSS/TSS (%)	50.5 ± 0.8	49.7 ± 1.6
COD (g L ⁻¹)	37.6 ± 1.9	37.9 ± 1.9
Total sulfide (mg L⁻¹)	824 ± 5	1129 ± 32

Table 3.2.4: Digested sludge composition in final period of experiment (average value and standard deviation)

Element	Microaerobic sludge [%]	Anaerobic sludge [%]	Ratio anaerobic/aerobic
Fe	20.68	20.48	1.01
Р	16.49	16.6	0.99
Са	11.37	11.62	0.98
S	10.49	8.85	0.84
Na	16.87	15.78	1.07
Al	9.47	9.38	1.01
Si	7.34	7.41	0.99

Table 3.2.5 Relative elemental composition of digested sludge from microaerobic and anaerobicreactor (major elements of mineral fraction) 300th day of operation

Foaming

During several test periods, the treated waste activated sludge contained increased levels of filamentous bacteria and so the risk of digester foaming was greater. Initially, when launching microaerobic digester operation, there was concern about the possible stimulation of aerobic bacteria from the activated sludge. The presence of oxygen might lead to the survival of aerobic filamentous bacteria in the microaerobic digester and consequently to the stimulation of foam production. Eventually, our concern about the possible undesirable behaviour of aerobic filamentous bacteria in microaerobic conditions was proven by the results to be unsubstantiated. The FP of the microaerobic digester was never higher than that of the reference digester during the whole experiment. The difference between the microaerobic and anaerobic digesters was distinct, especially during the period with a high FP. The maximum FP was 3.2 and 2.6 for the anaerobic and microaerobic reactor, respectively. In addition, the foam rising in the anaerobic reactor was much more stable in comparison to the foam originating from the microaerobic reactor. The index of stability IS was 96% in the anaerobic digester and 53% in the microaerobic digester. Better degradation of the activated sludge filaments (with dominance of Microthrix parvicella) during the microaerobic digestion can be illustrated by microscopic images of the digested sludge in Figure 3.2.3 with filament fragments being longer, more frequent and more compact in the anaerobic sludge.



Figure 3.2.3: Microscopic pictures of digested sludge. A - microaerobic and B - anaerobic, showing different abundance and morphology of filaments (Gram staining, direct light, enlargement 1250x magnification)

Evaluation of dewaterability by CST

Dewaterability was characterized by the periodic CST measurement of sludge samples from both digesters. The results are shown in **Figure 3.2.4** and indicate that dewaterability of the microaerobic sludge is slightly better especially at higher air doses. Relations between this result and higher concentration of soluble COD in R2 should be studied and explained in further studies. The CST values are relatively high due to the specific character of the treated sludge and due to high TSS and soluble salts concentrations of 30-33 g L⁻¹ and 8.0-9.5 g L⁻¹, respectively.





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3.2.4. Conclusions

The following conclusions can be drawn based on the presented results of sludge quality monitoring in anaerobic and microaerobic digesters:

- Sludge quality in both reactors is similar, only small differences were found in sludge composition.
- Slightly higher content of the total S and lower content of the S-S²⁻ in the microaerobic sludge indicates the accumulation of elemental S.
- The composition of the sludge liquor differs mainly in the soluble COD concentration, which is significantly lower, by 33% on average, in microaerobic digestion.
- The sludge digested under the microaerobic conditions manifested lower FP and foam stability. The dewaterability characterized by CST was also better for the microaerobic sludge.

3.3. MICROAERATION FOR HYDROGEN SULFIDE REMOVAL IN UASB REACTOR (Article 4)

Krayzelova, L., Bartacek, J., Kolesarova, N., Jenicek, P. (2014). "Microaeration for hydrogen sulfide removal in UASB reactor" Bioresource Technology 172(0): 297-302.

Abstract

The removal of hydrogen sulfide from biogas by microaeration was studied in Up-flow anaerobic sludge blanket (UASB) reactors treating synthetic brewery wastewater. A fully anaerobic UASB reactor served as a control while air was dosed into a microaerobic UASB reactor (UMSB). After a year of operation, sulfur balance was described in both reactors. In UASB, sulfur was mainly presented in the effluent as sulfide (49%) and in biogas as hydrogen sulfide (34%). In UMSB, 74% of sulfur was detected in the effluent (41% being sulfide and 33% being elemental sulfur), 10% accumulated in headspace as elemental sulfur and 9% escaped in biogas as hydrogen sulfide. The efficiency of hydrogen sulfide removal in UMSB was on average 73%. Microaeration did not cause any decrease in COD removal or methanogenic activity in UMSB and the elemental sulfur produced by microaeration did not accumulate in granular sludge.

Graphical abstract



Keywords

Microaeration, UASB reactor, elemental sulfur accumulation, sulfur balance, hydrogen sulfide removal

3.3.1. Introduction

Anaerobic treatment of wastewaters from industries such as pharmaceutical, paper, leather or food processing with high amount of sulfate in the influent often leads to high amount of hydrogen sulfide in biogas and sulfide (HS^- and S^{2-}) in liquid phase (Sawyer et al., 2003; Sinbuathong et al., 2007):

$$SO_4^{2-} + organic matter \xrightarrow{anaerobic bacteria} HS^- + H_2O + HCO_3^-$$
(Eq. 3.3.1)
$$S^{2-} \stackrel{H^+}{\longleftrightarrow} HS^- \stackrel{H^+}{\longleftrightarrow} H_2S$$
(Eq. 3.3.2)

High concentrations of hydrogen sulfide in biogas cause the corrosion of concrete and steel and emissions of odour and sulfur dioxide during the combustion of biogas (Buisman et al., 1990b; Hulshoff Pol et al., 1998). Moreover, sulfide in liquid phase is toxic to methanogens and causes the inhibition of anaerobic process (Khanal & Huang, 2003b; Zhou et al., 2007). For these reasons, the removal of sulfide from both gaseous and liquid phase is required and beneficial. Hydrogen sulfide can be removed from contaminated streams (liquid or gaseous) by physico-chemical processes such as adsorption, precipitation or reaction with alkaline substances (Abatzoglou & Boivin, 2009). Physico-chemical methods for hydrogen sulfide removal from biogas need additional chemicals and they work under high temperatures and pressures. This makes these methods energetically demanding and expensive (Appels et al., 2008). In contrast, biological methods have lower operational costs with lower or none utilization of chemicals (Syed et al., 2006). Biological methods are most often based on the biochemical oxidation of sulfide to sulfate, thiosulfate or elemental sulfur (Díaz et al., 2011b).

Microaeration (i.e. dosing of small amount of air or oxygen into anaerobic reactor) causes the oxidation of sulfide to elemental sulfur according to the following equation (Janssen et al., 1995):

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$$
 (Eq. 3.3.3)

It is an efficient and stable method for hydrogen sulfide removal in anaerobic digesters and biogas plants (Botheju & Bakke, 2011; Díaz & Fdz-Polanco, 2012; Jenicek et al., 2008; Jenicek et al., 2010; Ramos et al., 2013; Zhu et al., 2009). Unlike other biological methods, microaeration does not require separate reactor or complex control system (Janssen et al., 1995). So far, microaeration has been only few times reported in reactors for wastewater treatment such as Up-flow Anaerobic Sludge Blanket (UASB) reactor (Zheng & Cui, 2012; Zhou et al., 2007). Moreover, the focus of these studies was not the removal of hydrogen sulfide from biogas, but alleviation of sulfide toxicity (Zhou et al., 2007) or simultaneous COD removal and nitrification (Zheng & Cui, 2012).

In this work, the effect of microaeration in UASB reactor treating synthetic brewery wastewater was investigated and compared with a non-aerated UASB reactor. The fate of sulfur in both reactors including sulfur and COD balance was described. Moreover, the influence of microaeration on the methanogenic activity of the granular sludge present in the microaerobic reactor was evaluated.

3.3.2. Materials and Methods

Experimental set-up

Two reactors were operated in this study (Figure 3.3.1): Up-flow Anaerobic Sludge Blanket reactor (UASB) and Up-flow Microaerobic Sludge Blanket reactor (UMSB) as shown previously by Zheng et al. (2013). Both reactors (working volume 2.7 L) were kept at 37 °C with temperature controlled by resistance wire wrapped around the reactor. Reactors were inoculated with 1 L of granular sludge (particle size up to 0.5 cm) from a full scale UASB reactor treating wastewater from candy production. The average concentration of total suspended solids (TSS) was 50 g L⁻¹ of reactor. Both reactors were fed with synthetic brewery wastewater at loading rate 8 g COD L⁻¹ d⁻¹, and sulfate loading rate 0.084 g S L⁻¹ d⁻¹ (COD/S ratio of 95). The dosage of sulfate was artificially elevated compared to typical brewery wastewater in order to achieve high concentration of hydrogen sulfide in biogas. Hydraulic retention time (HRT) was 8 h, recycle flow 8 L h⁻¹ and superficial up-flow velocity 1.2 m h⁻¹. The operation of both, UMSB and UASB reactor began at the same time under the same anaerobic conditions. Air was dosed with a peristaltic pump into the recycled effluent of the UMSB reactor. Air dosing started after a start-up period (2.5 months) and the amount of air was 1 L d⁻¹. This amount corresponded to the stoichiometric molar ratio of O₂/S assuming that sulfate from the influent was first reduced to sulfide and subsequently oxidized to elemental sulfur (see Eq. 4.1 and 4.3). UASB reactor was kept under anaerobic conditions and was used as a control. Both UMSB and UASB reactors were operated for 373 days.



Figure 3.3.1: Scheme of UASB and UMSB reactor used in this study. (1) Influent, (2) distribution bed, (3) granular sludge, (4) effluent, (5) thermometer, (6) biogas, (7) gas-meter, (8) recycle, (9) temperature control, (10) air dosing.

Synthetic brewery wastewater

Synthetic brewery wastewater used in this study was prepared based on Peng et al. (2006) and Tarn (2002) with an extra addition of sulfate. It contained (per liter of influent): malt extract 5 g; yeast extract 2.5 g; peptone 0.75 g; maltose 4.3 g; 95% ethanol 6 ml; NH₄Cl 1.18 g; KH₂PO₄ 0.26 g;

 $MgSO_4$ 7H₂O 2.6 g; CaCl₂ 2H₂O 0.06 g and NaHCO₃ 5.6 g. This concentrated solution was diluted (1:5) by tap water before injection into the reactors. Trace elements solution was not added into the influent as all micronutrients were expected to be present in the malt extract. The average pH of the concentrated solution was, thanks to the creation of volatile fatty acids, between 5.7 and 5.8. Due to the sufficient buffering capacity of the dilution water, pH inside of the reactor was always between 7.0 and 7.6.

The assessment of specific methanogenic activity

The assessment of specific methanogenic activity (SMA) was carried out at mesophilic conditions in serum bottles (total volume of 120 mL) without mixing in triplicates according to the guidelines proposed by Angelidaki et al. (2009). The volume of liquid and gas phase was 80 and 40 mL, respectively. The synthetic brewery wastewater was used as a substrate with organic loading 2 g of substrate per g of volatile suspended solids (VSS) of inoculum. One gram (wet weight) of granular sludge was used as inoculum. The production of biogas was measured volumetrically using water displacement method (Angelidaki et al., 2009). The biogas composition was determined by GC 8000^{TOP} (Fisons Instruments, USA).

In order to estimate potential trace metal limitation in the granular sludge, targeted SMA experiments with extra addition of trace metal mixture according to Fermoso et al. (2010) were carried out occasionally.

Analytical methods

Influents and effluents of both reactors were sampled for COD_{Cr}, pH, VSS, TSS and sulfide analysis. Granular sludge was sampled and analysed for TSS, VSS and elemental composition. Analysis of COD_{Cr}, VSS and TSS were done according to the Standard Methods (American Public Health Association, 1997) as well as the determination of total sulfide, dissolved sulfide and hydrogen sulfide by iodometric titration. The amount of methane and carbon dioxide in biogas was measured by GC 8000^{TOP} (Fisons Instruments, USA) equipped with a heat conductivity detector HWD 800. The elemental composition of sludge was assessed by Elemental Vario EL III (Elementar Analysensystem GmbH, Germany) and by X-ray fluorescence analysis using the ARL 9400 XP sequential WD-XRF spectrometer (THERMO ARL, Switzerland).

3.3.3. Results and discussion

The efficiency of hydrogen sulfide removal from biogas by microaeration

The concentration of hydrogen sulfide in biogas in UMSB reactor decreased from over 9.0 g m⁻³ to 1.0 g m⁻³ in less than 3 days upon the start of aeration (**Figure 3.3.2**). After 10 days, the concentration of hydrogen sulfide in the biogas produced in UMSB reactor decreased to almost zero and remained at that level for almost a month. After 30 days, the concentration of hydrogen sulfide in biogas in UMSB occasionally increased to up to 6.0 g m⁻³, but it remained significantly lower compared to UASB reactor for 299 days of operation. These peaks of hydrogen sulfide concentration can be attributed to the uneven actual biogas production which caused temporary oversaturation of the effluent by sulfide. This problem is intrinsic to the small-scale set-up and should be alleviated by the future scale-

up of the reactor. The average concentration of hydrogen sulfide in biogas of UASB reactor was 8.9 ± 1.4 g m⁻³ while in UMSB reactor it was only 2.2 ± 1.3 g m⁻³.



Figure 3.3.2: The concentration of hydrogen sulfide in biogas from UMSB (- - -) and UASB (—) reactor throughout the operation.

The dose of air corresponding to the stoichiometric ratio of O_2/S (0.5 mole O_2 per 1 mole of S^{2-}) needed for oxidation of sulfide to elemental sulfur removed 73% of hydrogen sulfide from the biogas of UMSB reactor (with fluctuations between 20 and 99%).

No negative effect on the methanogenic activity of the biomass was observed. The efficiency of desulfurization was not as high as in other studies (Díaz et al., 2011b; Jenicek et al., 2010), where the removal efficiency of 98-99% was observed. However, the dose of air or oxygen was much higher in those studies compare to present study. Further research will be focused on the optimization and control of the amount of air in order to ensure complete desulfurization without oxygen being present in biogas.

Sulfur balance

Microaeration had significant and fast effect on sulfur distribution in UMSB reactor (**Figure 3.3.3**). Five major streams of sulfur leaving UMSB and UASB were identified: (1) total sulfide in the effluent, (2) excess granular sludge (biomass growth), (3) sulfur in biogas (as hydrogen sulfide), (4) deposition in the headspace of the reactors (mainly gas-liquid-solid, G-L-S, separator), and (5) solids in the effluent (excluding sulfide). The main difference between UASB and UMSB reactors were in the amount of hydrogen sulfide in biogas, in the deposition of sulfur in the G-L-S separator, and solids in the effluent excluding sulfide.

Of all sulfur dosed into UASB reactor, 34% was found in biogas while only 9% of sulfur was detected in biogas from UMSB. The sulfide detected in the effluent from UMSB and UASB reactors accounted for 41% and 49% of the sulfur dosed, respectively. I.e. the addition of air into UMSB reactor decreased not only the concentration of hydrogen sulfide in biogas, but also the concentration of

sulfide in the effluent. The average effluent concentration from UASB and UMSB was 13.6 mg L⁻¹ and 11.5 mg L⁻¹, respectively. These results were consistent with Díaz et al. (2011) who found that, while microaeration with air dosed into the gas phase removed only hydrogen sulfide from biogas, dissolved sulfide was removed if air was partially dosed into the liquid phase. Only negligible amounts of sulfate and thiosulfate were found in the effluents. This could be explained by the very low concentration of dissolved oxygen (DO). Annachhatre and Suktrakoolvait (2001) observed that at DO concentration greater than 0.1 mg L⁻¹, sulfate was the main end product, while at DO concentration less than 0.1 mg L⁻¹, elemental sulfur was the main end product. DO concentrations above 0.1 mg L⁻¹ have never been observed in this study. Krishnakumar et al. (2005) controlled the treatment of sulfide and the creation of different sulfur species based on ORP. At ORP from -400 to - 350 mV, sulfide removal was nearly 100% with the sulfur recovery around 80% and sulfate around 2-3%. ORP has never exceeded -420 mV in UMSB reactor. The average ORP was -450 mV and -425 mV for UASB and UMSB reactor, respectively.



Figure 3.3.3: Sulfur balance calculated in UMSB and UASB reactor during days 74-373 of operation: (1) Total sulfide in the effluent; (2) Sulfur in excess sludge; (3) Hydrogen sulfide in biogas; (4) Elemental sulfur in G-L-S separator; and (5) Solids in the effluent (excluding sulfide).

Significant difference was also observed in sulfur detected in the effluent. Over 33% of sulfur was present in the solid phase of the effluent from UMSB reactor, while only 6% was in the effluent of UASB reactor. This amount did not include sulfide and most likely consisted mainly of elemental sulfur. Large part of the elemental sulfur oxidized during microaeration also accumulated in the G-S-L separator of UMSB reactor (10%). The accumulation of elemental sulfur in the gas space and tubes was clearly visible and identifiable as whitish solid material. This material was formed of 73% of sulfur and 20% of water. Residual 7% accounted for other elements such as P, Si, Ca, etc. The G-S-L separator of UASB reactor contained only 1% of sulfur flow with no visible accumulation in head space and it was probably present there since the start-up of the reactor.

The amount of sulfur present in the excess sludge was 8% for both UMSB and UASB reactors. No significant increase of sulfur concentration in granular sludge during the operation was observed in both reactors. I.e. elemental sulfur formed in UMSB reactor due to microaeration did not

accumulated in granular sludge. The amount of sulfur bound in organic and inorganic form inside the sludge (**Figure 3.3.4**) even slightly decreased by 0.5% and 2.0% in both reactors throughout the operation with the majority of sulfur bound in inorganic form (most likely as sulfide precipitates).



Figure 3.3.4: Mass percentage of organic (A) and inorganic (B) sulfur in granular sludge in UMSB (- - -) and UASB (-) reactor throughout the operation.

According to the sulfur balance calculated for 299 days for both reactors, microaeration caused the partial oxidation of inhibitory sulfide to harmless elemental sulfur in UMSB reactor. Nonetheless, no positive effect of this decrease of sulfide concentration was observed probably due to the fact that the effluent concentrations in both reactors were relatively low (Khanal & Huang, 2003b).The effluent pH of 7.3 was observed in both reactors indicating that the same chemical species of sulfide (mainly HS⁻) prevailed in both reactors.

The influence of microaeration on the methanogenic activity of granular sludge

Microaeration did not impair the quality of granular sludge in terms of its physical properties, the activity of methanogenic bacteria, biogas production and the efficiency of COD removal.

The efficiency of COD removal was 89±5% and 90±4% for UASB and UMSB reactor, respectively. COD balance was calculated for both UASB and UMSB reactors including four main effluent streams: COD in biogas, residual COD in the effluent, COD of excess biomass and COD present in the effluent in the form of sulfide and hydrogen sulfide. The fifth stream, COD consumption by oxygen dosing, was considered in UMSB reactor only. Of all COD dosed in the influent, 80% was detected in biogas from UASB while 79% of COD was detected in biogas from UMSB. The COD detected in the effluent from UASB and UMSB reactors accounted for 10% and 9% of COD dosed, respectively. COD necessary for the biomass growth accounted for 1% in both UASB and UMSB reactor. COD present in the form of sulfide and hydrogen sulfide was approximately 2% in both reactors. Air dosed into UMSB reactor accounted for 1% loss of COD. There are 7% and 8% gaps in the COD balance in UASB and UMSB reactor, respectively.

The production of biogas was 8.5 ± 2.0 and $9.6\pm1.6 \text{ L d}^{-1}$ for UASB and UMSB reactor, respectively (**Figure 3.3.5**). The higher production of biogas in UMSB reactor (8% increase) was caused by the

nitrogen dosed with air (0.8 L d⁻¹) which was inert to any processes taking place in UASB and therefore escaped in biogas. Nitrogen dilution is a disadvantage of using air instead of pure oxygen for microaeration. On the other hand, by using pure oxygen, one should consider the increased possibility of forming a dangerous and explosive gas mixture of oxygen with methane (Appels et al., 2008; Wase & Forster, 1984). However, this was not the case of this study since no oxygen was detected in the biogas. The decrease of biogas production in UASB reactor during days 280 and 320 was caused by a technical problem with the pump for substrate (smaller amount of substrate was pumped into UASB reactor).



Figure 3.3.5: The production of biogas in UMSB (- - -) and UASB (-) reactor throughout the operation.

Biogas from UMSB and UASB contained 75% and 77% of methane (after subtracting nitrogen) with the average methane production of 6.6 and 6.5 L d⁻¹, respectively. The SMA of the sludge from both reactors was measured in batch experiments performed in regular intervals (**Figure 3.3.6**) and no significant difference was observed. The SMA of UMSB and UASB reactors were 0.389 and 0.336 mL CH_4 g⁻¹ TSS d⁻¹, respectively, and did not significantly change during the operation. Targeted SMA experiments did not indicate trace metal limitation in either reactor (data not shown). This showed that even the high amount of sulfide formed in the reactors did not precipitate trace metals rendering them biologically unavailable.

TSS of granular sludge was 49.8 and 50.2 g L⁻¹ for UMSB and UASB reactors, respectively, and did not changed significantly since the beginning of this study as the newly grown biomass was removed in regular intervals. Granules from both reactors remained compact and unchanged throughout the operation. Shen and Guiot (1996) reported that methanogens in granular sludge were more tolerant to the presence of oxygen than methanogens in flocculent sludge. Based on the multilayer structure of anaerobic granular sludge proposed by these authors, facultative anaerobes were predominant in the periphery of the granules, while oxygen-sensitive methanogens were located in the deeper layers, protected from the air. This explained the fact, that anaerobic organic degradation and production of methane was not affected by the dosing of air. Granules from UMSB also remained with no visible structure change. The color of the granules remained unchanged despite the findings

of Zitomer and Shrout (1998), who observed the changing of color in the aerated culture from black to brown, indicating less reduction of sulfate and/or iron to ferrous sulfide.



Figure 3.3.6: Specific methane production of UMSB (- - -) and UASB (—) reactor after 3 months of microaerobic conditions in UMSB measured in a batch experiment.

Additional advantages of microaeration

Desulphurization of biogas is not the only advantage of microaeration. Elemental sulfur from G-L-S separator can be recovered as potential valuable compound. It is suitable for the production of sulfuric acid or it can be applied in bioleaching processes (Tichý et al., 1994). However, it is necessary to find a way how to collect elemental sulfur from UMSB reactor without the inhibition of anaerobic treatment. For that, further research of sulfur collection is needed most likely in full-scale. Elemental sulfur present in the effluent of UMSB reactor in the form of suspended solids can be used for sulfur oxidizing autotrophic denitrification (Zhou et al., 2011) since biologically produced elemental sulfur is more available than chemically produced sulfur (Kleinjan et al., 2003).

Microaeration can also enhanced hydrolysis by increasing hydrolytic production of enzymes (Johansen & Bakke, 2006). Since hydrolysis is often considered as the bottleneck for the anaerobic digestion of solids (Myint et al., 2007), optimizing this step could enhanced the whole process. Dosing small amount of air can also be a good strategy to deal with accumulation of volatile fatty acids in the system (Zitomer & Shrout, 1998) or can enhance the treatment of persistent, eco-toxic or resistant organic waste which are difficult to treat either by aerobic or anaerobic treatment itself (Botheju & Bakke, 2011). This, however, was not confirmed in this study. Last but not least, oxygen in anaerobic digestion can improve the stability of the process during unbalanced conditions (Ramos & Fdz-Polanco, 2013).

3.3.4. Conclusions

- Microaeration has the potential to remove large quantities of hydrogen sulfide from biogas formed by the anaerobic treatment of sulfur rich wastewater in 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 and in G-L-S separator.
- No negative effect on methanogenic activity of granular sludge or production of biogas was observed in UMSB reactor.
- Elemental sulfur did not accumulate in the granular sludge of UMSB reactor.

3.4. MODEL-BASED OPTIMIZATION OF MICROAERATION FOR BIOGAS DESULFURIZATION IN UASB REACTORS (Article 5)

Pokorna-Krayzelova, L., Mampaey, K.E., Vannecke, T.P.W., Bartacek, J., Jenicek, P., Volcke, E.I.P.: "Model-based optimization of microaeration for biogas desulfurization in UASB reactors". *Accepted in Biochemical Engineering Journal (June 2017)*

Abstract

During anaerobic treatment of sulfate-rich wastewater, biogas with a high concentration of hydrogen sulfide (H_2S) is produced. Since H_2S is toxic to humans and can cause corrosion of concrete and steel, it needs to be removed before using the biogas for energy and heat production. Biogas desulfurization can be achieved by blowing small amount of air into the anaerobic reactor, a process which is termed "microaeration". In this study, the generally accepted Anaerobic Digestion Model No. 1 (ADM1) was extended with sulfate reduction and sulfide oxidation to optimize the microaeration process during the anaerobic treatment of sulfate-rich wastewater. The resulting model, termed ADM1-S/O, was validated against experimental data from two reactors operated under anaerobic and microaerobic conditions, showing a good description of H_2S concentrations in the biogas. The biomass composition in both reactors was not significantly affected by microaeration. Additionally, scenario analyses were carried out to assess the effect of the influent S:COD ratio and the aeration intensity (O_2 :S ratio) on the steady state reactor behavior.

Keywords

ADM1; biogas desulfurization; microaeration; modeling and simulation; UASB; wastewater treatment

3.4.1. Introduction

Wastewater from industrial processes such as the production of paper, textile, pharmaceuticals and explosives may contain high concentrations of sulfate. While sulfate emissions are not a direct threat to the environment, during anaerobic treatment of these wastewaters sulfate is reduced to sulfide, including gaseous hydrogen sulfide (H_2S). Sulfide causes the inhibition of anaerobic digestion, because of its toxicity to methanogens (Hulshoff Pol et al., 1998). Moreover, high concentrations of H_2S in the biogas cause the corrosion of concrete and steel and are toxic to humans. Therefore, sulfide has to be removed from the biogas.

Microaeration, the dosing of limited amount of air or oxygen into the anaerobic reactor allowing sulfide oxidizing bacteria (SOB) to oxidize sulfide to harmless elemental sulfur, has been demonstrated as an efficient desulfurization method (Díaz et al., 2010; Jenicek et al., 2011a; Krayzelova et al., 2014a; Ramos & Fdz-Polanco, 2014b). However, the basic mechanisms involved in microaerobic sulfide oxidation are not sufficiently understood and control strategies for microaeration are not yet very much developed (Krayzelova et al., 2015). Mathematical modeling of microaeration is expected to increase our knowledge on microaerobic processes and improve our ability to control the process.

The Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002) has been generally accepted and widely applied for mathematical modeling of anaerobic wastewater treatment, but it does not include sulfate reduction processes. The competition between sulfate-reducing bacteria (SRB) and methanogens for acetate in UASB reactors was modeled by Kalyuzhnyi and Fedorovich (1998) and by Kalyuzhnyi et al. (1998) for a dispersed plug flow UASB reactor. Ristow et al. (2002) modeled the anaerobic digestion process including sulfate reduction in a Recycling Sludge Bed Reactor (RSBR) treating acid mine drainage. Knobel and Lewis (2002) developed a model for the treatment of molasses and acid mine drainage in a packed bed, UASB, and gas-lift reactors under steady state and dynamic conditions. Also, Poinapen and Ekama (2010) developed a model including sulfate reduction processes and validated it with experimental results. The most comprehensive approach to modeling sulfate reduction in anaerobic digestion is the ADM1 extension reported by Fedorovich et al. (2003), who described the competition between SRB on the one hand and acidogenic bacteria and methanogenic archaea on the other hand for butyrate, propionate, acetate and hydrogen. All these studies addressed the competition among acetogenic bacteria, methanogenic archaea, and SRB and the resulting sulfate removal, focusing on model calibration and/or assessing the influence of operational parameters and influent concentrations. Less work has been done concerning the prediction and validation of H₂S transfer to the gas phase, even though it constitutes an important aspect in the anaerobic treatment of high-sulfate wastewater. Barrera et al. (2015) included H₂S transfer to the gas phase while modeling anaerobic digestion including sulfate reduction of canemolasses vinasse, a very high strength and sulfate-rich wastewater. Carrera-Chapela et al. (2016) set up a model to describe H₂S formation and transfer during anaerobic digestion of sewage sludge, which was calibrated and validated with experimental data from two pilot-scale reactors.

While a number of (above mentioned) studies has been devoted to the incorporation of sulfate reduction in anaerobic digestion models, chemical and/or biological oxidation of sulfide through microaeration during anaerobic wastewater treatment has not yet been modeled (Batstone et al., 2015; Krayzelova et al., 2015). Botheju et al. (2009) developed a model describing oxygen effects in anaerobic digestion, focusing on aerobic oxidation of soluble carbon, the improvement of organic

matter solubility and the inhibition of obligatory anaerobic organisms. Models describing biochemical oxidation of sulfide to elemental sulfur have only been set up for dedicated processes in biofilters or biotrickling filters (Martin Jr et al., 2004; Oyarzún et al., 2003; Santos et al., 2009; Santos et al., 2006).

In this contribution, 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 resulting model, termed ADM1-S/O, was validated on available experimental data for a strictly anaerobic UASB reactor and an Up-flow Microaerobic Sludge Blanket (UMSB) reactor previously published by Krayzelova et al. (2014).

3.4.2. Modelling microaeration in UASB reactors

Biological conversion processes – ADM1-S/O

The ADM1 was extended with four additional processes of sulfate reduction (Batstone, 2006; Fedorovich et al., 2003) and one additional process of sulfide oxidation (Jensen et al., 2011) (**Table 3.4.1** and **3.4.2**): conversion of butyrate and sulfate to acetate and hydrogen sulfide by X_bSRB (process 9a); conversion of propionate and sulfate to acetate and hydrogen sulfide by X_pSRB (process 10a); conversion of acetate and sulfate to carbon dioxide and hydrogen sulfide by X_aSRB (process 11a); conversion of hydrogen and sulfuric acid to hydrogen sulfide by X_hSRB (process 12a); and oxidation of hydrogen sulfide and oxygen to elemental sulfur by X_SOB (process 12b).

For each process, the stoichiometric coefficients were calculated by closing the COD, carbon, nitrogen and sulfur balances (**Table 3.4.1**). The kinetic expressions (**Table 3.4.2**) describe substrate limitation of both the electron donor (organic substrate or H_2 for SRB and H_2S for SOB) and the electron acceptor (SO₄²⁻ for SRB and O₂ for SOB) through a Monod-type function, as in Fedorovich et al. (2003).

Δ	Components i →	5	6	7	8	9a	9b _A	9b _B	9c	9d	10 _A	10 _B	11	13	20a	2 1a	22a	23a	23b
	Processes j ↓	S _{Tbu}	S _{Tpro}	S _{Tac}	S _{h2}	S _{so4}	S _{HS-}	S _{H2S}	S _{o2}	Ss	S _{HCO3-}	S _{CO2}	S _{TIN}	X _c	X _{bSRB}	X _{pSRB}	X _{aSRB}	X _{hSRB}	X _{SOB}
9a	Uptake of Butyrate by bSRB	-1		(1-Y _{bSRB}) [.] 0.8		$\frac{-(1-Y_{bSRB})}{64}$		$\frac{(1-Y_{bSRB})}{64} \cdot 0.2$				C _{bu} -C _{ac} (1- Y _{bSRB}) 0.8- C _{biom} Y _{bSRB}	-N _{biom} Y _{bSRB}		Y _{bSRB}				
10a	Uptake of Propionate by pSRB		-1	(1-Y _{pSRB}) ⁻ 0.57		$\frac{-(1-Y_{pSRB})}{64} \cdot 0.43$		$\frac{(1-Y_{pSRB})}{64}$ $\cdot 0.43$				C _{pro} -C _{ac} (1- Y _{pSRB}) 0.57 -C _{biom} Y _{pSRB}	-N _{biom} Y _{pSRB}			Y _{pSRB}			
11a	Uptake of Acetate by aSRB			-1		$\frac{-(1-Y_{aSRB})}{64}$		$\frac{(1-Y_{aSRB})}{64}$				C _{ac} - C _{biom} Y _{aSRB}	-N _{biom} Y _{aSRB}				\mathbf{Y}_{aSRB}		
12a	Uptake of Hydrogen by hSRB				-1	$\frac{-(1-Y_{hSRB})}{64}$		$\frac{(1-Y_{hSRB})}{64}$				-C _{biom} Y _{hSRB}	-N _{biom} Y _{hSRB}					Y _{hSRB}	
12b	Uptake of H ₂ S by X _{SOB}							-1	$\frac{-(16-Y_{SOB})}{32}$	1		-C _{biom} 'Y _{SOB}	-N _{biom} Y _{SOB}						Y _{SOB}
16a	Decay of X _{bSRB}											C _{biom} -C _{Xc}	N _{biom} - N _{Xc}	1	-1				
17a	Decay of X _{pSRB}											C _{biom} -C _{Xc}	N _{biom} - N _{Xc}	1		-1			
18a	Decay of X _{aSRB}											C _{biom} -C _{Xc}	N _{biom} - N _{Xc}	1			-1		
19a	Decay of X _{hSRB}											C _{biom} -C _{Xc}	N _{biom} - N _{Xc}	1				-1	
19b	Decay of X _{SOB}											C _{biom} -C _{Xc}	N _{biom} - N _{Xc}	1					-1
A1	H ₂ S acid-base reaction						-1	1											
A2	CO ₂ acid-base reaction										-1	1							

Table 3.4.1: Stoichiometric matrix A_{ij} and composition matrix for the sulfate reduction and sulfide oxidation processes considered in ADM1-S/O

Composition matrix																		
g COD per unit	1	1	1	1	0	64	64	-32	48	0	0	0	1	1	1	1	1	1
mole N per unit	0	0	0	0	0	0	0	0	0	0	0	1	N _{xc}	N _{biom}	N _{biom}	N _{biom}	N _{biom}	N _{biom}
mole S per unit	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0
mole C per unit	C _{bu}	C _{pro}	C_{ac}	0	0	0	0	0	0	1	1	0	C _{Xc}	C _{biom}	C _{biom}	C _{biom}	C _{biom}	C _{biom}
	Total butyrate (kg COD/m ³)	Total propionate (kg COD/m ³)	Total acetate (kg COD/m ³)	Hydrogen gas (kg COD/m ³)	Total sulfate (kmole S/m ³)	Hydrogen sulfide ion (kmole S/m ³)	Hydrogen sulfide (kmole S/m ³)	Oxygen (kmole O₂/m³)	Elemental sulfur (kmole S/m ³)	Bicarbonate (kmole C/m³)	Carbon dioxide (kmole C/m³)	Inorganic nitrogen (kmole N/m ³)	Composites (kg COD/m ³)	SRB butyrate degraders (kg COD/ m^3)	SRB propionate degraders (kg COD $/m^3$)	Acetotrophic SRB (kg COD/m ³)	Hydrogenotrophic SRB (kg COD/m ³)	SOB degraders (kg COD/m ³)

^	Components i →	$\operatorname{Process}_{rate} \left(c kg \operatorname{COD} m^{-3} d^{-1} \right)$
Aij	Processes j 🗸	Process rate (p;,kg COD III 'u')
9a	Uptake of Butyrate by bSRB	$\rho_{9a} = k_{m,bSRB} \cdot \frac{S_{bu}}{K_{S,bSRB} + S_{bu}} \cdot X_{bSRB} \cdot \frac{S_{SO4}}{K_{S,SO4,bSRB} + S_{SO4}}$ $\cdot I_{pH,biom} \cdot I_{N,lim} \cdot I_{h2S}$
10a	Uptake of Propionate by pSRB	$\rho_{10a} = k_{m,pSRB} \cdot \frac{S_{pro}}{K_{S,pSRB} + S_{pro}} \cdot X_{pSRB}$ $\cdot \frac{S_{SO4}}{K_{S,pSRB} + S_{SO4}} \cdot I_{pH,biom} \cdot I_{N,lim} \cdot I_{h2s}$
11a	Uptake of Acetate by aSRB	$\rho_{11a} = k_{m,aSRB} \cdot \frac{S_{ac}}{K_{S,aSRB} + S_{ac}} \cdot X_{aSRB}$ $\cdot \frac{S_{SO4}}{K_{S,aSRB} + S_{ac}} \cdot I_{pH,ac} \cdot I_{N,lim} \cdot I_{h2s}$
12a	Uptake of Hydrogen by hSRB	$\rho_{12a} = k_{m,hSRB} \cdot \frac{S_{h2}}{K_{S,hSRB} + S_{h2}} \cdot X_{hSRB}$ $\cdot \frac{S_{SO4}}{K_{S,hSRB} + S_{h2}} \cdot I_{pH,h2} \cdot I_{N,lim} \cdot I_{h2s}$
12b	Uptake of H_2S by X_{SOB}	$\rho_{12b} = k_{m,SOB} \cdot \frac{S_{h2s}}{K_{S,h2s,SOB} + S_{h2s}} \cdot X_{SOB} \cdot \frac{S_{O2}}{K_{S,O2,SOB} + S_{O2}} \cdot I_{pH,biom} \cdot I_{N,lim}$
16a	Decay of X _{bSRB}	$\rho_{16a} = k_{dec,XbSRB} \cdot X_{c4SRB}$
17a	Decay of X _{pSRB}	$\rho_{17a} = k_{dec, XpSRB} \cdot X_{pSRB}$
18a	Decay of X _{aSRB}	$\rho_{18a} = k_{dec,XaSRB} \cdot X_{aSRB}$
19a	Decay of X _{hSRB}	$\rho_{19a} = k_{dec,XhSRB} \cdot X_{hSRB}$
19b	Decay of X _{SOB}	$\rho_{19b} = k_{dec,XSOB} \cdot X_{SOB}$
A1	H ₂ S acid-base reaction	$\rho_{A1} = K_{AB,H_2S} \cdot \left(S_{HS^-} \cdot S_{H^+} - \frac{K_{a,H_2S}}{S_{H_2S}} \right)$
A2	CO_2 acid-base reaction	$\rho_{A2} = K_{AB,CO_2} \cdot \left(S_{HCO_3^-} \cdot S_{H^+} - \frac{K_{a,CO_2}}{S_{CO_2}} \right)$

Table 3.4.2: Kinetic expressions for the sulfate reduction and sulfide oxidation processes considered in ADM1-S/O

Inhibition by undissociated H_2S (I_{H_2S}) was described for acetogens, methanogens, and all SRBs following Han and Levenspiel (1988):

$$I_{H_2S} = \left(1 - \frac{S_{H_2S}}{K_{I,H_2S}}\right)^n \quad for S_{H_2S} < K_{I,H_2S}$$
Eq. 3.4.1a
$$I_{H_2S} = 10^{-6} \qquad for S_{H_2S} \ge K_{I,H_2S}$$
Eq. 3.4.1b

where S_{H_2S} is the concentration of hydrogen sulfide, K_{I,H_2S} is the hydrogen sulfide inhibition constant, which is set to 0.0161 M (Reis et al., 1992), and n is an empirical constant, equal to 0.401 (Reis et al., 1992). If $S_{H_2S} \ge K_{I,H_2S}$, complete inhibition due to hydrogen sulfide occurs, a small value was used to avoid numerical problems.

Oxygen inhibition on acidogens, acetogens (Botheju et al., 2009), hydrogenotrophic methanogens and acetotrophic methanogens (Ueki et al., 1997) was added compared to Fedorovich et al. (2003). It was described through non-competitive inhibition kinetics (Botheju et al., 2009):

$$I_{O_2} = \frac{K_{I,O_2}}{K_{I,O_2} + S_{O_2}}$$
 for $S_{O_2} \ge K_{I,O_2}$ Eq. 3.4.2a

$$I_{O_2} = 1$$
 for $S_{O_2} < K_{I,O_2}$ Eq. 3.4.2b

where S_{O_2} is the concentration of oxygen and K_{I,O_2} is the oxygen inhibition constant, which is set to 0.25 mM for granular sludge (Shen & Guiot, 1996). However, since the concentration of oxygen in the reactor is lower than the inhibition concentration, it had no effect on the results as such.

The overall ADM1-S/O model and the list of all parameter values applied in ADM1-S/O are in the appendix, section S.3.4.1. Note that the hydrolysis rates of carbohydrates, lipids and proteins were assumed unaffected by the small amounts of oxygen present. While enhanced hydrolysis in the presence of oxygen was observed by some authors (Botheju & Bakke, 2011; Johansen & Bakke, 2006), others found no evidence of it (Nguyen et al. 2007, Goel et al. 1997). According to Botheju and Bakke (2011), oxygen utilization would result in an additional hydrolysis of about 0.4 mg carbon per mg O₂. The average oxygen concentration of 1.92 10⁻⁵ mg O₂ L⁻¹ in this study (model validation case) thus corresponds with an additional amount of hydrolysed carbon of 0.768 10⁻⁵ mg C L⁻¹ or less than 0.001% of the total carbon amount in the reactor. The effect of O₂ on the hydrolysis rate was therefore neglected.

Liquid and gas phase mass balances

Liquid phase mass balances were set up for all state variables, including sulfate $(S_SO_4^{2-})$ and sulfide (S_H_2S) in addition to the ADM1 state variables (see appendix, **Table S3.4.1** and **S3.4.2**). The liquid mass balance of a component *i* is given by Eq. 3.4.3:

$$\frac{dS_{liq,i}}{dt} \cdot V_l = Q_{in}S_{liq,in,i} - Q_{out}S_{liq,i} + k_L a \cdot (S_{liq,i} - S_{liq,i,ss}) \cdot V_l + \sum_{j=1-19b} A_{ij} \cdot \rho_j \cdot V_l$$
 Eq. 3.4.3

where V_l is the volume of the liquid phase [m³], Q_{in} and Q_{out} are the influent and effluent flows, respectively, [m³ d⁻¹], $S_{liq,i}$ and $S_{liq,in,i}$ are the concentrations of component *i* in the liquid phase and in the influent [kmole m⁻³ or kg COD m⁻³], $S_{liq,i,ss}$ represents the equilibrium concentration of component *i* in the liquid phase corresponding with the prevailing gas phase concentration [kmole m⁻³ or kg COD m⁻³], k_La is the interphase mass transfer coefficient [d⁻¹], A_{ij} is the stoichiometric coefficient of component i in process j [kmole m⁻³ or kg COD m⁻³] and ρ_j denotes the rate of process j [d⁻¹].

Eq. 3.4.3 expresses that accumulation of a component *i* (left-hand side) is due to advective transport (in- and outflows), interphase transfer and biological conversions (respective terms on right-hand side).

Interphase transfer was considered for CH_4 , CO_2 , H_2S , H_2 , O_2 , and N_2 . For these components (*i*), a gas phase mass balance was set up as represented by Eq. 3.4.4:

$$\frac{\mathrm{d}S_{gas,i}}{\mathrm{d}t} \cdot V_g = Q_{gas}^{in} S_{gas,i}^{in} - Q_{gas}^{out} S_{gas,i} - k_L a \cdot \left(S_{liq,i} - S_{liq,i,ss}\right) \cdot V_l$$
 Eq. 3.4.4

where V_g denotes the volume of the gas phase [m³], Q_{gas}^{in} and Q_{gas}^{out} are the influent and effluent flows of gas, respectively, [m³ d⁻¹], $S_{gas,i}^{in}$ is the influent gas concentration of component *i* [kmole m⁻³] or [kg COD m⁻³] and $S_{gas,i}$ is the concentration of component *i* in the gas phase [kmole m⁻³] or [kg COD m⁻³].

It was assumed that no reactions take place in the gas phase. Details on the implementation of gasliquid transfer are given in the appendix, section S.3.4.4.

The pH was calculated at each time step from the electro-neutrality equation (charge balance method, see appendix, section S.3.4.3 Calculation of pH). Components involved in a chemical equilibrium and present only in the liquid phase, i.e. acetate (S_Tac), propionate (S_Tpro), butyrate (S_Tbu), valerate (S_Tva) and inorganic nitrogen (S_TIN) were assumed to reach their chemical equilibrium instantaneously. These components were characterized by their total concentrations as state variables; the concentrations of the individual equilibrium forms (S_ac, S_pro, S_bu, S_va, and S_nh3) were subsequently calculated from the total concentrations and the prevailing pH (see appendix, section S.3.4.3). For components involved in a chemical equilibrium and taking part in gas-liquid transfer, i.e. inorganic carbon (S_hco3-) and sulfide (S_hs-), liquid phase mass balances were set up for the unionized species (S_co2, S_h2s) involved in gas-liquid transfer, while the ionized forms were calculated from dynamic equations describing the chemical equilibria. Considering the typical pH operating range (7-7.5), only the dissociation reactions between H₂S and HS⁻ (pK = 6.9) and between CO₂ and HCO₃⁻ (pK = 6.4) were taken into account, neglecting the HS⁻ - S²⁻ (pK = 11.96) and HCO₃⁻ - CO₃²⁻ (pK = 10.25) equilibria, since they were far from the normal pH operating range. A detailed description is given as appendix, section S.3.4.3.

The total gas pressure in headspace ($P_{headspace}$) is calculated as the sum of all partial pressures [bar], which are related to the gas phase concentrations through the ideal gas law:

$$P_{headspace} = p_{T,H_2} + p_{T,CH_4} + p_{T,CO_2} + p_{T,H_2S} + p_{T,N_2} + p_{T,O_2}$$

$$3.4.5$$
Eq. (5)

$$= S_{gas,H_2} \cdot \frac{RT}{16} + S_{gas,CH_4} \cdot \frac{RT}{64} + S_{gas,CO_2} \cdot RT + S_{gas,H_2S} \cdot RT + S_{gas,N_2} \cdot RT + S_{gas,O_2} \cdot RT$$

RT

The biogas flow rate, Q_{gas}^{out} [m³d⁻¹] was calculated from the overpressure in the headspace, according to Batstone et al. (2002):

$$Q_{gas}^{out} = k_p \cdot \left(P_{headspace} - P_{atm} \right)$$
 Eq. 3.4.6

where P_{atm} is an atmospheric pressure [bar] and parameter k_p is the pipe resistance coefficient [m³ d⁻¹ bar⁻¹], which was adjusted to 1.6 to achieve a reasonable overpressure (around 10 bar) in the headspace.

Reactor configuration – simulation set-up

The model was validated on the experimental data described by Krayzelova et al. (2014), comparing the behavior of two UASB reactors, a strictly anaerobic UASB and a microaerobic one (termed UMSB). Both reactors consisted of a liquid phase (2.7 L) and a gas phase (0.3 L) and were assumed to

be completely mixed. The reactor cross section area, A_r , was 0.02 m². The operation temperature was kept constant at 35°C. The liquid phase recirculation rate was 8 L h⁻¹. The amount of VSS in biomass equaled to 50 kg VSS m⁻³, corresponding to 70 kg COD m⁻³, using a typical conversion factor of 1.4 g COD g⁻¹ VSS. The reactors were fed with synthetic wastewater mimicking brewery wastewater at a flow rate of 0.0082 m³ d⁻¹, containing average COD and sulfate concentrations of 2.32 g COD L⁻¹ and 0.072 g SO₄²⁻ L⁻¹, respectively (a concentrated synthetic wastewater was prepared and then diluted). The reactors were operated for 373 days. The influent flow of air (Q_{air}^{in}) was turned on the 74th day of experiment for UMSB reactor (0.001 m³ d⁻¹).

The reactor model was implemented in Aquasim 2.0 (Reichert, 1998), based on the Aquasim implementation of ADM1 for UASB reactors of Batstone et al. (2004). The total liquid phase of the reactor, consisting of the bulk liquid volume and the biofilm matrix, was assumed constant, so a growing biofilm implied a decreasing bulk liquid volume. The biofilm matrix corresponded to experimental data and consisted of 1 L of granular sludge with uniform granules with a diameter of 7 mm. The amount of granules ($n_{sp} \approx 5$ 600) was calculated based on the predefined total granule volume, $V_{tot} = 1L$. Since granular sludge is usually quite dense and contains extremely small pores it was assumed that granule structure had no diffusive solid transport (rigid biofilm matrix) and had no suspended solids within the pores (pore volume contains only liquid phase). External mass transfer limitation was neglected. The biomass porosity, ε_W , was assumed to be constant at 0.70 and was calculated based on the initial biomass volume fraction ($\varepsilon_{ini} = 0.3$).

The influent concentrations fed to the model (*Feed*ⁱⁿ) were set up dynamically according to the experimentally measured influent flow rate, which was fluctuating with the influent pump operation, and taking into account the fixed component fractions in the influent: $X_{li}^{in} = 4.94\%$; $X_{pr}^{in} = 4.94\%$; $X_{ch}^{in} = 55.85\%$; $S_{Tva}^{in} = 0.026\%$; $S_{Tbu}^{in} = 1.97\%$; $S_{Tpro}^{in} = 2.44\%$; $S_{Tac}^{in} = 2.70\%$; $S_{TIC}^{in} = 25.20\%$; $S_{TIN}^{in} = 1.92\%$; $S_{SO_4^{2-}}^{in} = 0.028\%$. The concentration of cations/anions ($S_{Z^+}^{in}$) was calculated based on the charge balance. The influent hydrogen ion ($S_{H^+}^{in}$) reflected an influent pH of 7. The influent flow (Q^{in}) corresponded to (dynamic) experimental data. For the simulation of microaerobic conditions (UMSB reactor), a small amount of air was supplied to the reactor in ADM1-S/O while for the simulation of anaerobic conditions (UASB reactor) aeration was completely turned off.

The initial conditions were defined for biofilm matrix as well as for the bulk liquid volume. All 12 bacterial species were considered to have equal initial concentrations of 5.55 kg COD m⁻³ in the biofilm matrix; the initial biofilm thickness was set at 0.03 mm. The initial bulk liquid concentrations were set at 2.15 kg COD m⁻³ for carbohydrates (X_ch); 0.19 kg COD m⁻³ for proteins (X_pr), and 0.19 kg COD m⁻³ for lipids (X_li); 10⁻⁶ kg COD m⁻³ for VFAs; 10⁻⁷ kmole m⁻³ for hydrogen ion (pH = 7) according to the wastewater composition, and 10⁻⁵ kg COD m⁻³ for all bacterial species.

The granular sludge from UASB and UMSB reactor (Krayzelova et al. 2014) was analysed by PCR and DGGE. SOBs were found in the UMSB reactor proving the biologically mediated oxidation of sulfide to elemental sulfur (unpublished results). Nevertheless, the presence of SOBs was the motivation to model the biological sulfide oxidation in the present manuscript.

The model was first validated upon the dynamic experimental data of Krayzelova et al. (2014), characterized by a dynamic influent flow, Q^{in} , an influent S:COD ratio (mole SO₄²⁻-S : kg COD) of approximately 0.0003 and an influent molar O₂:S ratio (for UMSB reactor) of 0.5. The model was then

applied for steady state scenario analyses concerning the effect of the influent S:COD ratio and of the aeration intensity (O_2 :S ratio), for a constant influent flow rate ($Q^{in} = 0.0082 \text{ m}^3 \text{ d}^{-1}$) and constant influent COD concentration (*Feed*ⁱⁿ = 2.32 kg m⁻³). In a first series of simulations, the influent S:COD ratio was varied by varying the influent sulfate concentration (5-15.5 mole SO₄²⁻-S m⁻³) while the oxygen concentration was kept fixed at 0.03 mole O₂ m⁻³. In a second series of simulations, the influent O₂:S ratio was varied by varying the dissolved oxygen concentration (0.03-2.32 mole O₂ m⁻³) for a constant influent S concentration of 1.16 mole SO₄²⁻S m⁻³ (corresponding with a S:COD ratio of 0.0003).

3.4.3. Results and discussion

Validation of ADM1-S/O

The simulated biogas flow rate and the H_2S concentration in the biogas were compared to the experimental data for both the UASB reactor (without microaeration) and the UMSB reactor (with microaeration) (**Figure 3.4.1**). An average biogas flow rate of 8.5 L d⁻¹ and 9.6 L d⁻¹ was measured experimentally for the UASB reactor and for the UMSB reactor, respectively. The simulated biogas flow rate showed a good fit (**Figure 3.4.1A**), 8.5 L d⁻¹ and 9.5 L d⁻¹ on average for the UASB and the UMSB reactor, respectively, corresponding with a Root-Mean-Square Error of 1.371 for UASB and 0.993 for UMSB. The experimentally measured methane content in the biogas reached 77% and 75% in UASB and UMSB reactor, respectively, while in the simulation, the fraction of methane reached 77% in both reactors (see **Figure S3.4.1** in appendix, section S3.4.5). The pH of both reactors was on average 7.3 and 7.1 in the experiments and in the simulations, respectively. The average COD removal measured experimentally was on average 87% in the UASB and 89% in the UMSB reactor, while the simulated value was 90% for both. Some fluctuations were observed during the experiments (Krayzelova et al., 2014a), caused most probably by the irregular pumping of feed and by experimental measurement inaccuracies, which also caused dynamics in the simulated behavior.



Figure 3.4.1: Model validation concerning the biogas flow (A) and the concentration of hydrogen sulfide in biogas (B) over time in the UASB and UMSB reactors. Markers denote experimental data; full lines denote simulation results.

The experimentally measured average H_2S concentration in the biogas of the UASB reactor was 8.9 g H_2S m⁻³, the simulated one amounted to 8.3 g m⁻³. With microaeration (UMSB reactor), the average H_2S concentration in the biogas was brought down to 2.2 g H_2S m⁻³ measured experimentally and 1.3

g m⁻³ simulated, corresponding to the removal efficiency of 75% for experiments and 84% for simulation. Overall, a very good match was obtained between experimental and simulated H_2S concentration in the biogas, even though some of the fast changes in H_2S concentrations were not fully captured because of the resolution of the available influent data (**Figure 3.4.1B**).

Table 3.4.3 summarizes the sulfur balance over the experimental data and the simulation results. The simulation showed a good fit for the decrease of H_2S from biogas in UASB reactor compare to the UMSB reactor, 73.5% (34% of H_2S in UASB and 9% of H_2S in UMSB) for experiments and 73.9% for simulation (23% of H_2S in UASB and 6% of H_2S in UMSB.

experimental data was not closed						
_	UASB		UM	SB		
	experiment	simulation	experiment	simulation		
Sulfate in the influent [%]	100	100	100	100		
Sulfate in the effluent [%]	0	2	0	3		
Sulfide in the effluent [%]	49	75	41	20		
Hydrogen sulfide in biogas [%]	34	23	9	6		
Elemental sulfur [%]	7 ^a	0	43 ^a	71		

Table 3.4.3.: Sulfur balance based on experimental data (Krayzelova et al., 2014a) and simulation results for UASB and UMSB reactors between days 74 – 200. Note that the sulfur balance based on experimental data was not closed

^a The elemental sulfur in experiments is taken as the sum of elemental sulfur found in the gas and liquid phase.

n.c.

0

8

-1

n.c.

0

8

2

n.c. not considered in the model

Sulfur in granular sludge [%]

Gap [%S_{in}-%S_{out}]

While elemental sulfur in experimental UMSB accounted only for 43%, in the modeled UMSB it reached 71%. The sulfur uptake (accumulation) in the granules was not modelled. The sulfur composition in the granular sludge after a year of operation was the same (8%) for the UASB and UMSB reactors (Krayzelova et al. (2014a). No significant increase of the sulfur concentration in the granular sludge was observed during the operation in any of the reactors. In other words, elemental sulfur formed in UMSB reactor due to microaeration did not accumulate in granular sludge.

The simulated decrease in dissolved sulfide concentration in the effluent, i.e. 73% (calculated as the decrease of 75% dissolved sulfide in UMSB reactor compare to 20% dissolved sulfide in UASB reactor) was much higher than the experimentally observed one, only 15% (calculated as the decrease of 49% of dissolved sulfide in the model of UMSB reactor compare to 41% of dissolved sulfide in the model of UASB reactor). The most probable reason for the higher sulfide removal in the simulations is the lack of competing reactions for O₂. In the model, oxygen was used only for sulfide removal while in reality, oxygen is consumed by other processes such as the oxidation of organic matter (Díaz et al., 2011b; Jenicek et al., 2014) thereby decreasing the sulfide removal efficiency in UMSB. A second explanation for the poor agreement between the experimental and predicted values could be the uncertainty regarding the exact location of the sulfide oxidation process. In this study, sulfide oxidation was assumed to take place in the liquid phase, while no reactions took place in the gas phase. Some authors observed oxidation of both gaseous and liquid sulfide (Díaz et al., 2011a;

Jenicek et al., 2014; Krayzelova et al., 2014a; van der Zee et al., 2007; Zhou et al., 2007), others did not observe the oxidation of dissolved sulfide and assumed the oxidation occurs only in gas phase (Díaz & Fdz-Polanco, 2012; Díaz et al., 2010) or at the gas-liquid interphase (Ramos et al., 2014b). If hydrogen sulfide removal would occur only in the gas phase, its simulated removal efficiency could be lower and thus closer to the experimental values. However, if the reactions in both the liquid and the gas phase would be assumed in the model, the hydrogen sulfide removal as well as the difference with the experimental results would be even higher.

A third possible explanation for the higher sulfide removal efficiencies in the model compared to the experimental results could be the reverse reduction of elemental sulfur back to sulfide which was not modeled. Since oxygen is limited in the UMSB reactor, the conditions are still "anaerobic" and SRB might be able to take the elemental sulfur and reduced it to sulfide again.

Microbiological composition of anaerobic granular sludge

The composition of granular sludge in terms of active biomass fractions for both the UASB and UMSB reactors is given in Table 3.4.4. Sulfate reduction was mainly performed by hydrogenotrophic sulfate reducing bacteria, which were able to compete with hydrogenotrophic methanogens. Sulfate reducing bacteria growing on butyrate, propionate and acetate (X_bSRB, X_pSRB and X_aSRB, respectively) only accounted for trace quantities of the active biomass. Although the influent sulfate was almost completely converted, its amount was not high enough for SRB to out-compete other butyrate, propionate and acetate degraders. According to the literature, SRB will out-compete methanogens for the substrates acetate (Chou et al., 2008; Omil et al., 1998), butyrate and propionate (Omil et al., 1996) when excess sulfate is present and/or at COD/SO₄²⁻ ratios lower than 1.3 g COD/g SO₄²⁻. Zhou et al. (2014) observed that SRB out-competed methanogens for ethanol even at COD/SO₄²⁻ ratios around 4.0. In this study, the influent COD/SO₄²⁻ ratio amounted to 32.2 g COD/g SO₄²⁻ (\approx 1 mole SO₄²⁻S/kg COD). But even though SRB growing on butyrate, acetate and propionate do not play a significant role in this study (the growth and decay rates of bSRB, aSRB and pSRB could be turned off in the model without visible effect on the simulations results – not shown) it was preferred to keep the model sufficiently general for application to other influent conditions. In the study of Carrera-Chapela et al. (2016) only VFA was used as electron donor (the ratio between 53 and 104 g COD/g SO₄²⁻). Barrera et al. (2015) used propionic acid, acetic acid and hydrogen as the electron donors (with 10-20 g COD/g SO₄²⁻ ratio) for the sulfate reduction, leaving only butyric acid behind.

When applying microaeration, SOB (X_SOB) accounted for 0.05% of the active biomass. Overall, the biomass fractions of the major biomass populations were hardly affected by the application of microaeration.

Active biomass	Fraction	UASB reactor	UMSB reactor
X_su	[%]	30.28	31.20
X_aa	[%]	5.47	5.45
X_fa	[%]	0.29	0.29
X_c4	[%]	6.48	6.27
X_bSRB	[%]	0.00	0.00
X_pro	[%]	7.13	6.88
X_pSRB	[%]	0.00	0.00
X_ac	[%]	21.79	21.03
X_aSRB	[%]	0.00	0.00
X_h2	[%]	9.90	9.72
X_hSRB	[%]	18.65	19.11
X_SOB	[%]	0.00	0.05

Table 3.4.4: Simulated active biomass composition of the granular sludge of UASB and UMSB reactoron day 200

Figure 3.4.2 shows bacterial distribution profiles in the granules of the UMSB reactor at day 200. The sugar degraders (**Figure 3.4.2A**) had the highest concentration on the periphery of granular sludge followed by acetotrophic (X_ac) and hydrogenotrophic (X_h2) methanogens (**Figure 3.4.2B**). The high amount of carbohydrates in the (brewery) wastewater (brewery wastewater), containing supported these bacterial groups and resulted in a high methane concentration in the produced biogas. SRB inside the granular sludge were mostly hydrogen-consuming (**Figure 3.4.2C**). Since hydrogen sulfide and oxygen had relatively high diffusion coefficients compared to other components of the model ($1.38 \cdot 10^{-3}$ (Cunningham et al., 2011) and $2.09 \cdot 10^{-4}$ (Lide, 2003), respectively), SOB grew inside of the granular sludge (**Figure 3.4.2D**). Nevertheless, this is not consistent with the findings of Shen and Guiot (1996), even though they used comparable diffusion coefficients of methane and oxygen (i.e. $1.29 \cdot 10^{-4}$ and $2.09 \cdot 10^{-4}$ m² d⁻¹, respectively (Lide, 2003). They simulated the oxygen penetration inside the multilayer structure of anaerobic granular sludge with aerobic and facultative bacteria (such as X_SOB) predominant at the periphery and oxygen-sensitive methanogens located in the deeper layers, protected from the air.



Figure 3.4.2: Bacterial distribution profile along the granule in UMSB reactor on day 200. A – Sugar, amino acids, and long chain fatty acids degraders (X_su, X_aa, X_fa); B – Methanogens (X_ac, X_h2, X_pro, X_c4); C – Sulfate reducing bacteria (X_hSRB, X_bSRB, X_pSRB, X_aSRB); D – Sulfide oxidizing bacteria (X_SOB). Note the different scales of the Y-axes.

Scenario analysis

The effect of the influent S:COD ratio

The effect of influent S:COD ratio on the percentage of H_2S in the biogas of the UASB and UMSB reactors is shown in **Figure 3.4.3**. For a UASB reactor, the fraction of H_2S in the biogas increased almost linearly, from 0.02% to 0.26%, with an increasing influent S:COD ratio of 0. 2 to 2.5. The H_2S concentration reached a constant value of 0.32% for an influent S:COD ratio of 0.003 or higher. This plateau corresponded to hydrogen depletion in reactor. Indeed, only hydrogenotrophic SRB (X_hSRB) played an important role in H_2S formation; sulfate reducing bacteria growing on butyrate, propionate and acetate were not able to outcompete methanogens growing on these substrates, not even at the highest simulated S:COD ratio of 6.7. When applying microaeration (UMSB reactor) the H_2S formation in biogas stabilized at lower values and for lower S:COD concentrations: the maximum H_2S fraction in biogas was 0.14% and 0.002%, applying an influent O_2 :S ratio of 0.25 and 0.5, respectively, for an influent S:COD ratio 0.003-0.007.



Figure 3.4.3: Influence of influent S:COD ratio (for a fixed COD concentration of 2.32 kg m⁻³) on the percentage of H_2S in biogas without (UASB) and with (UMSB) microaeration, in the latter case applying and influent O_2 :S ratio of 0.25 or 0.5.

Comparing the performance of the UASB and UMSB reactors, the addition of oxygen resulted in a decrease of the maximum H_2S concentration in biogas by 56% for an influent O_2 :S ratio of 0.25 and a decrease of 99% for an influent O_2 :S ratio of 0.5 at an influent S:COD ratio of 3.3 and higher. Díaz et al. (2010) reached 99% removal of H_2S for an influent S:COD ratio of 0.001 applying an influent O_2 :S ratio 1.27. This agreed very well with our simulation results, which showed a 97% removal efficiency of H_2S for an influent S:COD ratio of 0.001 and an O_2 :S ratio of 1.25. The simulated amount of oxygen in the biogas was 0.9%, which also matched the experimentally measured value (1%) of Diaz et al. (2010).

The effect of the influent O₂:S ratio

The effect of aeration intensity in terms of the influent O_2 :S ratio on the biogas quality and on the H_2 S removal efficiency is displayed in **Figure 3.4.4.** For an influent O_2 :S ratio of 0.5 the H_2 S removal efficiency amounted to 91.2% with 0.24% of O_2 in biogas. When increasing the influent O_2 :S ratio, the H_2 S removal efficiency increased, up to 96.5% (corresponding with H_2 S concentration of 0.4 g m⁻³) for an influent O_2 :S ratio of 3. However, the increasing H_2 S removal efficiency could only be realized at the expense of a linearly increasing leftover of oxygen in the biogas, up to 4% for an influent O_2 :S ratio of 3. An oxygen content below 1% is required for biogas application in fuel cells and below 3% (after carbon dioxide removal and upgrading) for application as vehicle fuel or injection of biogas into the natural gas grid (Deublein and Steinhauser, 2011). Since oxygen can create a dangerous and explosive gas mixture with methane (Appels et al., 2008; Wase & Forster, 1984), increasing the amount of oxygen could endanger the whole process. However, in some studies O_2 fractions in biogas of 4% were reported (Díaz & Fdz-Polanco, 2012; Rodriguez et al., 2012).



Figure 3.4.4: Influence of influent O_2 :S ratio (UMSB reactor, for a fixed sulfate concentration of 1.16 mole $SO_4^{2^2}$ -S m⁻³) on (A) the percentage of H_2S and O_2 in biogas and on (B) the H_2S removal efficiency.

Comparing the simulation results with experimental data, at an influent O_2 :S ratio of 0.5, 91.2% H₂S removal was simulated with 0.24% O_2 left in biogas, while in the experiments, only 73% H₂S from biogas was removed with less than 0.02% O_2 in biogas (Krayzelova et al., 2014a). The higher H₂S removal efficiency simulated than for the experimental data can be explained by the microaeration model considering oxygen to be used for the oxidation of sulfide to elemental sulfur, while in reality other components such as organic matter may be oxidized as well. Comparing simulation with experiments where oxygen was blown into the gas phase (i.e. oxidation of organic matter can be omitted), a very good match was obtained. At an O_2 :S ratio of 1.25, 96.2% of H₂S was removed with 1.34% O_2 left in biogas. Díaz et al. (2010) injected the air into the headspace at the O_2 :S ratio 1.23 (omitting the oxidation of organic matter) and removed 97.5% H₂S from biogas with 1.5% O_2 in biogas. It is clear that aerobic carbon oxidation needs to be considered in the model when oxygen is blown through the liquid phase.

3.4.4. Conclusions

An anaerobic digestion model with sulfur and oxygen (ADM1-S/O) was set up to describe and control sulfate reduction and sulfide oxidation in anaerobic and microaerobic environments:

- The model validation showed a good fit in terms of H₂S emissions and biogas flow. The results of sulfur balance showed the limitations of the present model as it predicted higher H₂S removal (lower H₂S concentrations in the effluent) than observed experimentally. In case microaeration is realized by blowing oxygen into the liquid phase, aerobic carbon oxidation and re-reduction of elemental sulfur back to sulfide need to be considered in the model.
- The simulated composition of active biomass in the microaerobic reactor was not significantly affected by microaeration. Sulfur oxidizing bacteria only made up a small fraction of the active biomass (0.05% of active biomass for an influent COD:SO₄²⁻ ratio of 32.3 g g⁻¹).
- Hydrogen sulfide in biogas proportionally increased with increasing influent S:COD ratio. Maximum H₂S concentrations of 0.32 and 0.14% were observed in the biogas from UASB and UMSB reactor, respectively, for S:COD ratio of 3.3 g g⁻¹ and O₂:S ratio of 0.25.
- The highest H₂S removal efficiency from biogas was obtained for a O₂:S ratio 0.5 kmole O₂ kmole SO₄²⁻-S. Increasing the O₂:S ratio to over 0.5 did not significantly improve H₂S removal.

3.5. SIMPLE BIOGAS DESULFURIZATION BY MICROAERATION – A FULL SCALE EXPERIENCE (Article 6)

Jenicek, P., Horejs, J., Pokorna-Krayzelova, L., Bindzar, J., Bartacek, J.: "Simple biogas desulfurization by microaeration - full scale experience". Accepted in Anaerobe in January 5, 2017

Abstract

Hydrogen sulfide in biogas is common problem during anaerobic treatment of wastewater with high sulfate concentration (breweries, distilleries, etc.) and needs to be removed before biogas utilization. Physico-chemical desulfurization methods are energetically demanding and expensive compare to biochemical methods. Microaeration, i.e. dosing of small amount of air, is suitable and cost effective biochemical method of sulfide oxidation to elemental sulfur. It has been widely used in biogas plants, but its application in anaerobic reactors for wastewater treatment has been rarely studied or tested. The lack of full-scale experience with microaeration in wastewater treatment plants has been overcome by evaluating the results of seven microaerobic digesters in central Europe. The desulfurization efficiency has been more than 90% in most of the cases. Moreover, microaeration improved the degradability of COD and volatile suspended solids.

Graphical abstract



Keywords

Anaerobic digestion, biogas, hydrogen sulfide removal, microaeration, sludge stabilization

3.5.1. Introduction

Anaerobic digestion of sludge is the key process for municipal wastewater treatment plants aiming to exploit the energy potential of organic pollution of wastewater through biogas (Jenicek et al., 2013b; Spinosa et al., 2011). The suitable quality of biogas is the limiting condition for effective power production and also for all other types of biogas utilization. The elevated concentration of hydrogen sulfide is the most frequent problem.

Many physico-chemical methods of biogas desulfurization are available, such as alkali washout, sorption, precipitation, stripping, etc. Operation at high temperature and pressure, as well as the need for additional equipment and chemicals, make physico-chemical methods energetically demanding and expensive (Appels et al., 2008; Muñoz et al., 2015). In contrast, biological methods based on the biochemical oxidation of sulfide to sulfate, thiosulfate and elemental sulfur involve lower operational costs with lower or no need for chemical addition (Buisman et al., 1989; Syed et al., 2006). Among biological methods, microaeration is the simplest one. It can be performed inside the anaerobic reactor without requirements to build a new separate desulfurization unit.

Microaeration has recently proved to be highly efficient biological method of sulfide removal regarding anaerobic digestion of wastewater and sludge (Díaz et al., 2010; Jenicek et al., 2008; Khanal & Huang, 2003b; Krayzelova et al., 2014a; van der Zee et al., 2007; Zitomer & Shrout, 2000). This process is based on the controlled dosing of a limited amount of air or oxygen into the digester to ensure the oxidation of sulfide into elemental sulfur. Precipitated elemental sulfur is removed from the digester together with the digested sludge. The presence of oxygen does not negatively influence the activity of anaerobic bacteria because it is consumed quickly and almost completely (Jenicek et al., 2011a).

A variable air/oxygen dosing rate is necessary as the consequence of the feed composition and loading rate variations resulting in the varying production of sulfide. Besides, residual oxygen in the biogas must meet the requirements of the biogas utilization technology that will be employed afterwards. Optimal process control is the key parameter to the successful microaeration in such cases. When the digestion process is stable as regards biogas production and H₂S concentration, the air/oxygen flow rate can be constant for certain period. However, when it is necessary to cope with the changes of H₂S concentration and biogas flow, oxygen supply must be controlled (Krayzelova et al., 2015).

Until now, the lack of full-scale experience in wastewater treatment plant digesters was reported as a disadvantage of the microaerobic desulfurization (Krayzelova et al., 2015). Present work describes the experiences obtained during long-term operation (in the period of years 2003 to 2015) of 7 microaerobic digesters in central Europe in terms of H_2S removal and changes in the quality of sludge and sludge liquor.

3.5.2. Material and Methods

Microaeration has been applied on seven municipal wastewater treatment plants (WWTPs) in central Europe. Their characterization is described in **Table 3.5.1**.

Digester	Population equivalent	Anaerobic reactor volume	Type of sludge	The start of microaeration
_	[-]	[m³]	[-]	[year]
Р	19 000	1 600	primary + waste activated	2012
Μ	29 000	2 600	primary + waste activated	2012
К	19 000	1 900	primary + waste activated	2010
Ko	35 000	3 200	primary + waste activated	2010
L	27 000	3 000	primary + waste activated	2005
В	350 000	30 000	primary + waste activated	2005
U	36 000	2 100	primary + waste activated	2003
Р	19 000	1 600	primary + waste activated	2012
М	29 000	2 600	primary + waste activated	2012
К	19 000	1 900	primary + waste activated	2010

Table 3.5.1: Characterization of selected municipal WWTP

In each of them a desulfurization unit (**Figure 3.5.1**) has been built to blow air inside of the digester. The desulfurization unit consisted of the source of pressurized air (such as air compressor), and the control unit which was able to monitor and control the air flow. This simple technology does not need changes in the digester construction and can be implemented without interruption of the digestion process. In all evaluated digesters, air has been dosed into the recirculation stream (**Figure 3.5.2**).



Figure 3.5.1: Technological scheme of full-scale desulfurization unit (designed by K&K Klatovy)



Figure 3.5.2: Air dosing point into the sludge recirculation pipe

3.5.3. Results and Discussion

Start-up

Start-up period of microaerobic digestion took usually 3 to 12 weeks as shown in **Table 3.5.2**. Nevertheless, our experiences from laboratory and pilot experiments indicated, that the start-up period can be much shorter if necessary (data not shown). Following aims must be fulfilled during start-up period: (1) the optimization of air dose, and (2) the adaptation and growth of sulfide oxidizing bacteria. **Figure 3.5.3** shows the start-up of digester U. Microaeration was turned on in the week 29 and the start-up took approx. 12 weeks.

Digester	Biogas production	Air dose	Time of start-up*	H ₂ S without microaeration**	H ₂ S with microaeration**	H₂S removal efficiency
	[m³d⁻¹]	[m³h⁻¹]	[weeks]	[mg m ⁻³]	[mg m ⁻³]	[%]
Р	750	1.00	7	3710	449	87.9
Μ	950	0.34	8	771	31	96.0
К	520	0.28	4	890	48	94.6
Ko	1000	1.00	3	3823	198	94.8
L	900	1.20	10	4383	21	99.5
В	9600	6.00	8	2633	690	73.8
U	830	1.20	12	7580	72	99.1

Table 3.5.2: Start-up results of microaerobic desulfurization in selected WWTP

*the time of the air dose optimization, **annual average



Figure 3.5.3: Relation between hydrogen sulfide concentration of biogas and air flow rate (digester U)

As expected, the dose of air depends strongly on H_2S concentrations in both gaseous and liquid phase. In evaluated digesters the air doses were in the range of hundreds and thousands of liters per hour in general, no cleaning or pretreatment of air was necessary.

At higher air doses nitrogen from air will dilute biogas and decrease the methane concentration. In microaerobic digesters monitored in present work the volumetric dose of air ranged between 1-3% of biogas production. In such cases the effect of the remaining N_2 on the biogas composition was negligible – maximum decrease of methane concentration did not exceed 2%. Therefore it was not necessary to consider replacement of air by expensive pure oxygen (Díaz et al., 2010).

Desulfurization efficiency

The achieved desulfurization efficiency was between 74% and 99% (**Table 3.5.2**). Requested reduction of H_2S concentration was reached in all cases. In addition long-term operation brought usually further decrease of the H_2S concentration.

The typical course of H_2S concentration in biogas when microaeration technology is applied (for digester K) illustrates **Figure 3.5.4**. The desulfurization efficiency is effective even with strong fluctuation of initial sulfide and/or H_2S concentration which is a common problem at wastewater treatment plants. From operational point of view high efficiency and stability of desulfurization is important. Hydrogen sulfide compromises the functions of cogenerations unit, causes the corrosion of concrete and steel, and is toxic to humans.





Quality change of digested sludge and sludge liquor

It was indicated that microaeration is able to improve not only the biogas quality but the quality of digested sludge and sludge liquor as well (Jenicek et al., 2014). The most important changes were found in the soluble COD concentration of sludge liquor. The substantial significant decrease of the soluble COD was probably observed because of the combination of anaerobic and aerobic conditions which allows combining the degradation activity of anaerobic and aerobic microorganisms. The decrease of soluble COD was observed in all cases (**Table 3.5.3**) and varied between 18 to 33%. The combination of aerobic and anaerobic conditions can improve the hydrolysis; however, Johansen and Bakke (2006) observed that the extra hydrolyzed products were oxidized to carbon dioxide.

	Soluble COD (average and standard deviation)							
Digester	Without microaeration	With microaeration	Decrease					
	$[mg L^{-1}]$	[mg L ⁻¹]	[%]					
К	373 ± 27	307 ± 32	17.7					
L	430 ± 37	337 ± 36	21.6					
Μ	340 ± 24	232 ± 20	31.8					
U	778 ± 43	522 ± 39	32.9					

Table 3.5.3: Change of average soluble COD concentration in sludge liquor due to microaeration

Concerning N_{ammon} concentration in the sludge liquor the results are rather ambiguous. It was observed both, either slight decrease in concentration or statistically unchanged concentration (**Table 3.5.4**). The influence of microaeration is not very strong in this case and it is combined with other factors such as pH changes, increased N_{ammon} release if degradation of organic matter is deeper, and the different specific gas loading rate of digester surface. Relatively low N_{ammon}

concentration caused by the low extent of sludge thickening before digestion could also affect the results. Celis (2012) reported significant decrease of N_{ammon} concentration (about 10 %) at values between 2000-2700 mg L⁻¹.

	N _{ammon}							
Digester	Without	With	Change					
Digester	microaeration	microaeration	Change					
	[mg L ⁻¹]	$[mg L^{-1}]$	[%]					
К	685 ± 28	690 ± 35	+ 0.7					
L	740 ± 57	718 ± 42	- 3.0					
Μ	521 ± 34	487 ± 41	- 6.5					
U	856 ± 32	784 ± 26	- 8.4					

 $\label{eq:stable} \textbf{Table 3.5.4:} Change of average N_{ammon} \ concentration \ in \ sludge \ liquor \ due \ to \ microaeration$

Another important finding is better volatile suspended solids (VSS) degradation during microaeration resulting in the decreasing ratio of volatile suspended solids to total suspended solids (VSS/TSS) in digested sludge (**Table 3.5.5**). Although this parameter is very good and sensitive indicator of digestion process, due to seasonal changes of the raw sludge quality, however, the fluctuation of this parameter occurs in the range of 2-5% in monitored digesters. The decrease is then strictly speaking statistically not significant. Given this context, we consider these changes in annual averages VSS/TSS as remarkable. Deeper degradation of sludge can be related to a potential inhibition of methanogens by sulfide. The oxidation of sulfide should eliminate such inhibition. This hypothesis could be supported by the fact that the highest decrease of VSS/TSS ratio was achieved at WWTP with the highest H₂S concentration (digester U).

Table 3.5.5: Change of average VSS/TSS ratio of a	digested sludge
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	VSS/TSS							
Digester	Without microaeration	With microaeration	Decrease					
	[mg L ⁻¹]	[mg L ⁻¹]	[%]					
К	0.629	0.612	2.7					
L	0.566	0.546	3.5					
Μ	0.515	0.507	1.6					
U	0.658	0.597	9.3					

Even at the lowest decrease of VSS/TSS ratio presented in digester M it was possible to find the significant increase of biogas production. Because of the substantial yearly fluctuation of sludge quality and biogas production the respective monthly average production is compared in **Figure 3.5.5**. The average increase of biogas production was 17%. On the top of it the average increase of methane production was 16 %.



Figure 3.5.5: Comparison of the biogas production before (2011) and after (2012) the application of microaeration technology in digester M from June to September.

Operational remarks

According to some authors, microaeration takes place almost solely in reactor headspace (Díaz et al., 2011b; Kobayashi et al., 2012; Ramos et al., 2014c; Rodriguez et al., 2012). The yellow-whitish deposition of elemental sulfur on the walls and pipes can clog the system resulting in headspace overpressure and biogas leakage. In the digesters monitored and described in present work no problems with clogging of the gas pipes were observed (Jenicek & Horejs, 2013). The reason is probably strict dosing into the liquid phase where most of the oxygen is consumed and sufficient digester headspace where the rest of oxygen is consumed.

The air dosing point is often discussed topic of technological aspects of microaeration (Krayzelova et al., 2015). In present paper, all digesters applied microaeration with air dosed in the recirculation stream. In such case a bigger amount of air is required as a certain part of oxygen is consumed by other oxidation processes in addition to H2S removal. While applying the air into the gas phase of the digester, the advantage is the lower amount of air required, because of the lower extent of dissolved sulfide oxidation. On the other hand, problems with the precipitation of the elemental sulfur in gas pipes and other improper places may occur frequently (Ramos et al., 2012).

In general, oxygen or air is not allowed to mix with the digester biogas because of the explosion risks of methane/oxygen mixture. However, the inflammable range is typically 5-15% by volume of methane with air (Appels et al., 2008; Wase & Forster, 1984). Therefore, the leakage of biogas in air should be considered as the higher threat compare to the mixing of a small amount of air/oxygen with biogas. During common microaerobic desulfurization the amount of oxygen in biogas can never reach these values. In the evaluated digesters, the O_2 maximum concentration in biogas was 0.3 %, however the amount was often below detection limit.

Lastly, it is necessary to say that present work does not intend to argue the application of the microaerobic process every time in each anaerobic process. Only well substantiated benefit and
improvement of process such as the desulfurization of biogas, the suppression of sulfide toxicity, the improved removal of specific toxicants, the enhanced quality of digested sludge or another should be sufficient reason for the application of microaerobic conditions at anaerobic digestion of sludge.

3.5.4. Conclusions

The results of long-term full-scale operation of sludge digesters in microaerobic conditions confirm that the biogas desulfurization by microaeration become mature technology and the lack of operating experience has been overcome:

- Main benefits from microaerobic sulfide removal are the ability to remove sulfide inside the anaerobic reactor without requirements to build a new separate desulfurization unit and without additional chemicals.
- The application of the microaerobic conditions is a simple, highly efficient and stable method for hydrogen sulfide removal from the biogas.
- The start-up of the microaerobic biogas desulfurization takes a few weeks due to the necessary air dose optimization and sulfide oxidizing bacteria adaptation.
- The hydrogen sulfide removal efficiency of about 99% can be achieved at a high initial H2S concentration (above 4,000 mg m⁻³). The achievement of hydrogen sulfide concentration in tens of mg m-3 is realistic.
- The decrease of the methane content in the biogas due to the presence of surplus nitrogen from the dosed air is lower than expected (maximum 2%). Therefore it is not necessary to use pure oxygen instead of air dosing.
- The VSS/TSS ratio of the digested sludge decreased in most of the cases with the microaerobic conditions due to the better efficiency in VSS degradation.
- A decrease of the soluble COD concentration in the sludge liquor was observed in all systems where the microaerobic conditions were applied.

4. MICROAERATION IN BIOMEMBRANE

4.1. Biomembrane I – Lab-scale experiences: The use of a silicone-based biomembrane for microaerobic H₂S removal from biogas (Article 7)

Lucie Pokorna-Krayzelova, Jan Bartacek, Dana Vejmelkova, Ana A. Alvarez, Petra Slukova, Jindrich Prochazka, Eveline I.P. Volcke, Pavel Jenicek. "The use of a silicone-based biomembrane for microaerobic H₂S removal from biogas." *Submitted in Separation and Purification Technology Journal in March 19, 2017*

Abstract

A lab-scale bio-membrane unit was developed to improve H_2S removal from biogas through microaeration. Biomembrane separated biogas from air and consisted of a silicone tube covered by microaerobic biofilm. This setup allowed efficient H_2S removal while minimizing biogas contamination with oxygen and nitrogen. The transport and removal of H_2S , N_2 , O_2 , CH_4 and CO_2 through bare membrane, wet membrane and biomembrane was investigated. Membrane allowed the transfer of gases through it as long as there was enough driving force to induce it. H_2S concentration in biogas decreased much faster with the biomembrane. The permeation of gases though the membranes decreased in order: $H_2S > CO_2 > CH_4 > O_2 > N_2$. H_2S removal efficiency of more than 99% was observed during the continuous experiment. Light yellow deposits on the membrane indicated the possible elemental sulfur formation due to biological oxidation of H_2S . *Thiobacillus thioparus* were identified by FISH and PCR-DGGE.

Keywords

Biomembrane; Hydrogen sulfide removal; Microaeration; Oxygen; Sulfur oxidizing bacteria

4.1.1. Introduction

During anaerobic treatment of wastewater with high sulfate concentration, sulfate reducing bacteria (SRB) degrade sulfur-containing compounds to corrosive and toxic sulfide (Nayono, 2010; Ramos et al., 2013; Russell, 2006). Its elevated concentration in both gaseous and liquid phase can cause many problems regarding health, environmental, operational and maintenance issues. Most of the commercial and well-established sulfide removal technologies used in full-scale applications rely on physico-chemical processes such as adsorption on activated carbon and absorption in alkaline solutions (Díaz et al., 2011b; Ramos et al., 2013). Although these processes are rapid and efficient, their large capital and operational costs (high pressures or temperatures), chemicals requirement and production of secondary pollutants are unfavorable, especially for medium-low productions (Díaz et al., 2011b; Ho et al., 2013; Janssen et al., 1999). Thus, the search for more economical methods has led to biological methods based on the biochemical oxidation of sulfide to elemental sulfur and sulfate by sulfur oxidizing bacteria (SOB) (Janssen et al., 1999). Biological methods impose lower operational costs with lower or no need for chemical addition; they require only oxygen (Buisman et al., 1989; Syed et al., 2006). Among the biological methods, microaeration (controlled dosing of small amount of air/oxygen into anaerobic digesters) has recently gained growing attention for its high efficiency, reliability, simplicity and economic efficiency (Díaz & Fdz-Polanco, 2012; Jenicek et al., 2011a; Krayzelova et al., 2014a; Ramos et al., 2014d).

When H₂S concentration in biogas is too high (several thousands of ppm), microaeration may introduce too high amounts of nitrogen gas and/or may cause undesirable sulfur deposits in biogas pipes. Therefore, we introduced the novel concept of biomembrane, which serves as biofilm support and provides surface for sulfur precipitation thus avoiding its accumulation in the pipeline. Moreover, the separation of biogas and air decreases biogas contamination by nitrogen.

In the present paper, the efficiency of microaeration with silicone-based biomembrane for the removal of H₂S from biogas was tested, in batch as well as continuous system. Transport and removal of H₂S, N₂, O₂, CH₄ and CO₂ through the biomembrane was measured for three different setups: bare membrane, wet membrane and biofilm-covered membrane (biomembrane). The growth of SOB biofilm in biomembrane unit was observed and the presence of SOBs was determined by FISH and PCR-DGGE analyses.

4.1.2. Materials and Methods

Experimental set-up

The biomembrane unit (BMU) shown in **Figure 4.1.1** was designed to simulate the placement of the membrane into the headspace of an anaerobic reactor. The BMU consisted of a plexi-glass reactor and a membrane. The membrane was made from silicone rubber (poly-dimethyl siloxane, PDMS), the inner and outer diameters were 10 mm and 12 mm, respectively, the length was 0.9 m, and surface area was 0.034 m². Air reservoir was added to the air side to increase the air-to-biogas ratio. The volume of biogas and air side was 5.27 and 1.45 L, respectively, including all tubes and connections. The flow of gases was countercurrent. Biogas flowed bottom to top inside the reactor (at a flow rate of 16.2 L h⁻¹) and air flowed top to bottom inside the membrane (at a flow rate of 16.2 L h⁻¹). Due to its relatively constant composition, a synthetic biogas with a volumetric composition of 64.1% of methane, 35.5% of carbon dioxide and approximately 2.5 - 5 mg L⁻¹ (0.2 - 0.4%) of hydrogen sulfide

was used for all the experiments. This biogas was obtained by mixing these three gases from separate tanks to the desired composition using mass flow controllers controlled by a program developed in-house using National Instruments software LabVIEW 2012 running on Compact RIO system (National Instruments, US).



Figure 4.1.1: The scheme of BMU. 1 - biomembrane, 2 - reactor, 3 - air reservoir, 4 7 - pumps, S1 - H_2S sensors, S2 - gases sampling points, A-D - microbiological sampling points.

Batch experiments

Three experimental setups were studied in the BMU: Setup I – bare membrane, Setup II – wet membrane, and Setup III – biofilm membrane (biomembrane). During Setup I, the transfer of gases through the membrane was studied without the interference of liquid or biomass. Both air and biogas were kept completely separated, each running in its own loop. The only possible exchange of components was through the membrane. At the start of each experiment biogas side was flushed with fresh biogas from the mixing system and air side was flushed with the fresh air. After that both sides were closed and biogas and air were continuously recirculated. Setup II and Setup III were similar to Setup I with a third liquid loop added to the system. Tap water (at a flow rate of $1.33 \text{ L} \text{ h}^{-1}$) was used in Setup II to study the effect of liquid surface for gases transfer, while sludge and reject water (at a flow rate of $1.33 \text{ L} \text{ h}^{-1}$) were used in Setup III to allow the SOB biofilm growth on the membrane surface and to study the biochemical sulfide oxidation.

The inoculum was taken from a mesophilic anaerobic stabilization tank of a municipal wastewater treatment plant (WWTP) in Česká Lípa (Czech Republic) while reject water was from the central municipal WWTP in Prague (Czech Republic). The characteristics are summarized in **Table 4.1.1**.

Parameter	Units	Sludge	Reject water	
рН	-	7.4	7.9	
Total COD	g L⁻¹	17.34	2.46	
Dissolved COD	g L⁻¹	0.94	0.89	
TS	g L⁻¹	23.7	n.a.	
TSS	g L⁻¹	22.1	n.a.	
VS	g L⁻¹	13.2	n.a.	
Total sulfur	% dry mass	4.78	n.a.	
Total sulfide	mg L ⁻¹	n.a.	11.2	
Total ammonia	g L ⁻¹	n.a.	1.08	

 Table 4.1.1: Characterization of sludge and reject water

n.a. – not available

Continuous experiment

The continuous experiment was also studied with Setup III. Real biogas (average flow of 3.1 L d⁻¹) from lab-scale UASB reactor treating brewery wastewater (average H₂S concentration of 6.9 mg m⁻³) was connected to the biogas side of BMU. Air side was flushed with the fresh air at the beginning, closed and recirculated (at a flow rate of 16.2 L h⁻¹).

The concentration of H_2S and the composition of gases (CH₄, CO₂, N₂, and O₂) were measured regularly on both sides.

Calculation of permeability

The permeability of each gas through the membrane was determined using a model obtained by performing a molar balance. This balance took into account all the flows in and out of each side. In general, the change in molar mass of each gas during a time step can be expressed as function of the number of (1) moles that are in the side at the beginning of the time step, (2) moles that are released (air side) or incorporated (biogas side) to compensate the pressure, and (3) moles that are transferred through the membrane.

The number of moles of gas present in each side (1) or released/incorporated to compensate the pressure (2) was determined using the ideal gas law equation (Eq. 4.1.1):

$$n_i = \frac{p_i * V}{R * T}$$
 Eq. 4.1.1

Where:

 p_i : Partial pressure of the gas in the side at the beginning of a particular time step (i), [atm].

V: Volume [m³].

- *R*: Ideal gas constant, $[0.00008205 \text{ m}^3 \text{ atm mol}^{-1} \text{ K}^{-1}]$.
- *T*: Ambient temperature, [298.15 K].

The moles transferred through the membrane (3) were calculated using Eq. 4.1.2.

$$n_{m_i} = \frac{P*A}{x} * (p_{F_{i-1}} - p_{P_{i-1}}) * t$$
 Eq. 4.1.2

Where:

P: Permeability of the gas through the membrane, [mol m $m^{-2} s^{-1} Pa^{-1}$].

A: Surface area of the membrane, $[m^2]$.

x: Thickness of the membrane, [m].

 $p_{F_{i-1}}$: Partial pressure of the gas on the feed side in the previous time step (*i*) [Pa]. (For O₂ or N₂ on the air side, for CH₄, CO₂ and H₂S on the biogas side)

 $p_{P_{i-1}}$: Partial pressure of the gas on the permeate side in the previous time step (*i*) [Pa]. (For O₂ or N₂ on the biogas side, for CH₄, CO₂ and H₂S on the air side)

t: Duration of the time step [s].

Based on the experimental data, the permeability of the gas (P) was determined for each setup. The least squares method was used to find the best fit of the model to the experimental data.

Chemical analyses

Hydrogen sulfide concentration in the gas was measured using an online electrochemical gas sensor (Membrapor H₂S sensor type H₂S/S-10000-S). Other gases (CH₄, CO₂, N₂, and O₂) were measured by the GC Shimadzu 2014 equipped with a thermal conductivity detector (CH₄, CO₂, air) and by the GC 8000^{TOP} (Fisons Instruments, USA) equipped with a heat conductivity detector HWD 800 (O₂, N₂, CH₄). Analysis of COD, pH, solids, ammonia and sulfide were done according to the Standard Methods (American Public Health Association, 1997). The sulfur composition of sludge was assessed by Elemental Vario EL III (Elementar Analysensystem GmbH, Germany) and by X-ray fluorescence analysis using the ARL 9400 XP sequential WD-XRF spectrometer (THERMO ARL, Switzerland).

Microbiological analyses

Samples for microbiological analyses were taken from sampling points A-D (**Figure 4.1.1**) in two sets. First set of samples (A1-D1) was taken the second day of the biofilm growth experiment (E1, discussed in section 4.1.3 Results - Biofilm growth), while the second set of samples (A2-D2) was taken at the end of the continuous experiment.

FISH analysis

Samples for fluorescence *in-situ* hybridization were processed according to Nielsen et al. (2009). Samples were fixed for both Gram negative and Gram positive cells except for samples A1 and B1 which were fixed for Gram negative only (low amount of biofilm). Samples fixed according to Gram positive procedure were used together with HGC69A probe; all other probes were applied to Gram negative fixed samples. After hybridization the cells were stained with DAPI staining $(1 \ \mu g \ m l^{-1}, 15 \ m in)$. Then Vectashield was applied and samples were analyzed on epifluorescence microscope Olympus BX51 under 400x magnification. The specific probes used in this study are listed in **Table 4.1.2**. All of them were labeled with Cy3.

Probe	Specificity	FA [%]	Reference
ARC915	Archaea	20	Bryukhanov et al. 2011
ALF1B	Alphaproteoobacteria, some Deltaproteobacteria, Spirochaetes	20	Manz et al. (1992)
ALF968	Alphaproteoobacteria, except of Rickettsiales	20	Greuter et al., 2015
BET42a	Betaproteobacteria	35	Manz et al. (1992)
GAM42a	Gammaproteobacteria	35	Manz et al. (1992)
DELTA495a	Most Deltaproteobacteria and most Gemmatimonadetes	35	Lücker et al. (2007)
DELTA495b	some Deltaproteobacteria	35	Lücker et al. (2007)
DELTA495c	some Deltaproteobacteria	35	Lücker et al. (2007)
SRB385	Desulfovibrionales and other SRB	35	Amann et al. (1990)
CFB560	subgroup of Bacteroidetes	30	O'Sullivan et al. (2002)
HGC	Actinobacteria	25	Roller et al. (1994) Roller et al. (1995)
PAR651	genus Paracoccus	40	Neef et al. (1996)
TMD131	Thiomicrospira denitrificans	35	Fernandez et al. (2008)
TBD1419	Thiobacillus denitrificans	50	Fernandez et al. (2008)
TBD121	Thiobacillus denitrificans, T. thioparus	20	Fernandez et al. (2008)

Table 4.1.2: Specific probes used for the FISH analysis in this study. Probes BET42a, GAM42a, all DELTA495 and HGC were used together with corresponding competitors (Greuter et al., 2015).

FA...Concentration of formamide used in this study

PCR-DGGE analysis

Total DNA was extracted from all samples using PowerSoil[®] DNA Isolation Kit (MOBIO Laboratories, USA). PCR was performed with general bacterial primers 341F-GC and 907R (Schäfer and Muyzer, 2001) from Sigma-Aldrich and FastStart[™] High Fidelity PCR System, dNTPack (Roche). Mastermix per one reaction consisted of: 10x Buffer without MgCl₂ 2.5 μl, MgCl₂ 1.7 μl, dNTP mix 1 μl, PCR water 16.2 μ l, and polymerase 0.4 μ l). Then 0.5 μ l of each (25 μ M) primer, 1 μ l of BSA (Sigma-Aldrich) and 1.2 µl of extracted DNA were added. Cycling conditions were following: pre-denaturation for 5 min at 95 °C, followed by 34 cycles of denaturation for 30 sec at 95 °C, annealing for 40 sec at 57 °C, and extension for 40 sec at 72 °C, and finished with extension for 30 min at 72 °C. DGGE was performed according to Schäfer and Muyzer (2001) using Ingeny PhorU system (Ingeny, Leiden, NL). The denaturing gradient used was 30 - 60 % and the electrophoresis run at 100 V for 16 hours. After that the gel was stained with SYBR Green I staining solution for 1 hour and chosen bands were excised with sterile scalpel. DNA was eluted in 40 µl of PCR H₂O for 24 h. Subsequently 1.2 µl of eluted DNA was used for re-PCR, which was done as described before with only differences in number of cycles (27 instead of 34) and using primer 341F without GC clamp. After PCR products confirmation by agarose electrophoresis they were purified with Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced at the Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine UK, Prague. The obtained sequences were processed with Chromas and classified using RDP database.

4.1.3. Results

The content of nitrogen, oxygen, methane and carbon dioxide in biogas and air side in all three setups is given in **Figure 4.1.2**. In general, the concentrations of the gases followed the same behavior in all setups. Only O_2 did not increase in the biogas side as it was consumed by SOB. Content of N_2 and O_2 in the air side (**Figure 4.1.2A** and **4.1.2B**, resp.) decreased with time, while increasing in the biogas side. Regarding CH₄ and CO₂ (**Figure 4.1.2C** and **4.1.2D**, resp.), their concentration gradually decreased in the biogas side, while increasing in the air side. **Figure 4.1.2E** shows the concentration of hydrogen sulfide in biogas and air side in all three Setups. It decreased in the biogas side. Only in Setup III, the concentration in the air side increased to 0.32 mg L^{-1} and after two hour decreased close to zero. The experimental data for the H₂S concentration in the biogas side was approximated to a first order equation; a linear fit was applied to determine the rate at which it changed with time. The results obtained are -0.4; -0.5; and $-1.2 \text{ mg L}^{-1} h^{-1}$ for Setup I, II, and III, respectively. It is indisputable that the rate at which the concentration decreased in the biogas side was much faster in Setup III. The percentage of removal after 3 h of experiment was 55-70%. The presence of light yellow spots on the surface of the membrane appears to indicate that the H₂S in the biogas was oxidized to elemental sulfur by the biofilm.

Based on the model developed and the data collected, the permeability of each gas through the membrane was determined for the experiments performed in Setups I and Setup II (**Table 4.1.3**). It was not possible to fit the data of Setup III with the molar balance; therefore, permeability values for Setup III were not calculated.

Casa	Permeabili	Permeability [Barrer] ^a						
Case	N ₂	O ₂	CH₄	CO ₂	H₂S			
Setup I	214 ± 11	501 ± 36	801 ± 8	2545 ± 35	3410 ± 339			
Setup II	156 ± 47	486 ± 151	889 ± 99	2660 ± 14	3425 ± 64			

Table 4.1.3: Average permeability for each gas in Setup I and Setup II

^a Barrer = 10^{-10} cm³(STP) cm cm⁻² s⁻¹ cm Hg⁻¹



Figure 4.1.2: The composition of gases in biogas and air side (Setup I, Setup II, and Setup III). A - Nitrogen (in atm), B - Oxygen (in atm), C - Methane (in atm), D - Carbon dioxide (in atm), E - Hydrogen sulfide (in mg L-1). Note the different scale.

Biofilm growth

In Setup III, repeated measurements (E1-E4) were done over 41 days to test the biofilm growth for H_2S removal from biogas. The results of H_2S concentration in biogas and air side is shown in **Figure 4.1.3**. In the biogas side, H_2S concentration decreased from 2.5 mg L⁻¹ to less than 0.5 mg L⁻¹ in 5 hours for E1 and in 4 hours for E2-E4. In the air side, H_2S concentration firstly increased during the first 1 to 2 hours (in E1 up to 0.35 mg L⁻¹) and then decreased (for E2-E4 to less than 0.1 mg L⁻¹). O_2 and N_2 concentrations in the biogas side increased by 1% and 10%, respectively. CH_4 and CO_2 concentrations in the air side increased by 3% and 10%; respectively (data not shown). **Table 4.1.4** shows CH_4/CO_2 ratio at the beginning and at the end of the experiments as well as the specific H_2S removal rate. The CH_4/CO_2 ratio was close to 2 at the beginning of the experiments, while it increased to 2.1-2.2 at the end of the experiments. H_2S removal rate increased from 52.7 mg m⁻² h⁻¹ to 90.1 mg m⁻² h⁻¹ in 40 days.



Figure 4.1.3: The H₂S concentration in biogas and air side during biofilm growth experiments. Note the different scale.

Exp.	Day of experiment	CH_4/CO_2 ratio at the	CH_4/CO_2 ratio at the	Specific H ₂ S
-	[a]	-	-	$[g m^{-2} d^{-1}]$
E1	2	1.97	2.24	1.26
E2	27-28	1.94	2.11	1.57
E3	34-35	1.94	2.12	1.93
E4	40-41	1.92	2.08	2.16

Table 4.1.4: H₂S removal rate and CH₄/CO₂ ratio for biofilm growth experiments

Continuous experiment

The biogas from UASB reactor with high H_2S concentration (6.9 g m⁻³) was continuously blown into the BMU in order to test the effectiveness of the biomembrane. The experiment took 15 days. The air side was closed and oxygen was decreasing over the time. **Figure 4.1.4A** shows the total amount of removed hydrogen sulfide from UASB reactor and the amount of oxygen which stayed in the air side. **Figure 4.1.4B** shows the concentration of hydrogen sulfide in UASB reactor, and biogas and air side of BMU.



Figure 4.1.4: The results of continuous process. A - The removed amount of H_2S and O_2 . B - The concentration of H_2S in UASB and BMU.

The losses of methane and carbon dioxide from the biogas side to the air side accounted for 7% and 39%, respectively (data not shown). The contamination of biogas with oxygen and nitrogen accounted for 6% and 30%; however, the biogas production was quite low (approx. 3.1 L d⁻¹) resulting in higher biogas dilution. The efficiency of hydrogen sulfide removal was more than 99% and the specific H_2S removal was 0.98 g m⁻² d⁻¹ on average (with maximum of 1.10 g m⁻² d⁻¹).

Microbiological analyses

FISH analysis

The summary of FISH results using oligonucleotide probes with various specificity is showed in **Table 4.1.5**. Preliminary screening with less specific probes showed the dominance of Bacteria. No signal was detected with the probe specific for Archaea in all samples. Therefore PCR-DGGE was performed only with primers specific for Bacteria. Probes targeting the major classes of Proteobacteria (ALFmix, BET42a, GAM42a and DELTAmix) gave a positive signal for samples A1-D1. Furthermore *Thiobacillus thioparus* (positive TBD121 and negative TBD1419) was detected in these samples as the only known SOB. FISH analysis showed a significant decrease in diversity during the experiment as the only positive signal for samples A2-D2 was gained with probe HGC (Actinobacteria). Representative FISH pictures are shown in **Figure 4.1.5**.

Probe	A1	B1	C1	D1	A2	B2	C2	D2
ARC915	-	-	-	-	-	-	-	-
ALFmix	+	+	+	+	-	-	-	-
BET42a	+	+	+	+	-	-	-	-
GAM42a	+	+	+	+	-	-	-	-
DELTAmix	+	+	+	+	-	-	-	-
SRB385	+	+	+	+	-	-	-	-
CFB560	-	-	-	-	-	-	-	-
HGC	nd	nd	+	+	+	+	+	+
PAR651	+	+	+	+	-	-	-	-
TBD121	+	+	+	+	-	-	-	-
TBD1419	-	-	-	-	-	-	-	-
TMD131	-	-	-	-	-	-	-	-

Table 4.1.5: Results of FISH analysis; ALFmix = ALF1B + ALF968, DELTAmix = DELTA495a-c + competitors. nd = not determined. Samples A1-D1 were taken the second day of the biofilm growth experiment (E1), while samples A2-D2 were taken at the end of continuous experiment.



Figure 4.1.5: FISH signal of Cy3-labeled probes (pink) and DAPI stain (blue). A - Sample D1, probe TBD121, B - Sample D2, probe HGC69A.

There was no evidence of distinct diversity in four sampling points. There are no differences in presence/absence of specific group of bacteria among samples in one set. There might be difference in quantity, but quantification was not possible due to many sulfur deposits which gave interfering signal. The only visible difference was lower signal of most of the probes observed in sample D1 in comparison with A1-C1.

PCR-DGGE analysis

Bacterial DGGE profiles of two sets of samples are depicted in **Figure 4.1.6**. The bands which were successfully sequenced are assigned with numbers and the putative affiliation from RDP database is shown. There are significant differences between first (A1-D1) and second (A2-D2) set of samples as was also observed with FISH (**Table 4.1.5**). Samples taken at the beginning of the experiment show much more diversity than those from the end of the experiment. As for the bacteria of sulfur cycle,

M A1 B1 C1 D1 A2 B2 C2 D2	В.	Phylum	Class	Family	Genus	Species	Sim. [%]
	1	Bacteroidetes	Bacteroidia	Marinilabiliaceae	unclassified		99.0
	2	Bacteroidetes	Sphingobacteriia	Chitinophagaceae	unclassified		98.6
E E E E E E E E E E E	3	Bacteroidetes	Bacteroidia	Porphyromonadaceae	Proteiniphilum	-	96.0
	4	Bacteroidetes	Bacteroidia	Marinilabiliaceae	unclassified		98.0
E C S C S S S S S S S S S S S S S S S S	5	Proteobacteria	Gamma-	Methylococcaceae	Methylobacter	-	99.8
1.	6	Proteobacteria	Beta-	Hydrogenophilaceae	Thiobacillus	T. thioparus	99.4
	7	Proteobacteria	Gamma-	Xanthomonadaceae	unclassified		99.8
	8	Proteobacteria	Gamma-	Xanthomonadaceae	Rhodanobacter	-	99.6
5.	9	Proteobacteria	Gamma-	Xanthomonadaceae	unclassified		99.8
·12	10	Proteobacteria	Gamma-	Xanthomonadaceae	unclassified		100
	11	Bacteroidetes	unclassified				99.8
12	12	Firmicutes	Clostridia	Ruminococcaceae	Clostridium III	-	99.8
	13	Actinobacteria	Actinobacteria	Mycobacteriaceae	Mycobacterium	M. parascrofulaceum	99.8
14	14	Actinobacteria	Actinobacteria	Microbacteriaceae	Humibacter	H. albus	98.4

sequence number 6 showed the closest similarity (99.4 %) with *Thiobacillus thioparus*. This SOB was detected only in the samples from the first set, which is in accordance with the results of FISH.

Figure 4.1.6: DGGE profile of bacterial community in two sets of samples. Left: Numbers with dots indicate successfully sequenced excised bands. M = marker. The table: Identification of DNA sequences obtained from DGGE by RDP. B. = Band, Sim. = Similarity.

There is one band (number 13) with substantial higher intensity than other bands visible in samples A2-D2. This band corresponds to genus *Mycobacterium*.

4.1.4. Discussion

This paper shows the ability of biomembrane to effectively remove H₂S from biogas while restricting biogas contamination with nitrogen. Biomembrane was used in BMU for both, batch and continuous experiments. While in batch experiments (E4) the specific H₂S removal was as high as 2.16 g H₂S m⁻² d⁻¹, the maximum specific H₂S removal was only 1.10 g H₂S m⁻² d⁻¹ in the continuous experiment. This shows that the potential of the BMU was not fully used because the UASB reactor connected to the BMU did not produce enough biogas with H₂S. However, H₂S was completely removed from the biogas with the H₂S removal efficiency of more than 99% during the continuous experiment.

Biofilm growth in the biomembrane unit

SOB biofilm grew and improved its H_2S removal abilities during the biofilm growth experiment. During the experiments the creation of light yellowish deposits of most probably elemental sulfur were observed on the surface of the membrane. However, the amount was not sufficient for elemental analysis. More than 96% of H_2S was removed after 5 hours in E4 with the biomembrane.

Few studies comparable to this research have been published so far. Camiloti et al. (2016) used External Silicone Membrane Reactor (ESMR) to remove sulfide from wastewater. Silicone membrane was connected to the continuously stirred tank reactor and wastewater was recirculated through the membrane. Oxygen was dosed solely through the membrane wall and it successfully (chemically and

biochemically) oxidized sulfide to elemental sulfur and sulfate proving the applicability of membrane for desulfurization. In that case, membrane served as a barrier between air and the wastewater and sulfide was removed in the liquid phase. In the present paper, bio-membrane served as a barrier between air and the biogas with a thin biofilm layer on the membrane surface at the biogas side for H_2S removal in the gas phase.

The transfer of other gasses across the membrane was decreasing with biofilm growth which was caused by the covering of the membrane with the biofilm. While at the beginning of the biofilm growth experiment (E1) the covered area of membrane was about 60%, at the end (E4) it was about 90%. The biofilm served as a barrier decreasing the contamination of biogas with nitrogen and oxygen and preventing the losses of methane from biogas to air. In E1, the concentration of methane in the biogas side decreased by 9%, while in E4 it was only by 2%. It can be assumed that better membrane coverage with biofilm will assure still smaller methane losses.

The contamination of biogas with nitrogen and oxygen is one of the disadvantages of direct microaeration, where oxygen or air is blown directly into the gas or liquid phase of an anaerobic reactor (Krayzelova et al., 2015). Indeed, even small dilution of biogas may complicate its further use in cogeneration unit (Appels et al., 2008; Wase & Forster, 1984). Nitrogen dilution of biogas in this study was 15% in E1 but it decreased to less than 6% in E4. Since the membrane was not totally covered with biofilm, even lower nitrogen dilution can be expected after complete membrane coverage with biofilm is achieved.

The amount of oxygen in biogas during the direct microaeration can reach up to 4% (Díaz & Fdz-Polanco, 2012; Díaz et al., 2010; Rodriguez et al., 2012). In this paper, the amount of oxygen in biogas side reached 2% in E1, but after 40 days of biofilm growth it was less than 1%. Compared to the direct microaeration, biomembrane with adapted and active SOB biofilm can prevent oxygen contamination of biogas due to microaeration.

Bacterial diversity in the biofilm

The only known SOB detected in the biofilm by both FISH and PCR-DGGE was *Thiobacillus thioparus*. However, this species was found only on second day (E1). This set of samples showed also positive signal with the probe specific for genus *Paracoccus*, which includes species capable of sulfur oxidation (Kelly et al., 2006). In samples from day 56, genus *Mycobacterium* (phylum Actinobacteria) was detected as dominant (**Figure 4.1.5**). Recently, strains of *Mycobacterium* capable of S⁰ oxidation were isolated from deteriorated sandstone (Kusumi et al., 2011). The suggestion that *Mycobacterium* might be part of S-cycle processes in the reactor is supported by the presence of massive S⁰ deposits. *Mycobacteria* were also found in other reactors removing H₂S under similar conditions (Ramos et al., 2014c).

For more detailed analysis of biofilm development and SOB diversity, more samples should have been taken during the experiment. However, in the given scale, this could negatively affect the experiment by interrupting microaerobic conditions and disrupting the biofilm.

Permeability of the membrane for different biogas components

The permeability order of the components in Setup I and II was $H_2S > CO_2 > CH_4 > O_2 > N_2$ with H_2S being the fastest component to move through the membrane and N_2 the slowest. The permeation

order of $H_2S > CO_2 > CH_4$ coincides with the findings reported by Kraftschik et al. (2013). Moreover, permeability values for N_2 , O_2 , CH_4 and CO_2 in PMDS reported in the literature (**Table 4.1.6**) follow the same permeation order ($CO_2 > CH_4 > O_2 > N_2$), with the exception of Tremblay et al. (2006) who reported a higher permeation for N_2 than for CH_4 .

		N ₂	02	CH₄	CO ₂	H₂S
Merkel et al. (2000)		400	800	1200	3800	-
Javaid (2005)		460	-	1452	-	-
Tremblay et al. (2006)		180	-	90	1300	-
Basu et al. (2010)		250	500	800	2700	-
This study	Setup I	210	500	800	2550	3410
	Setup II	160	490	890	2660	3430

Table 4.1.6: The comparison of N₂, O₂, CH₄, and CO₂ permeability [Barrer] in PMDS membranes

As shown in **Table 4.1.6**, the values reported for the permeability of gases through silicone rubber vary greatly from one researcher to another. It has been reported that transport properties of a membrane can change depending on whether the experiment was carried out with a pure gas or a mixture of two or more gases (Raharjo et al., 2007). This fact could have caused the difference observed between the permeability values reported in previous works and the ones obtained in this one. Calculations in the present paper were done based on the behavior of a mixture of gases, while in previous papers the values were most often calculated from experiments with pure gases.

Further challenges

Many researchers have identified biofilm control as the most challenging aspect of operating applications using biomembrane. Excessive biofilm growth will not only cause non-uniform flow distribution and channeling, but also the inhibition of substrate or gas diffusion, eventually deteriorating the system performance (Hwang et al., 2009). To determine the effect of a thicker biofilm layer or sulfur accumulation on the membrane on the transfer of gases must be examined. The control of the elemental sulfur deposition on the membrane and sulfur harvesting is another research challenge.

4.1.5. Conclusions

The ability of biomembrane unit to remove H₂S from biogas has been shown:

- In batch experiments, specific H₂S removal was 2.16 g m⁻² d⁻¹.
- In continuous experiment, specific H_2S removal rate reached 0.98 g m⁻² d⁻¹ on average with H_2S removal efficiency of more than 99%. Methane losses accounted for 7%.
- Methane losses and nitrogen and oxygen biogas contamination decrease with increasing membrane coverage with biofilm.
- Light yellow deposits on the membrane indicated elemental sulfur formation.
- Thiobacillus thioparus was identified by FISH and PCR-DGGE.
- Gases permeation through membrane decreased in order: $H_2S > CO_2 > CH_4 > O_2 > N_2$.

4.2. Biomembrane II – Pilot-scale experiences: Microaeration through biomembrane for biogas desulfurization: lab-scale and pilot-scale experiences (Article 8)

Pokorna-Krayzelova, L., Bartacek, J., Theuri, S., Segura Gonzalez, C.A., Prochazka, J., Volcke, E.I.P., Jenicek, P. "Microaeration through biomembrane for biogas desulfurization: lab-scale and pilot-scale experiences" *Submitted in Applied Microbiology and Biotechnology (June 2017)*

Abstract

Microaeration, i.e. dosage of limited amount of air or oxygen into an anaerobic reactor, is a biological method to remove hydrogen sulfide oxidation from biogas by converting it into elemental sulfur. However, dosing air directly into the reactor results in the dilution of biogas with nitrogen and oxygen and could also lead to the blocking of pipes by elemental sulfur. These disadvantages can be overcome by the use of biomembrane i.e. membrane covered with biofilm that separates air and biogas. Experiments with bare membrane, wet membrane and biological oxidation of hydrogen sulfide. The membrane was then placed into the gas phase of a pilot-scale anaerobic digester. Different amounts of air were dosed through the biomembrane to determine the optimum air-to-biogas ratio, to evaluate methane losses through the biomembrane and to evaluate biogas contamination with nitrogen and oxygen. H₂S content was decreased from 3000 ppm to less than 100 ppm within two days. The loss of methane was 3.7% of the total methane production and the specific H₂S removal rate was 32 mg m⁻² d⁻¹.

Keywords

Biomembrane; Desulfurization; Hydrogen sulfide oxidation; Microaeration; Oxygen; Pilot-scale

4.2.1. Introduction

During the anaerobic treatment of wastewater with high sulfate concentrations, sulfate reducing bacteria produce a high amount of hydrogen sulfide (Nayono, 2010; Russell, 2006), which causes major technological problems such as the inhibition of anaerobic processes, corrosion of tanks, piping, engines and boilers, and emissions of sulfur dioxide from biogas combustion (Hulshoff Pol et al., 1998). Because of these negative effects, hydrogen sulfide has to be removed from biogas.

Available methods for desulfurization are physico-chemical (e.g. absorption, precipitation) or biological ones (e.g. biochemical oxidation of sulfide) (Petersson & Wellinger, 2009; Wellinger & Lindberg, 1999). Biological processes are often simpler and more cost-effective compared to physico-chemical methods (Hulshoff Pol et al., 1998). One of the options for biological H₂S removal from biogas, which has been used in full-scale (Jeníček et al., 2017), is microaeration, i.e. the controlled dosing of a small amount of air into the anaerobic reactor (Díaz & Fdz-Polanco, 2012; Jenicek et al., 2011a; Ramos & Fdz-Polanco, 2014a). In microaeration, sulfide is oxidized to elemental sulfur by sulfide oxidizing bacteria, at low oxygen concentrations. The elemental sulfur produced is insoluble and could be possibly removed from the system. Both the total concentration of sulfide in the effluent and the concentration of hydrogen sulfide in biogas can be significantly decreased with this process (Janssen et al., 1995).

The possible drawbacks of microaeration are the dilution of biogas with nitrogen and little or no control over the exact location where elemental sulfur is deposited. Elemental sulfur usually accumulates on the walls in the reactor headspace (Díaz et al., 2011a) or in the liquid effluent (Krayzelova et al., 2014a). However, sulfur could also accumulate on in the three-phase separator of UASB and similar anaerobic reactors or in gas pipes, which may cause serious clocking problems. These problems could be overcome by using biomembrane (Pokorna-Krayzelova et al., 2017a), where air needed for microaeration is delivered through the membrane, (which decreased the contamination of biogas by nitrogen) and the sulfide oxidizing biofilm needed for biological sulfide oxidation is growing on the surface of the membrane. Previous lab-scale study (Pokorna-Krayzelova et al., 2017a) with simple silicone tube used as a membrane revealed the ability to remove hydrogen sulfide from biogas in batch as well as continuous experiments.

In this paper, a commercial hollow fiber membrane was first tested in the lab-scale for hydrogen sulfide removal from biogas. Experiments with bare membrane, wet membrane and biofilm membrane were conducted in a separate batch reactor to measure the gas permeation and to detect the chemical and biological sulfide oxidation. Membrane was then placed into the headspace of a pilot-scale anaerobic digester to prove its ability to continuously remove large quantities of hydrogen sulfide during real operation. Different amounts of air were dosed through the biomembrane to determine the optimum air-to-biogas ratio, to evaluate methane losses through the biomembrane and to evaluate biogas contamination with nitrogen and oxygen.

4.2.2. Materials and methods

Experimental set-up

Lab-scale biomembrane unit

A120L biomembrane unit (BMU) including a commercial membrane (specifications in **Table 4.2.1**) was used to simulate the head space of an anaerobic reactor (**Figure 4.2.1**). Air reservoir has been connected to the air side to decrease the ratio between biogas and air volume. Gas permeation (the concentration of hydrogen sulfide, oxygen, nitrogen, methane and carbon dioxide) through the membrane was measured in three configurations: bare membrane (experiments with dry membrane), wet membrane (membrane was submerged for 30 minutes in the tap water before the experiment), and biomembrane (membrane was submerged for 30 minutes in the mesophilic sludge for biofilm attachment before the experiment). During the biomembrane experiments, biogas was humidified with concentrated sodium chloride at pH 4 to allow biofilm growth.

Specifications of membrane	Units	Value
Туре	-	LM-P2*
Membrane material	-	PVDF**
Specific surface area	m ²	20
Pore size	μm	0.10-0.40
Maximum working pressure	MPa	0.12
Working temperature	°C	5-45
Membrane inside volume	L	3.5
Biogas side to air side ratio	-	5.1
Biogas side volume	L	23.0
Air side volume	L	117.9

Table 4.2.1:	Membrane	and	BMU	specification
	memorane		0.0.0	speenieation

*Model number, **Polyvinylidene fluoride

At the beginning of each experiment, the air side was flushed with fresh air from the atmosphere and biogas side was flushed with synthetic biogas (64.1% CH_4 , 35.5% CO_2 , and approximately 0.2 % H_2S). Both systems were sealed and recirculated at a flow rate 13.2 L h⁻¹. Experiments were conducted under atmospheric pressure and ambient temperature. Hydrogen sulfide, oxygen, nitrogen, methane and carbon dioxide were measured hourly on both sides. The transfer of methane, carbon dioxide, nitrogen and oxygen was evaluated.

Before the start of each experiment, lab-scale BMU was tested for potential leakages and the experiment started only when BMU was found to be perfectly tight (data not shown).



Figure 4.2.1: Schematic overview of the lab-scale biomembrane unit: 1 - air reservoir, 2 - biomembrane unit, 3 - biogas humidifier (used only for experiments with biomembrane), 4 - recycle pumps, 5 - sampling point for gases (CH₄, CO₂, N₂, O₂), 6 - H₂S sensor.

Calculations of membrane parameters in the lab-scale biomembrane unit

CH₄, CO₂, N₂, and O₂ transfer

The oxygen, nitrogen, methane and carbon dioxide transfer rates (r_1) through the membrane were calculated for the membrane. At the start of the experiment, the concentrations of oxygen and nitrogen in the biogas were close to zero and so were the concentration of methane and carbon dioxide in the air side. This concentration gradient resulted in the transfer of gases through the membrane until the partial pressures on both sides equalized.

The increase in the molar amount of a gas at one side of the membrane equals its decrease at the other side, as well as the amount transferred through the membrane, as expressed through Eq. 4.2.1:.

$$V_b \frac{dc_b}{dt} = -V_a \frac{dc_a}{dt} = r_1 A_m (c_a - c_b)$$
 Eq. 4.2.1

where c_b and c_a denote the gas concentration at the biogas and air side, respectively [mol L⁻¹], A_m is the membrane surface area [m²], r_1 is the transfer rate [L m⁻² h⁻¹], and V_b and V_a are the volumes of biogas and air side, resp. [L].

H₂S transfer

The hydrogen sulfide parameters such as the transfer rate (r_1) , the chemical oxidation rate (r_{chemox}) , the biological oxidation rate (r_{bioox}) , and the half-saturation constant (K_s) were calculated for the membrane.

The concentration of hydrogen sulfide was high on the biogas side and close to zero on the air side. This caused a decrease in the concentration on biogas side and an increase on the air side. The concentration decrease on the biogas side during experiments with bare membrane and wet membrane was caused by the transfer across the membrane as well as by the chemical oxidation. The rate of transfer across the membrane and the chemical oxidation rate were modelled using a first order reaction. Considering the biomembrane, hydrogen sulfide was also used as a substrate for the bacteria growth which was modelled using the Monod's type equation.

The hydrogen sulfide concentrations on the biogas (Eq. 4.2.2) and air (Eq. 4.2.3) side were calculated according to the following equations:

$$V_b \frac{dc_b}{dt} = r_1 A_m (c_a - c_b) - V_b r_{chemox}(c_b) - V_b r_{bioox} \left(\frac{c_b}{c_b + K_s}\right)$$
Eq. 4.2.2

$$V_a \frac{dc_a}{dt} = r_1 A_m (c_a - c_b) - V_a r_{chemox}(c_a)$$
Eq. 4.2.3

Where c_b is the concentration of hydrogen sulfide on biogas side [mg L⁻¹], c_a is the concentration of hydrogen sulfide on air side [mg L⁻¹], A_m is the membrane surface area [m²], r_1 is the hydrogen sulfide transfer rate [L m⁻² h⁻¹], r_{chemox} is the hydrogen sulfide chemical oxidation rate [h⁻¹], r_{bioox} is the H₂S biological oxidation rate [h⁻¹], K_s is the H₂S half-saturation constant [mg L⁻¹], and dt is the time step [h]. Biological H₂S oxidation was not included in Eq. 4, as biofilm grew only on the side of membrane facing the biogas side.

Equations 4.2.1, 4.2.2 and 4.2.3 were solved for the parameters r_1 , r_{chemox} , r_{bioox} , and K_s by minimizing the sum of squared errors between experimental data and the data gained from the calculations, using the inbuilt Excel (Microsoft Office 2013) differential equation "solver".

Pilot-scale CSTR with biomembrane

The applicability of biomembrane for hydrogen sulfide removal was tested in a 250L pilot-scale continuous stirred tank reactor (CSTR) shown in **Figure 4.2.2**. Biomembrane (membrane 1 with 10 m² surface area) was placed into the gas space of 50 L. The reactor was inoculated with 200 L of mesophilic sludge from a municipal wastewater treatment plant and operated at 40 °C. Cheese whey was used as feed. The organic loading rate (OLR) initially amounted to 0.05 g COD g⁻¹ VSS d⁻¹ and was gradually increased to 0.30 g COD g⁻¹ VSS d⁻¹ (**Table 4.2.2**). The concentration of the mesophilic sludge was kept constant the whole time. The CSTR was operated in 30 minutes cycles (30 s feeding, 1260 s mixing, 500 s sedimentation, and 10 s sludge recirculation). The hydraulic retention time (HRT) of the CSTR was 21 days.



Figure 4.2.2: Schematic overview of the pilot-scale CSTR with biomembrane: 1 - feed reservoir, 2 - feed pump, 3 - feed flow meter, 4 - heating, 5 - feed recycle pump, 6 - settling zone, 7 - biomembrane, 8 - air blower, 9 - gas flow meter, 10 - liquid sampling, 11 - gas sampling.

Table 4.2.2: Overview of operating periods for the pilot-scale BMU. The characteristics of period A-D
(increasing OLR) and period I-VI (changing air/H ₂ S rate)

	OLR	Oxygen dose	
	[g COD g ⁻¹ VSS d ⁻¹]	[mL min⁻¹]	[relative to stoichiometric ratio]
Α	0.05	0	0
В	0.10	0	0
С	0.15	0	0
D	0.30	0	0
Ι.	0.30	400.0	× 1000
II.	0.30	40.0	× 100
III.	0.30	4.0	× 10
IV.	0.30	0.8	× 2
V.	0.30	1.2	× 3
VI.	0.30	2.0	× 5

Microaeration was turned on after the start-up period where an OLR of 0.30 g COD g⁻¹ VSS d⁻¹ was reached. The amount of air dosed was expressed relative to the stoichiometric $H_2S:O_2$ molar ratio of 2:1 required for the oxidation of sulfide to elemental sulfur. An excess amount of oxygen was dosed initially, its dosing was then gradually decreased to determine the proper amount for biogas desulfurization. In the diagrams, the dose of oxygen is shown with symbols I.-VI. (**Table 4.2.2**): I. - 1000x stoichiometry (400 mL air min⁻¹); II. - 100x stoichiometry (40 mL air min⁻¹); IV. - 2x stoichiometry (0.8 mL air min⁻¹); V. - 3x stoichiometry (1.2 mL air min⁻¹); and VI. - 5x stoichiometry (2.0 mL air min⁻¹).

Hydrogen sulfide, nitrogen, oxygen, methane, and carbon dioxide were measured in the biogas and air effluent, while total and dissolved COD, organic acids, sulfide, pH and ammonia were measured in the CSTR influent and effluent.

Analytical methods

Hydrogen sulfide concentration in the gas for lab-scale BMU was monitored using an online electrochemical gas sensor (Membrapor H_2S sensor type H_2S/S -10000-S). Hydrogen sulfide concentration in the gas for pilot-scale CSTR with bio-membrane unit was measured using the RAE H_2S gas detection tube. Gases (CH₄, CO₂, N₂, and O₂) were measured by GC Shimadzu 2014 equipped with a thermal conductivity detector (CH₄, CO₂, air) and by the GC 8000^{TOP} (Fisons Instruments, USA) equipped with a heat conductivity detector HWD 800 (O₂, N₂, CH₄). Analysis of COD, pH, VFA, solids, ammonia and sulfide were done according to the Standard Methods (American Public Health Association, 1997). The sulfur composition of sludge was assessed by Elemental Vario EL III (Elementar Analysensystem GmbH, Germany) and by X-ray fluorescence analysis using the ARL 9400 XP sequential WD-XRF spectrometer (THERMO ARL, Switzerland).

4.3. Results

Lab-scale biomembrane unit

Hydrogen sulfide transfer and removal

The concentration of hydrogen sulfide in biogas side and air side is shown in **Figure 4.2.3**. The experiment with dry membrane showed not only the transfer of H_2S from biogas side to air side, but also potential chemical sulfide oxidation (**Table 4.2.3**). The chemical sulfide oxidation accounted for 0.002 L h⁻¹ for the dry membrane, while for the experiment with wet membrane the chemical sulfide oxidation was 0.013 L h⁻¹. However, it could also be caused by the solubility of hydrogen sulfide in water. The last configuration with biomembrane showed also biological sulfide oxidation in addition to the other mention parameters with a ratio of approximately 20% for chemical and 80% for biological sulfide oxidation. The complete results of kinetic parameters are shown in **Table 4.2.3**.

Membrane	Units	Bare	Wet	
	Cinto	membrane	membrane	Biomembrane
O_2 transfer rate (r_{1,O_2})	$m^{3} m^{-2} h^{-1}$	0.097 ± 0.009	0.025 ± 0.004	0.044 ± 0.007
N_2 transfer rate (r_{1,N_2})	$m^3 m^{-2} h^{-1}$	0.106 ± 0.018	0.215 ± 0.005	0.026 ± 0.004
CH_4 transfer rate (r_{1,CH_4})	m ^³ m ⁻² h ⁻¹	0.102 ± 0.016	0.016 ± 0.004	0.016 ± 0.002
CO_2 transfer rate (r_{1,CO_2})	$m^{3} m^{-2} h^{-1}$	0.108 ± 0.018	0.070 ± 0.038	0.130 ± 0.027
H_2S transfer rate (r_{1,H_2S})	$m^{3} m^{-2} h^{-1}$	0.075 ± 0.012	0.044 ± 0.014	0.070 ± 0.013
H_2S chemical sulfide oxidation rate (r_{chemox})	L h⁻¹	0.002 ± 0.001	0.013 ± 0.001	0.016 ± 0.000
H_2S biological sulfide oxidation rate (r_{bioox})	L h⁻¹	-	-	0.067 ± 0.015
H_2S half-saturation constant (K_S)	$mg L^{-1}$	-	-	0.60 ± 0.20



Figure 4.2.3: Hydrogen sulfide composition in biogas side (A) and air side (B).

Transfer of methane, carbon dioxide, nitrogen and oxygen

Transfer of methane, carbon dioxide, oxygen and nitrogen (**Figure S4.2.1**, Supplementary material, Section 8.3) between biogas side and air side depended on their permeability through the membrane as well as on their solubility in water. While for the experiments with bare membrane the transfer of all gases was comparable (approx. $0.103 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$) as shown in **Table 4.2.3**, the wet membrane and biomembrane experiments showed differences which can be caused by the mentioned solubility in water. For wet membrane and biomembrane experiments, the highest transfer rate had nitrogen ($0.215 \pm 0.005 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$) and carbon dioxide ($0.130 \pm 0.027 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1}$), respectively.

Pilot-scale CSTR with biomembrane

COD removal efficiency

The CSTR was operated for over 250 days. Microaeration was turned on at day 83; following a 82 days start-up period. The COD removal efficiency (**Figure 4.2.4a**) was on average 93% (start-up excluded) and was not affected by microaeration. The pH of the feedstock fluctuated between 2.9 and 5.2. Despite the high acidity of the feedstock, the effluent pH was 7.3 \pm 0.4 on average.





Efficiency of hydrogen sulfide removal

Hydrogen sulfide concentration (**Figure 4.2.4b**) increased to up to 3,000 ppm during the start-up (periods A-D). Upon the start of microaeration (period I), the concentration of hydrogen sulfide decreased within one day from 3,000 ppm to less than 100 ppm. The rate of H₂S oxidation was approximately 2,800 ppm d⁻¹. After 40 days, microaeration was turned off and H₂S concentration in biogas increased to 4,000 ppm. This was repeated in periods II and III (100x and 10x stoichiometry, respectively). The results were identical to period I: H₂S concentration decreased within one day from 4,000 ppm to less than 100 ppm (H₂S oxidation rate in period II was approx. 2,700 ppm d⁻¹). In periods IV and V (2x and 3x stoichiometry, resp.) the amount of oxygen was not sufficient to completely remove H₂S from biogas resulting in approximately 500 ppm of H₂S left in biogas for both periods. During period VI (5x stoichiometry) enough oxygen to remove H₂S from biogas was supplied (concentration below 100 ppm) with minimum biogas contamination with O₂ and N₂.

Biogas side and air side composition

The composition of biogas and air leaving the pilot-scale CSTR is shown in **Figure 4.2.5** and **Table 4.2.4**. In period C and D where no oxygen was dosed, the amount of methane and carbon dioxide was 57 and 36%, respectively. Even though there was no microaeration at this time, some oxygen and nitrogen were observed in biogas during these two periods (approx. 2 and 5%, resp.). This is probably caused by the contamination of the samples by air. The composition of gasses in the biogas side for the periods III-VI was almost the same (around 52:41:1:6 for $CH_4:CO_2:O_2:N_2$). Periods I and II (with strong air overdose) had higher amount of oxygen and nitrogen. Only in period I, methane-to- CO_2 ratio was higher.

The decreasing amount of air blown into the reactor resulted in decreasing amount of oxygen and nitrogen and increasing amount of methane and carbon dioxide in the effluent air. Methane losses also decreased with the decreasing amount of air (**Table 4.2.4**). For period I methane losses were 9.5 liters per day while during period VI, the losses decreased to 0.4 L d⁻¹. Specific H₂S removal was almost equal for all periods (approx. 34 mg m⁻² d⁻¹).



Figure 4.2.5: The gasses composition during the experimental period, A - Biogas composition, B - Air composition. OLR increased during period A-D (**Table 4.2.2**) and air/H₂S ratio varied during period I-VI (**Table 4.2.2**).

	Biogas composition [%]				Air composition [%]			%]	Specific H ₂ S removal	CH ₄ losses	CH₄ production
	CH ₄	CO ₂	O ₂	N ₂	CH ₄	CO2	O ₂	N ₂	[g m ⁻² d ⁻¹]	[L d⁻¹]	[L d ⁻¹]
C-D	56.8	36.3	1.5	5.4	-	-	-	-			
Ι.	63.1	23.7	2.0	10.9	2.4	6.5	15.2	75.9	0.034	9.5	64.7
II.	54.9	34.3	3.2	7.6	9.2	18.8	9.2	62.7	0.062	5.0	71.2
III.	52.8	39.6	1.2	6.4	30.5	38.0	3.2	28.3	0.032	3.3	46.6
IV.	51.7	43.2	1.1	4.0	48.0	42.7	1.8	7.5	0.032	0.4	62.5
V.	53.7	40.1	1.1	5.1	43.1	34.7	3.7	18.5	0.036	2.0	60.1
VI.	51.6	41.6	1.3	5.5	36.8	33.6	8.7	20.9	0.036	1.9	50.5

 Table 4.2.4: Air composition, biogas composition, specific H₂S removal, and CH₄ losses

4.4. Discussion

Pilot-scale CSTR with biomembrane

A few studies comparable to this research have been published so far. Valdes et al. (2016) placed a silicon membrane placed inside a continuous anaerobic fixed bed reactor through which pure oxygen was dosed for the conversion of sulfide to elemental sulfur. In the set-up of Camiloti et al. (2016), wastewater was recirculated between a CSTR and an external silicone membrane. Oxygen was dosed only through the membrane wall and it successfully (chemically and biochemically) oxidized sulfide to elemental sulfur and sulfate proving the applicability of membrane for desulfurization. In both studies, the membrane through which air was dosed, was in direct contact with wastewater, such that sulfide was removed in the liquid phase. However, this can cause problems with the decrease in the membrane transfer capacity contributing extra resistance to oxygen sulfide concentration in biogas is controlled indirectly through sulfide concentration in liquid, almost all sulfide has to be removed in order to achieve sufficiently low hydrogen sulfide concentration in biogas. In this study, bio-membrane served as a barrier between air and biogas with a thin biofilm layer on the membrane surface at the biogas side for H_2S removal in the gas phase. Therefore, hydrogen sulfide content in biogas was controlled directly and with higher efficiency.

Hydrogen sulfide removal

The pilot-scale CSTR with biomembrane was capable to remove up to 98% of H_2S from biogas without affecting the production of methane. Specific H_2S removal rate did not change and remained around 34 mg m⁻² d⁻¹ regardless of the amount of air dosed. This is lower than its capacity. The previously published H_2S removal rate in BMU (980 mg m⁻² d⁻¹) was much higher (Pokorna-Krayzelova et al., 2017a). This is because hydrogen sulfide production in CSTR was much smaller than the capacity of the biomembrane used in this study. I.e. the rate of hydrogen sulfide removal was given by the rate of hydrogen sulfide production.

Oxygen transfer

The theoretical amount of oxygen needed for complete H_2S removal was shown to be insufficient and 5-times more oxygen was needed. When sufficient amount of oxygen was supplied, H_2S was removed almost completely (below 100 ppm) with minimum methane losses (less than 3.7%). Biogas was not diluted with nitrogen and oxygen. The amount of oxygen and nitrogen in biogas (for periods III to VI) was most probably caused by the manipulation and collection of samples. The same amount (approx. 1.5% of oxygen and 5.4% of nitrogen) was detected already before microaeration started (periods A-D). The dilution of biogas by nitrogen and oxygen is one of the disadvantages of direct microaeration (Krayzelova et al., 2015), where air is blown directly into the reactor. Thus, biomembrane is a significant improvement of the microaeration process.

Hydrogen sulfide oxidation in lab-scale biomembrane unit

Chemical and biological sulfide oxidation in the membrane tested accounted for 0.54 g d^{-1} and 2.27 g d⁻¹, respectively. According to Buisman et al. (1990a), at lower sulfide concentration (around 10 mg L⁻¹) the biological sulfide activity was 75 time faster than chemical sulfide oxidation and

according to Janssen et al. (1995), the chemical sulfide oxidation becomes important when biological activity of sulfide oxidizing bacteria is limited. However; this was not the case. Chemical sulfide oxidation occurred in all three experiments (bare, wet, and biofilm membrane) independently on the biological activity. Moreover, for the biofilm membrane the rate of chemical sulfide oxidation was the highest compared to the experiments with bare and wet membrane.

4.5. Conclusions

The efficiency of the pilot-scale CSTR with biomembrane for H_2S removal from biogas was tested in this paper:

- Both, chemical and biological sulfide oxidation were detected during the experiments with biological sulfide oxidation being four times higher.
- The proper amount of air needed for complete hydrogen sulfide removal from biogas with no oxygen and nitrogen leftovers in biogas equaled to 500% of stoichiometric amount based on sulfur present in the feed.
- The total methane production decreased by 3.7% because of microaeration.
- The maximum specific hydrogen sulfide removal was 34 mg m⁻² d⁻¹ and it was limited by the rate of hydrogen sulfide production inside the reactor.

5. POSSIBLE APPLICATION OF RECOVERED SULFUR: A TIRE-SULFUR HYBRID ADSORPTION DENITRIFICATION (T-SHAD) PROCESS FOR DECENTRALIZED WASTEWATER TREATMENT (Article 9)

Krayzelova, L., Lynn, T.J., Banihani, Q., Bartacek, J., Jenicek, P., Ergas, S.J. (2014). "A Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process for decentralized wastewater treatment." Water Research 61: 191-199.

Abstract

Nitrogen discharges from decentralized wastewater treatment (DWT) systems contribute to surface and groundwater contamination. However, the high variability in loading rates, long idle periods and lack of regular maintenance presents a challenge for biological nitrogen removal in DWT. A Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process was developed that combines nitrate (NO₃⁻) adsorption to scrap tire chips with sulfur-oxidizing denitrification. This allows the tire chips to adsorb NO₃⁻ when the influent loading exceeds the denitrification capacity of the biofilm and release it when NO₃⁻ loading rates are low (e.g. at night). Three waste products, scrap tire chips, elemental sulfur pellets and crushed oyster shells, were used as a medium in adsorption, leaching, microcosm and upflow packed bed bioreactor studies of NO₃⁻ removal from synthetic nitrified DWT wastewater. Adsorption isotherms showed that scrap tire chips have an adsorption capacity of 0.66 g NO₃⁻ -N kg⁻¹ of scrap tires. Leaching and microcosm studies showed that scrap tires leach bioavailable organic carbon that can support mixotrophic metabolism; resulting in lower effluent SO₄²⁻ concentrations than sulfur oxidizing denitrification alone. In column studies, the T-SHAD process achieved high NO₃⁻-N removal efficiencies under steady state (90%), variable flow (89%) and variable concentration (94%) conditions.

Keywords

Decentralized wastewater systems, denitrification, scrap tire chips, nitrate adsorption, sulfur oxidation

Graphical abstract



T-SHAD: Tire-Sulfur Hydrid Adsorption Denitrification



[mg h⁻¹]

ate 8

ing. 6

09

Nitrogen

10

5.1. Introduction

Decentralized wastewater treatment (DWT) systems make up one-third of the wastewater that is treated in the United States (Wakida & Lerner, 2005). DWT systems are often chosen over centralized systems, particularly in small communities, rural areas and developing countries, because they are inexpensive, easy to use and have relatively low maintenance requirements. However, the high variability in influent loading rates, long idle periods (e.g., during vacations) and lack of regular maintenance presents a challenge for biological nitrogen removal in DWT (Fagergren et al., 2004; Oakley et al., 2010; Scholes, 2006). Due to these challenges, effluent nitrogen standards that can be met by full-scale municipal wastewater treatment plants are rarely met in DWT (Associates, 2000; Scholes, 2007; Scholes, 2006). Thus, DWT systems significantly contribute to eutrophication of surface waters (Allen & Kramer, 1972) and human health problems, such as methemoglobinemia (Crittenden, 2005). This study introduces a Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process to enhance the removal of nitrate (NO_3^-) from nitrified wastewater in DWT systems. The T-SHAD process combines three waste products, scrap tire chips, elemental sulfur pellets and crushed oyster shells, as a medium in up-flow packed bed bioreactors. This medium works in synergy to increase NO₃⁻ removal under variable loading conditions and decrease the production of undesirable by-products (excess biomass, sulfate $[SO_4^{2-}]$ and carry-over of organic carbon).

In heterotrophic denitrification systems, the dosing rate of the organic substrate must be carefully matched to the NO_3^- loading rate. Incomplete denitrification occurs when the organic substrate is insufficient, while organic carbon carry-over to the effluent occurs when excess substrate is provided (Sengupta et al., 2007; Zhou et al., 2011). Sulfur oxidizing denitrifying bacteria eliminates this problem by using elemental sulfur as an electron donor according to the following equation (Batchelor & Lawrence, 1978):

$$55S^{0} + 20CO_{2} + 50NO_{3}^{-} + 38H_{2}O + 4NH_{4} \rightarrow 4C_{5}H_{7}O_{2}N + 55SO_{4}^{2-} + 25N_{2} + 64H^{+}$$
 (Eq. 5.1)

The low growth rates of these autotrophic bacteria also result in low excess biomass production and decreased maintenance requirements for backwashing (Sengupta et al., 2007; Sun & Nemati, 2012; Zhou et al., 2011). Prior studies have shown that heterotrophic and autotrophic denitrification processes can be combined and promoted simultaneously (mixotrophic metabolism) to increase denitrification rates, reduce $SO_4^{2^2}$ production and reduce alkalinity requirements (Oh et al., 2001; Sahinkaya & Dursun, 2012; Sengupta et al., 2007; Sierra-Alvarez et al., 2005).

The use of an adsorptive filter media as a biofilm carrier provides an advantage in DWT denitrification. When influent NO_3^- loading rates are higher than the maximum denitrification rate of the system, the medium can temporarily adsorb NO_3^- . Desorption and denitrification can occur during periods of low NO_3^- loading (e.g., at night). Previously studied materials for NO_3^- adsorption include grapheme (Ganesan et al., 2013), boron waste (Olgun et al., 2013), wheat residues (Wang et al., 2007; Xu et al., 2013a) and scrap tires (Lisi et al., 2004). Lisi et al. (2004) studied the adsorptive properties of tire chips for use as putting green drainage materials and observed a significant reduction in NO_3^- concentrations. Furthermore, several studies have investigated the use of scrap tires as a packing media for biofilm reactors. Shin et al. (1999) used scrap tire chips as a packing material in sequential anaerobic-aerobic biofilm reactors and observed that scrap tires did not inhibit biofilm formation and that sufficient surface area was available for microbial growth (Shin et al., 2013).

1999). Park et al. (1996) studied the adsorptive capacity of volatile organic compounds using various materials and observed that tire chips had 1.4 to 5.6% of the adsorptive capacity of granular activated carbon (Park et al., 1996). Ground rubber was also used to adsorb benzene and o-xylene from water contaminated with aromatic hydrocarbons and as a sorption media in in-situ reactive permeable barriers (Kershaw et al., 1997). However, none of the previous studies have investigated the combination of adsorption and biological denitrification.

The overall goal of this research was to develop a low cost and robust denitrification process that can be applied in DWT systems. The specific objectives of this study were to: (1) determine the adsorptive capacity of scrap tires for NO_3^- in nitrified wastewater, (2) characterize the leachate from scrap tires and its potential use as a carbon source for mixotrophic sulfur oxidizing denitrification and (3) investigate a hybrid adsorption and mixotrophic sulfur utilizing denitrification process in column studies under steady and variable loading conditions.

5.2. Materials and Methods

5.2.1. Materials

• Synthetic wastewater

Experiments were carried out using synthetic nitrified wastewater with the following composition $(mg L^{-1})$: KH_2PO_4 (43.9); NH_4Cl (7.6); $MgCl_2 \cdot 6H_2O$ (33.0); $FeCl_2 \cdot 4H_2O$ (0.4); $NaHCO_3$ (50.0) and trace element solution (2 mL L⁻¹). The trace element solution contained (mg L⁻¹): $ZnCl_2$ (0.0189); ethylenediamine-tetraacetic acid (EDTA) (0.5760); $CaCl_2 \cdot 2H_2O$ (0.0700); $MnCl_2 \cdot 4H_2O$ (0.0472); $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.0100); $CuSO_4 \cdot 5H_2O$ (0.0281) and $CoCl_2 \cdot 6H_2O$ (0.0200). NO_3^- was added as KNO_3 to achieve the desired NO_3^- -N concentration for the various experiments, as described below. The final pH was adjusted to 7.0-7.2 with NaOH.

• Biomass carriers

Scrap tire chips were obtained from Liberty Tire Recycling (Rockledge, Florida) and hand-sorted based on size to between 1.0-1.5 cm. The specific weight of the material was 1,040 g L^{-1} and the bulk density was 330 g L^{-1} . Elemental sulfur pellets (0.4-0.6 cm) were obtained from Martin Midstream Partners (Seneca, Illinois). Crushed oyster shells were added as an alkalinity source. They were obtained from Myco Supply (Pittsburgh, Pennsylvania) and sieved to remove fines smaller than 0.6 cm.

5.2.2. Nitrate adsorption study

A standard batch test was performed (Ganesan et al., 2013) to determine the adsorption capacity of scrap tires for NO_3^- . Four series of seven 250-mL glass Erlenmeyer flasks each received 0, 2.5, 5, 10, 20, 30, and 40 g of scrap tires. The first series of seven bottles were filled with 200 mL of deionized water (DI), while the other three series were filled with 200 mL of the synthetic wastewater described above spiked with a stock KNO₃ solution to achieve an initial nitrate as nitrogen (NO_3^- -N) concentration of 100 mg L⁻¹. Scrap tires were baked at 105°C for four hours to avoid biological activity. This temperature was selected because it did not appear to damage the structure of the tire chips; however some volatile components may have volatilized. The bottles were agitated at room temperature (20 ± 2°C) using a Thermo Scientific multi-purpose rotator (Dubuque, Iowa, U.S.A.) at

120 rpm. Samples were collected for anion and cation analysis, as described below, every ten days for 30 days. Samples were collected for metal analysis at the end of the study.

Langmuir (Eq. 5.2) and Freundlich (Eq. 5.3) adsorption equations were used to determine adsorption behavior of scrap tires for NO_3^- (Foo & Hameed, 2010; Roy et al., 1992).

$$Q_e = \frac{Q_{max} \cdot b \cdot c_{eq}}{1 + b \cdot c_{eq}}$$
(Eq. 5.2)

$$Q_e = K \cdot c_{eq}^{1/n} \tag{Eq. 5.3}$$

where Q_e is the amount of NO₃⁻-N per the mass of scrap tires [mg g⁻¹], c_{eq} is the equilibrium aqueous phase concentration [mg L⁻¹], Q_{max} is the maximum surface loading capacity [mg g⁻¹], *b* is the equilibrium constant between adsorption and desorption, and *K*, and 1/n are constants.

A short-term adsorption/desorption study was carried out at room temperature ($20 \pm 2^{\circ}$ C) with shaking using a HS 260 basic rotator (IKA-Werke GmbH, Germany) at 120 rpm. Three 250-mL Erlenmeyer flasks were filled with 40 g of scrap tires (200 g tire L⁻¹) and 200 mL of the synthetic wastewater described above, which was spiked with a stock KNO₃ solution to achieve an initial NO₃⁻ - N concentration of 50 mg L⁻¹. After three days of adsorption, the solution was drained from the tire chips and the flasks were re-filled with 200 mL of the synthetic wastewater without nitrate addition. The flasks were allowed to desorb for three days. Samples were collected at the beginning and at the end of the adsorption and desorption stages, and NO₃⁻, pH and COD were measured in the samples.

5.2.3. Leaching study

A leaching study was conducted to determine the concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD₅), total organic carbon (TOC), total nitrogen (TN), sulfur, iron and specific anions, cations, and metals that can be leached from the tires. Two hundred grams of baked scrap tires were added to 1.5 L of the synthetic wastewater describe above (no NO_3^- addition) in 2L glass bottles. The bottles were agitated at room temperature ($20 \pm 2^{\circ}$ C) using a Thermo Scientific multi-purpose rotator (Dubuque, Iowa, U.S.A.) at 120 rpm. Another 2L glass bottle containing only synthetic wastewater (without tire chips) was used as a control. Samples were collected after 72 hours, and filtered using 0.45 µm mixed cellulose membranes. The time of exposure (72 hours) was chosen based on a preliminary experiment (data not shown), which showed that after 72 hours the concentration of COD in the leachate stabilized.

5.2.4. Microcosm Study

Microcosms were set up at room temperature ($20 \pm 2^{\circ}$ C) in triplicate in 160 mL glass serum bottles containing 90 mL of the synthetic wastewater described above. A factorial experimental design was used to compare sulfur oxidizing denitrification (6 g elemental sulfur, 2 g crushed oyster shells), scrap tire chip denitrification (10 g tire chips) and T-SHAD (10 g scrap tire chips, 6 g elemental sulfur, 2 g crushed oyster shells). The amount of sulfur added was arbitrary as these experiments were used to provide qualitative data on the impact of scrap tire addition to the sulfur oxidizing denitrification process. Ten mL of activated sludge with a volatile suspended solids (VSS) concentration of 2.69 g L⁻¹ from the Hillsborough County Northwest Regional Wastewater Reclamation Facility (NWRWRF) in Tampa, Florida was added as inoculum into each bottle. The NWRWRF employs a 5-Stage BardenPho process to treat domestic wastewater and achieves effluent total nitrogen (TN) concentrations below 5 mg L⁻¹. A microcosm with synthetic wastewater and activated sludge was used as a control for endogenous decay. A microcosm with no activated sludge or electron donor was used as a control for abiotic removal mechanisms. All bottles were purged with nitrogen gas to remove dissolved oxygen and spiked with KNO₃ to achieve an initial NO₃⁻ -N concentration of 50 mg L⁻¹. Samples were collected over time from the microcosms and water quality measurements were carried out as described below.

5.2.5. Column study

Two 0.5 L acrylic Koflo calibration columns (Cary, Illinois) were used as up-flow packed bed reactors at room temperature ($20 \pm 2^{\circ}$ C). The T-SHAD column was filled with 250 g of scrap tire chips, 40 g of elemental sulfur pellets and 13 g of crushed oyster shell. The amount of elemental sulfur pellets was calculated based on the stoichiometry of sulfur oxidizing denitrification (Batchelor & Lawrence, 1978) and the amount of NO₃⁻ removed from the wastewater projected over one year of operation of the columns (50 mg L⁻¹ NO₃⁻ -N with a flow rate of 2 L d⁻¹) and multiplied by a safety factor 1.3. The amount of crushed oyster shell was calculated based on the ratio of elemental sulfur pellets to crushed oyster shell of 3:1 based on prior research in our laboratory (Sengupta et al., 2007). A second column was filled with 250 g of scrap tire chips to understand the role of the tire chips in denitrification.

The column study was divided into four phases. Phase 1 (20 d) was a start-up period. Both columns were inoculated with activated sludge from the NWRWRF and operated with a closed recirculation system. Each time the NO₃⁻ -N concentration decreased, the columns were re-spiked with NO₃⁻ to enhance attachment and acclimation of the biofilm. Phase 2 (46 d) was a steady flow period. Both reactors were operated with the synthetic wastewater described above with an influent NO₂⁻ -N concentration of 50 mg L⁻¹. The reactors were set at an Empty Bed Contact Time (EBCT) of 6 h by maintaining and influent flow rate 2 L d⁻¹. Phase 3 (13 d) was set-up with a stable NO_{2}^{-1} -N concentration of 50 mg L⁻¹ and a varying flow rate. Two pumps were connected to the column with a timer, which controlled the flow rate over a 24-hour cycle to mimic a DWT system. During the morning and evening hours (6 am-10 am and 6 pm-10 pm) the flow rate was 4.5 L d⁻¹, while during the work hours (10 am-6 pm) the flow rate was 3.0 L d⁻¹ and during the night (10 pm-6 am) the flow rate was 1.5 L d⁻¹. Phase 4 (10 days) was set-up with a stable flow rate of 2 L d⁻¹ and a varying influent NO₃⁻ concentration. One pump and two different influent reservoirs were connected to the column with a timer which controlled the concentration during a day. During the morning and evening hours (6 am-10 am and 6 pm-10 pm) the influent NO_3^- -N concentrations were 100 mg L⁻¹, while during the day and night (10 am-6 pm and 10 pm-6 am) NO_3^- -N concentrations were 35 mg L⁻¹. Phases 1 and 2 were operated with both the T-SHAD and tire only columns, while Phases 3 and 4 were operated with only the T-SHAD column. During Phases 1 and 2, samples were collected daily, while during Phases 3 and 4, samples were collected hourly over three 24-hour cycles.

5.2.6. Analytical Methods

Chloride (Cl⁻), nitrite (NO₂⁻), nitrate (NO₃⁻), phosphate (PO₄³⁻), sulfate (SO₄²⁻), sodium (Na⁺), ammonium (NH₄⁺), potassium (K⁺), calcium (Ca²⁺), and magnesium (Mg²⁺) concentrations were measured by ion chromatography (United States Environmental Protection Agency, USEPA, 1997)

using an 850 Professional Ion Chromatograph (Metrohm AG, Herisau, Switzerland). An Orion 5 Star (Thermo Scientific Inc., Beverly, Massachusetts) meter with a calibrated probe was used to measure pH. *Standard Methods* (APHA, 2012) were used to measure TOC (Method 5310B), TN (4500-N), COD (5220D), BOD₅ (5210B) and alkalinity (2320B). Total iron (Fe^{total}) and ferrous iron (Fe^{2+}) concentrations were measure by the 1-10 phenanthroline method (3500-Fe B). Sulfide (S^{2-}) was measured by the Methylene Blue Method (4500D). The elemental metal composition of the samples was measured using a Perkin-Elmer Elan DRC II Quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS). A semi quantitative analysis (Total Quant Internal Standard Mode) was initially performed to establish the composition of the samples, after which a quantitative analysis was performed.

5.3. Results and Discussion

5.3.1. Nitrate adsorption study

Adsorption of NO_3^- -N to scrap tires is shown in **Figure 5.1**. The rate of adsorption varied with time. For example, with 200 g of scrap tires L⁻¹ the average adsorption rate was 2.6 mg NO_3^- -N L⁻¹ d⁻¹ over the 30 days of the experiment; however, during the first 10 days the adsorption rate was 4.5 mg NO_3^- -N L⁻¹ d⁻¹. The slow, variable NO_3^- removal rates observed in this study may indicate that slow diffusion into the tire material is required prior to adsorption of NO_3^- to interior sites (Smallman & Ngan, 2014). A combination of diffusion, adsorption and slow surface reaction (Zhang & Stanforth, 2005) may also be occurring.



Figure 5.1: NO₃⁻-N adsorption kinetics in synthetic nitrified wastewater with varying amount of scrap tire chips with an initial NO₃⁻-N concentration of 100 mg L⁻¹.

Langmuir and Freundlich equations were used to fit the data from the adsorption experiments using simple linear regression (**Figure 5.2**). For the Freundlich equation, the constants *K* and 1/n were equal to 0.160 and 0.287, respectively. For the Langmuir equation, the equilibrium constant between adsorption and desorption, *b*, was determined to be 0.066, with a maximum capacity, Q_{max} , of 0.657 mg NO₃⁻ -N per g⁻¹ of scrap tires. The sum of squared residuals was 0.004 and 0.001 for
Langmuir and Freundlich isotherms, respectively, resulting in slightly better fit for Freundlich equation.



O Measured Data — Langmuir Isotherm - - - Freundlich Isotherm

Figure 5.2: Results of synthetic nitrified wastewater (initial NO_3 -N concentration = 100 mg L⁻¹) adsorption experiment with Langmuir and Freundlich isotherm and final Langmuir and Freundlich equation.

Results from the short term adsorption/desorption study showed that over three days, 22.5 (± 1.5) mg L^{-1} of NO₃⁻N was removed from the synthetic wastewater, which initially contained 50 mg L^{-1} . After draining the tire chips and refilling the flasks with synthetic wastewater without NO_3^{-} , the liquid phase NO₃⁻-N concentration increased to 10.6 (\pm 0.1) mg L⁻¹ over three days. Although based on our previous tests, the system was not in equilibrium, the concentrations obtained during the short term adsorption/desorption study were consistent with the isotherm results (Figure 5.2). Detailed investigation of adsorption/desorption mechanisms was outside the scope of this research; however, additional experiments and development, calibration and verification of a mathematical model of the T-SHAD process is currently underway in our laboratory. The adsorption capacity achieved in this study with synthetic wastewater was high compared with the results of Lisi (2004), who reported a maximum adsorption capacity of 0.337 mg NO_3^- -N g⁻¹ tire chips using a DI water medium and a seven day contact time. When DI water was used in our study, a maximum adsorption capacity of only 0.024 mg NO_3^- -N g⁻¹ tire chips was observed. There are conflicting reports in literature on the effect of ionic strength on adsorption (Fike, 2001). Increasing ionic strength can decrease adsorption (Borrok & Fein, 2005; Liu et al., 2013; Petruzzelli et al., 2013; Srivastava & Singh, 2010), increase it (Al-Degs et al., 2008) or have no or a very small influence on it (Tan et al., 2009). Since the use of scrap tires as an adsorption media for NO₃⁻ has not been studied extensively, further research is necessary to determine how ionic strength influences adsorption.

During the NO_3^- adsorption study, phosphate was slightly adsorbed to the scrap tires (0.022 mg g⁻¹ of scrap tires), while sulfate was released (0.032 mg g⁻¹ of scrap tires). Ammonium removal was also observed; however, the initial concentration of ammonium was too low to calculate the amount of ammonium as nitrogen (NH_4^+ -N) adsorbed per gram of tire. Calcium was released from the tires

(0.074 mg g⁻¹ of scrap tires) while magnesium was adsorbed (0.079 mg g⁻¹ of scrap tires). Concentrations of nitrite, chloride, sodium, and potassium did not change significantly during the adsorption study. An ion balance showed that while the cations were almost in balance (2.1 mmoL L⁻¹ adsorbed and 1.3 mmoL L⁻¹ released), an anion balance was not achieved (35.9 mmoL L⁻¹ adsorbed and 2.1 mmoL L⁻¹ released).

5.3.2. Leaching study

The initial and final (after leaching for 72 hours) composition of the synthetic wastewater are shown in **Table 5.1**. The mass of each compound released or adsorbed per gram of tire was also calculated. Scrap tire chips slightly consumed alkalinity, resulting in a decrease of pH from 7.4 to 6.9. The concentration of COD in the leachate reached 45.5 mg L⁻¹ (0.34 mg g⁻¹ of scrap tire chips), of which 11.7 mg L⁻¹ was measured as BOD₅ (0.087 mg g⁻¹ of scrap tire chips) and 17.24 mg L⁻¹ was measured as TOC (0.118 mg g⁻¹ of scrap tire chips). The duration of the COD release is discussed in the results from the column study (Section 3.4). The release of bioavailable organic carbon, measured as BOD₅, supports the hypothesis that under the right environmental conditions and in the presence of denitrifying bacteria scrap tire chips can support heterotrophic denitrification. Although specific organic compounds were not measured in this study, Nelson (1994) tested for the presence of a number of toxic organic compounds in tire leachate (118 organic compounds) and found that none were detected at concentrations greater than 1.0 ng L⁻¹ (Nelson et al., 1994). Kellough (1991) found that tires leached very little in the way of metals (28 metal elements), PCBs (28 compounds) or PAHs (18 compounds).

Anion (Cl⁻, NO₂⁻, NO₃⁻, and SO₄²⁻) and cation (Fe²⁺, NH₄⁺, K⁺, and Mg²⁺) concentrations did not significantly change during the three day leaching experiment. Na⁺ and Ca²⁺ concentrations both increased by 0.018 mg per g of tire, while PO₄³⁻ decreased by 0.030 mg per g of tire. Fe³⁺ and S²⁻ concentrations did not increase, indicating, that inorganic electron donors most likely did not play a role in denitrification. Zinc, selenium, manganese, antimony and cobalt were detected by ICP-OES at concentrations of (mg L⁻¹): 0.79, 0.11, 0.05, 0.01, and 0.01, respectively. Zinc has been described by several authors as a potentially toxic component in tires (Camatini et al., 2001; Camponelli et al., 2009; Turner & Rice, 2010). Lead, strontium, boron, uranium, vanadium, barium, iron and arsenic were below detection limits. Metal solubility increases with decreasing pH (Elik, 2007; Gäbler, 1997; Chuan et al., 1996; Martínez & Motto, 2000); however, as discussed previously, little change was observed in pH in this study.

Although toxicity tests were not performed in this study, a number of prior studies have been conducted on the toxicity of scrap tires to aquatic organisms. The sensitivities of organisms differ depending on species tested, type/source of tires, particle sizes and experimental conditions (Day et al., 1993). In general, toxicity increases with decreasing particle size (Gualtieri et al., 2005). Abraded tires have been shown to be toxic to some species (Associates, 2000; Wik et al., 2009), while whole or scrap tire chips similar to the material used in our study resulted in no or low toxicity (Day et al., 1993; Scholes, 2007; Scholes, 2006). The reproduction of *Ceriodaphia dubia* was the most sensitive endpoint tested, while no consistent toxicity was observed in the nematode, *Danio rerio*, or fathead minnows (Day et al., 1993; Scholes, 2006; Wik et al., 2009). Wik (2006) observed the toxicity of abraded tire leachate to *Daphnia magna*, while no toxicity to this species was observed by Kellough (1991) or Day (1993). Kellough (1991) found out there may exist a factor associated with scrap automobile tires that is acutely toxic to Rainbow Trout but due to the type of the chemical analysis

the exact nature of this factor remains unclear. However, mortality among goldfish was very low (Kellough, 1991).

	Synthetic	Scrap Tire	Amount per gram of scrap
Parameter*	wastewater	Leachate	tire
-	mg L⁻¹	mg L ⁻¹	mg g ⁻¹
pH (no units)	7.4	6.9	
Alkalinity	32	32	0.0
(mg CaCO ₃ L) COD	BDL	46	0.34
BOD₅	0.10	12	0.087
тос	1.4	17	0.12
Cl	120	120	0
PO4 ³⁻ -P	7.6	6.3	-0.010
Na⁺	140	140	0
NH4 ⁺ -N	2.9	0.13	-0.020
Ca ²⁺	0.47	2.9	0.018
Mg ²⁺	45	41	-0.031
Zn	0.022	0.82	0.0059
Se	0.0026	0.11	0.00080
Sb	0.00010	0.013	0.000090
Mn	0.018	0.064	0.00034
Со	0.010	0.021	0.000080

Table 5.1: Composition of leachate after 72 hours of exposure of synthetic wastewater to 133 g L^{-1} of scrap tire chips

BDL = below detection limit (3 mg L⁻¹ for COD) *Amounts of other parameters per gram of scrap tire chips in synthetic wastewater (Fe²⁺, Fe^{total}, S²⁻, NO₃⁻⁻N, NO₂⁻⁻N, SO₄²⁻⁻S, TN, K⁺) were too low or undetectable after 72 hours of exposure.

5.3.3. Microcosm study

Concentrations of NO_3^- -N in all microcosms and controls over the 30 day study microcosm are shown in **Figure 5.3a**. The initial NO_3^- -N concentration of 50 mg L⁻¹ decreased to almost zero in the sulfuroyster shell, tire only, and T-SHAD amended microcosms after five days. The microcosms were respiked with NO_3^- between days 5 and 6; both sulfur-oyster shell and T-SHAD microcosms reduced $NO_3^$ to almost zero after 4 days, compared with 7 days for the tire only microcosm. On day 12, the microcosms were again re-spiked with NO_3^- . The NO_3^- -N removal rate in the sulfur-oyster shell and T-SHAD microcosms increased (from 13.76 mg L⁻¹ d⁻¹ to 16.25 mg L⁻¹ d⁻¹ for sulfur-oyster shell microcosm and from 13.28 mg L⁻¹ d⁻¹ to 16.23 mg L⁻¹ d⁻¹⁻ for T-SHAD microcosm), most likely due to acclimation of the microbial population to elemental sulfur and biomass growth. In the tire only microcosms, the NO_3^- removal rate decreased, most likely due to depletion of the limited amount of bioavailable organic carbon that leached from the tires and the attainment of adsorption capacity. A small decrease in NO_3^- -N, from 50 mg L⁻¹ to approximately 44 mg L⁻¹, was observed in the endogenous decay controls. NO_3^- concentrations in the abiotic control microcosms (without activated sludge addition) did not change over 30 days.

Concentrations of sulfate as sulfur $(SO_4^{2^2}-S)$ in all microcosms and controls over the 30 day study microcosm are shown in **Figure 5.3b**. The concentration of $SO_4^{2^2}-S$ in the sulfur-oyster shell microcosm increased with NO_3^- -N removal. The observed $SO_4^{2^2}$ production was slightly lower (approximately 75%) of the production predicted by the stoichiometry reported by Batchelor and Lawrence (1978; Eq. 5.1). This may have been due to organics present in the scleroprotein matrix of the oyster shells serving as an electron donor for partial heterotrophic denitrification (Sengupta et al. 2007) and is consistent with our previous work (unpublished data). In the T-SHAD microcosm, the first 50 mg L⁻¹ of NO_3^- -N was removed without $SO_4^{2^2}$ -S production. After re-spiking NObetween days 5 and 6, an increase in $SO_4^{2^2}$ -S concentrations was observed; however, $SO_4^{2^2}$ production was lower than in the sulfur-oyster shell medium, indicating that mixotrophic metabolism was occurring. In the tire only, endogenous and abiotic control microcosms, $SO_4^{2^2}$ -S concentrations did not change significantly.



Figure 5.3: Denitrifying microcosm study results with synthetic wastewater: a) NO_3^-N concentration over time; b) $SO_4^{2^-}$ -S concentration over time. Discontinuous vertical lines on days 5 and 12 show the dates of re-spiking the microcosm bottles with KNO₃.

5.3.4. Column study

Phase 1 of the column study was carried out with a closed recirculation loop with periodic NO₃⁻ spiking to promote attachment and acclimation of the microbial biofilm. Consistent NO₃⁻ removal was observed in both tire only and T-SHAD columns within the first 20 days of operation (**Figure 5.4**). High COD concentrations were observed in both columns (178 and 201 mg L⁻¹, for the tire only and T-SHAD columns, respectively), most likely due to the accumulation of COD leached from the scrap tires during the closed loop phase of operation.

During Phase 2, both columns were operated under steady influent loading conditions. The average NO_3^- removal efficiencies were 18% and 90% for the tire only and T-SHAD column, respectively (**Figure 5.4** and **Table 5.2**). The average effluent NO_3^- -N concentration in the T-SHAD column was 5.4

mg L⁻¹, which is below the US EPA drinking water standard of 10 mg L⁻¹. COD concentrations decreased dramatically in the effluent during Phase 2 (**Table 5.2**), indicating a lower level of COD leaching from the scrap tires over time under flow through conditions. The average effluent $SO_4^{2^2}$ -S concentration in the T-SHAD column was 46.0 mg L⁻¹. Based on the stoichiometry observed during microcosm study with no tire chips (1.9 g S/g N), 50% of NO₃⁻⁻ was removed by sulfur/oyster shell denitrification, with the balance of the NO₃⁻⁻ removal due to biosynthesis, adsorption and/or heterotrophic denitrification using organics leached from the tires chips. The influent and effluent pH remained near-neutral in both columns. Although sulfur oxidizing denitrification consumes alkalinity, the crushed oyster shells were an effective pH buffer, as has been shown previously (Sengupta et al., 2007).



Figure 5.4: Concentrations of $NO_3^{-}N$ and $SO_4^{2^{-}}S$ in the influent and effluent of T-SHAD and tire only columns: a) $NO_3^{-}N$ in T-SHAD; b) $NO_3^{-}N$ in tire; c) $SO_4^{2^{-}}S$ in T-SHAD; d) $SO_4^{2^{-}}S$ in tire. Vertical line on day 20 separates Phases 1 and 2.

Phases 3 and 4 were set up to simulate the variations in NO_3^- loading rates observed from wastewaters generated from single family homes over the course of day. Only the T-SHAD column was used in these experiments due to the low NO_3^- removal observed with the tire only column in Phase 2. Even with the highly variable loading rates applied, the average effluent NO_3^- -N concentrations in the T-SHAD column during Phases 3 and 4 were 5.6 and 3.7 mg L⁻¹, respectively. None of the measured samples exceeded the US EPA drinking water standard for NO_3^- (**Figure 5.5**). Effluent COD concentrations decreased to 0.1 mg L⁻¹ (**Table 5.2**), indicating that the COD leached from the scrap tires was consumed by biological activity. Effluent SO_4^{2-} concentrations were relatively low (**Figure 5.5**), with the average effluent SO_4^{2-} -S concentrations of 51 and 77 mg L⁻¹, during Phases 3

and 4, respectively. These values are well below the US EPA drinking water guideline of 250 mg L⁻¹, as well as below the prior results achieved in our laboratory with sulfur oxidizing denitrification without tire chips (Sengupta et al., 2007).

Parameter	Influent	Tire Effluent	T-SHAD Effluent
Phase 2 results			
NO ₃ ⁻ -N [mg L ⁻¹]	51.5	42.1	5.4
SO4 ²⁻ -S [mg L ⁻¹]	0.0	0.2	46.0
NO ₂ ⁻ -N [mg L ⁻¹]	0.5	0.5	0.4
рН	7.8	7.6	7.2
COD [mg L ⁻¹]	0.0	17.8	8.3
Phase 3 results			
NO ₃ ⁻ -N* [mg L ⁻¹]	51.0		5.6
SO4 ²⁻ -S* [mg L ⁻¹]	0.2		51.5
NO ₂ ⁻ -N* [mg L ⁻¹]	0.3		0.3
рН	7.5		7.2
COD [mg L ⁻¹]	0.0		0.1
Phase 4 results			
NO ₃ ⁻ -N* [mg L ⁻¹]	57.9		3.7
SO4 ²⁻ -S* [mg L ⁻¹]	0.1		77.1
NO ₂ ⁻ -N* [mg L ⁻¹]	0.3		0.3
рН	6.9 and 7.0**		7.0
COD [mg L ⁻¹]	0.0		0.1

Table 5.2: Summary of average concentrations of $NO_3^{-}N$, SO_4^{-2} -S, pH and COD in the influent, tire column effluent and T-SHAD column effluent during Phases 2, 3 and 4.

* flow weighted averages

**pH in the first and second reservoir with NO₃⁻N concentration of 100 and 35 mg L^{-1} .



Figure 5.5: Concentrations of NO_3^- -N and $SO_4^{2^-}$ -S in the influent and effluent in the T-SHAD column under variable flow (Phase 3) and NO_3^- -N concentrations (Phase 4): a) NO_3^- -N in Phase 3; b) NO_3^- -N in Phase 4; c) $SO_4^{2^-}$ -S in Phase 3; d) $SO_4^{2^-}$ -S in Phase 4. (Note the different scales of diagram b and d)

A mathematical model that combines adsorption and biological denitrification under dynamic loading conditions is currently under development in our research group to investigate the removal mechanisms in the T-SHAD system further and to provide guidance for design engineers.

5.4. Conclusions

The T-SHAD process is a promising alternative for denitrification of nitrified wastewater in DWT systems. The low effluent NO_3^- concentrations observed under highly variable loading conditions applied in this study indicated that the combination of adsorption and denitrification will help buffer the variations in loadings observed in typical DWT systems. During periods of high NO_3^- loading, both adsorption and denitrification will remove NO_3^- while during low loading periods desorption of NO_3^- can prevent starvation conditions for the denitrifying community. The adsorption capacity for NO_3^- onto scrap tire observed in this study of 0.657 mg NO_3^- -N g⁻¹ was higher than the capacity observed by other authors. Although organic carbon leaching was not high enough to completely denitrify wastewater via heterotrophic metabolism, the bioavailable organic carbon leached from the tires could promote mixotrophic denitrification, resulting in reduced effluent $SO_4^{2^-}$ concentrations in the T-SHAD effluent. Preliminary results and a review of the literature indicate that leaching of zinc and other potential toxicants are unlikely to pose a problem to aquatic systems.

6. CONCLUSIONS & RECOMMENDATIONS

Microaeration, i.e. dosing of a limited amount of air inside of an anaerobic reactor to oxidize gaseous and liquid sulfide to elemental sulfur, is the central topic of this PhD thesis. Two types of microaeration have been tested: microaeration applied directly in anaerobic reactor (where air was dosed inside a CSTR or UASB reactor), and microaeration in biomembrane (where air was dosed in the gas space of an anaerobic reactor with biomembrane placed inside).

Although the interest in microaeration for hydrogen sulfide removal from biogas in full-scale has been steadily growing, only over 50 papers on this topic have been published during the last decade (searched April 2017). Microaeration has been widely applied in full-scale anaerobic digesters of the CSTR type aiming at biogas production (biogas plants). However, microaeration in high-rate anaerobic reactors for wastewater treatment (such as UASB reactors) has been rarely studied or applied. Microaeration through a biomembrane is a completely new topic and only a few papers on this topic have been published so far, dealing with biomembranes for microaerobic sulfide removal in the liquid phase. To the author's best knowledge, biomembranes for microaerobic sulfide removal in the gas phase have not yet been discussed in literature.

6.1. Application of microaeration directly in the anaerobic reactor

H₂S removal efficiencies and sulfur balance

Microaeration has the potential to remove large quantities of hydrogen sulfide from biogas formed by the anaerobic treatment of sulfur-rich waste streams. Microaeration can be applied to UASB reactors, containing granular sludge and treating wastewater streams containing merely soluble organic matter, as well as to CSTRs with suspended biomass treating waste streams with a larger amount of particulate matter.

In lab-scale microaerobic UASB reactor (Section 3.3), the efficiency of hydrogen sulfide removal from biogas was on average 73% (with the oxygen dose corresponding to the stoichiometric amount) for an initial concentration of H_2S in biogas as high as 9,000 mg m⁻³. When dosing double of the stoichiometrically required amount of oxygen, the efficiency increased to 92% (data not published).

Microaeration significantly affects the sulfur distribution. In general, sulfur from the gas phase (as H_2S) and liquid phase (as HS^{-}) was transformed into solid sulfur (S^{0}). Sulfur removed from the biogas and the liquid in a microaerobic UASB reactor was present as inorganic suspended solids in the effluent and partly accumulated on the wall of the head space and in the G-L-S separator. Sulfur deposition in the granular sludge was not significant.

In a microaerobic CSTR (Section 3.2), more than 99% of H_2S was removed compared to a completely anaerobic CSTR (from 17.600 mg H_2S m⁻³ in an anaerobic CSTR to 52 mg H_2S m⁻³ in a microaerobic CSTR on average). Slightly higher content of the total sulfur and lower content of the sulfide in the microaerobic sludge compare to anaerobic sludge indicated the accumulation of elemental sulfur.

Other technologically important aspects

For UASB reactors, no negative effect of microaeration on the methanogenic activity of granular sludge was observed in terms of methane production. The production of biogas and the efficiency of COD removal was not affected either.

Granules from both reactors remained compact and unchanged throughout the operation.

In the CSTR, no negative effect of microaeration on the methanogenic activity was observed in terms of methane production.

Sludge quality in anaerobic and microaerobic reactors was similar, only small differences were found in sludge compositions (i.e. the total sulfide concentration in microaerobic CSTR was lower compare to strictly anaerobic CSTR). The sludge digested under the microaerobic conditions had lower foaming potential and foam stability. Dewaterability was better for microaerobic sludge. The composition of sludge liquor differed mainly in soluble COD concentration, which was significantly lower (by 33% on average) in microaerobic digester.

Modeling microaeration

Literature review (Chapter 2) pointed out that a mathematical model describing microaeration for biogas desulfurization was not yet available. Since such model could greatly improve the understanding of the process, an anaerobic digestion model with sulfur and oxygen (ADM1-S/O) was set up to describe and control sulfate reduction and sulfide oxidation in anaerobic and microaerobic environments (Section 3.4).

The model showed a good fit to the experimental data in terms of H_2S emissions and biogas flow. The composition of active biomass in the microaerobic reactor was not significantly affected by microaeration according to the model. Sulfur oxidizing bacteria only made up a small fraction.

However, the results of sulfur balance revealed the limitations of the present model as it predicted higher H_2S concentration in the effluent. For microaeration, where oxygen is blown directly into the liquid phase, aerobic carbon oxidation and re-reduction of elemental sulfur back to sulfide should be considered.

Full-scale operation

The results of long-term full-scale operation of seven sludge digesters in Europe (Section 3.5) confirmed that the biogas desulfurization by microaeration has become a mature technology. The application of the microaerobic conditions is a simple, highly efficient and stable method for hydrogen sulfide removal from biogas. The main benefit from microaeration is the ability to remove sulfide inside the anaerobic reactor without needing to build a separate desulfurization unit and without additional chemicals.

The start-up of full-scale microaerobic biogas desulfurization takes a few weeks due to the adaptation of sulfide oxidizing bacteria. A hydrogen sulfide removal efficiency of about 99% can be achieved even at high influent H_2S concentrations (above 4,000 mg m⁻³). The achievement of hydrogen sulfide concentrations as low as in tens of mg m⁻³ is realistic.

Microaeration at full-scale caused the decrease of the VSS/TSS ratio of the digested sludge due to the better efficiency in VSS degradation. Also, a decrease of soluble COD concentration in the sludge liquor was observed in all microaerobic systems.

6.2. Application of microaeration in biomembranes

Direct microaeration in CSTR or UASB reactors can have certain disadvantages such as high amounts of nitrogen in the biogas and/or undesirable elemental sulfur deposits in biogas pipelines. The alternative, novel concept of microaeration through biomembranes was examined in Chapter 4. Biomembranes serve as a support for biomass growth (biofilm) and provide a surface for elemental sulfur precipitation thus avoiding its accumulation in the pipeline. Moreover, the separation of biogas and air decrease biogas dilution by nitrogen. Possible disadvantage of biomembrane are the loss of methane through the membrane and the higher cost of the overall desulfurization equipment.

The ability of biomembrane unit to remove H_2S from biogas was demonstrated at lab-scale (Section 4.1) as well as at pilot scale (Section 4.2). In the lab-scale system, selective gas permeation through membrane decreased in the following order: $H_2S > CO_2 > CH_4 > O_2 > N_2$. In the continuous lab-scale experiment, H_2S removal efficiency was more than 99%. Methane losses accounted for 7%, while the biomembrane was not fully covered with biofilm. Methane losses and nitrogen and oxygen biogas contamination decreased with increasing membrane coverage with biofilm. Light yellow deposits on the membrane indicated elemental sulfur formation. PCR and DGG proved the presence of sulfide oxidizing bacteria, *Thiobacillus thioparus*.

In the pilot-scale system, commercial PVDF membrane appeared to have the best features (strength, durability, and ability to retain liquid) to serve as a biomembrane. Both chemical and biochemical sulfide oxidation were observed, the biological sulfide oxidation rate being four times faster. The amount of air needed for complete H₂S removal from biogas with no oxygen and nitrogen leftovers in biogas was found five times larger than the theoretical stoichiometry amount. Methane losses were 3.7% of the total methane production. The specific H₂S removal was not affected by the amount of air blown into the CSTR's gas space.

6.3. Possible application of recovered sulfur

One of the advantages of microaeration is the production of elemental sulfur, which could be recovered from the system.

In this thesis, the possible application of recovered elemental sulfur was tested through its use as an electron donor for autotrophic, sulfur oxidizing denitrification (with the combination of the adsorption process) (Chapter 5). The Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process turned out to be a promising alternative for the denitrification of nitrified wastewater in decentralized wastewater treatment systems.

6.4. Research perspectives

Identification of reaction mechanisms and affecting factors

The exact biological and chemical reaction mechanisms involved in sulfide oxidation under microaerobic conditions have not yet been completely elucidated. Besides, it is necessary to identify and evaluate the factors affecting these reactions and the H₂S removal as such: mixing, reactor geometry and mass transfer phenomena.

To find out how to recover elemental sulfur from the system

One of the advantages of microaeration is the production of elemental sulfur, which can be recovered for further use and its possible recovery and usage. According to Kleinjan et al. (2003), the biologically produced elemental sulfur is more available for other biological processes than chemically produced elemental sulfur. However, the exact method for recovery was not yet developed. For that, further research of elemental sulfur collection is needed most likely in full-scale.

Mathematical modeling

A mathematical model describing microaeration in a UASB reactor was set up in this thesis (Section 3.4). In this model, only biological sulfide oxidation to elemental sulfur was taken into account, while chemical sulfide oxidation was neglected. Also additional reactions are missing and should be taken into account such as: further oxidation of elemental sulfur to sulfate if oxygen level exceeds certain limit, re-reduction of elemental sulfur and/or sulfate back to sulfide, enhanced hydrolysis with higher level of oxygen and aerobic oxidation of soluble carbon.

Also mathematical model of microaeration in different systems (such as CSTR with suspended solids sludge) should be addressed.

The mathematical model of microaeration in biomembranes is completely missing and constitutes an important research gap.

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8. APPENDIX

8.1. Supplementary material of Section 3.1.

S.3.1.1 Lowry calibration

The quantification (concentration in $\mu g m L^{-1}$) of *Sulfuricurvum kujiense* in samples was measured by Lowry's method - the spectrophotometric measurements of protein (Waterborg & Matthews, 1984). The calibration curve is presented in Figure S1. The COD concentration of 1 mg *Sulfuricurvum kujiense* corresponds to 485 mg.



Figure S3.1.1: The calibration curve for Sulfuricurvum kujiense

S.3.1.2 Maximum sulfide uptake rate

The maximum sulfide uptake rate (482 mmoL S.mg COD^{-1} .h⁻¹) was determined based on the experiments where the maximum uptake of sulfide by *Sulfuricurvum kujiense* over period of time was measured (**Table S3.1.1**).

Time	Sulfuricurvum kujiense			S ²⁻	dil.	S ²⁻	k _{m.H2S.SOB}
h	Abs.	μg mL ⁻¹	mg COD L ⁻¹	mg L ⁻¹	-	mmoL L ⁻¹	mmoL S ²⁻ mg ⁻¹ COD h ⁻¹
1	0.0266	4.4	2135	15.95	500	249	
2	0.0274	4.8	2330	10.81	500	169	514
3	0.0280	5.1	2477	8.29	500	130	447
4	0.0288	5.5	2673	6.82	250	53	486
							482

Table S3.1.1: Maximum sulfide uptake rate determination

S.3.1.3 Decay rate

Decay rate constant (0.24 h^{-1}) was calculated based on the experiment where the decrease of *Sulfuricurvum kujiense* concentration over period of time was measured (**Figure S2**).



Figure S3.1.2: Decay rate determination

S.3.1.4 Yield coefficient

Yield coefficient (10.37 mg COD mmoL⁻¹ S²⁻) was determined based on the growth of *Sulfuricurvum kujiense* over the decrease of sulfide concentration during the period of time (**Table S3.1.2**).

time h	с _{ѕов} mg COD L ⁻¹	C _{S2-} mg L ⁻¹	SOB growth mg COD L ⁻¹ h ⁻¹	S ²⁻ removed mg L ⁻¹ h ⁻¹	Yield mg COD mg ⁻¹ S ²⁻
0	23.435	0.190	0.0000	0.0000	0.00
1	23.445	0.142	0.0102	0.0480	0.21
2	23.464	0.100	0.0183	0.0420	0.44
3	23.512	0.100	0.0481	0.0000	
					0.32

Table S3.1.2: Yield coefficient determination

8.2. Supplementary material of Section 3.4.

S.3.4.1 Biological conversion processes - ADM1-S/O

The stoichiometric matrix, composition matrix and kinetic expressions for ADM1-S/O are summarized in **Tables S3.4.1** and **S3.4.2**. **Tables S3.4.3**, **S3.4.4** and **S3.4.5** list the corresponding stoichiometric, kinetic and physicochemical parameters, respectively.

pH inhibition (I_{pH}) was described the same as in ADM1 (Rosen & Jeppsson, 2006):

$$I_{pH} = e^{-3 \cdot \left(\frac{pH - pH_{UL}}{pH_{UL} - pH_{LL}}\right)^2}$$
Eq. S3.4.1

where pH is the actual value in UASB reactor, pH_{UL} is the pH value with no inhibition and pH_{LL} is the pH value with full inhibition.

Nitrogen "inhibition" ($I_{N,limit}$) was also described analogously to ADM1, with a lower limit of 10^{-6} to avoid numerical problems when nitrogen is limiting. (Batstone et al., 2002):

$$I_{N,limit} = \frac{S_{TIN}}{K_{S,IN} + S_{TIN}} \quad for \ S_{TIN} \ge K_{S,IN}$$
Eq. S3.4.2a

$$I_{N,limit} = 10^{-6}$$
 for $S_{TIN} < K_{s,IN}$ Eq. S3.4.2b

where S_{TIN} is the concentration of inorganic nitrogen and $K_{s,IN}$ is the inorganic nitrogen concentration at which the growth ceases, 0.0001 M (Batstone et al., 2004).
Chapter 8 – Appendix

Components i →	1	2	3	4	5	6	7	8	9	9a	90 _A	ap ^B	90	9e	भ	10	11	
Processes j↓	S_su	S_aa	S_fa	S_Tva	S_Tbu	S_Tpro	S_Tac	S_h2	S_ch4	S_so4	S_hs-	S_h2s	\$_o2	S_s	S_hco3-	S_co2	S_TIN	-
Disintegration	200							1						-		Cxo-Cxo. bd.xc-Cost.fot.xc-Caa.Tpr.xo-Cau.Toh.xc-Ca.fi.xo	N _{X0} -N _{X1} ,T _{X1,X0} -N _{S1} ,T _{S1,X0} -N _{B0} ,T _{pr}	38
Hydrolisis of Carbohydrates	1													_		0 (C ₅₀ -C ₅₀)	0	_
lydrolysis of Proteins		1														0 (C _{aa} -C _{aa})	0 (N _{aa} -N _{aa})	
Hydrolysis of Lipids	1-f _{l.xe}		filxe													$C_{ii}\text{-}C_{si}^{'}(1\text{-}f_{i},\chi_{0})\text{-}C_{is}^{'}f_{ii}\chi_{0}$	0	
Uptake of Sugars	:-1				(1-Y _{su}).f _{bu.su}	(1-Y _{su}).f _{pro,su}	(1-Y _{su}).f _{so,su}	(1-Y _{su}).f _{r2.su}								$C_{su} - C_{bu}, (1 - Y_{su}).f_{bu,su} - C_{pro}, (1 - Y_{su}).f_{pro,su} - C_{so}(1 - Y_{su}).f_{so,su} - C_{bom}, Y_{su}$	-Noom-Ysu	_
Uptake of Amino Acids		-1		(1-Y _{aa}).f _{v.a,aa}	(1-Y _{aa}).f _{ou,aa}	(1-Y _{aa}).f _{pro,aa}	(1-Y _{aa}).f _{so,aa}	(1-Y _{aa}).f _{*2,aa}								$C_{aa} - C_{va} - (1 - Y_{aa}) \cdot f_{va, aa} - C_{bv} \cdot (1 - Y_{aa}) \cdot f_{bu, aa} - C_{pro} \cdot (1 - Y_{aa}) \cdot f_{pro, aa} - C_{ac} (1 - Y_{aa}) \cdot f_{ac, aa} - C_{borr} \cdot Y_{aa} - Y_{a$	N _{aa} -Y _{aa} .N _{biom}	
Uptake of LCFA			-1				(1-Y _{fa}).0.7	(1-Y _{fa}).0.3								C_{f_3} - C_{a_5} .(1- Y_{f_3}).0.7- C_{blors} . Y_{f_3}	-Nhom-Yfa	
Uptake of Valerate				÷-1		(1-Y _{c4}).0.54	(1-Y _{c4}).0.31	(1-Y _{o4}).0.15								$C_{va}\text{-}C_{po}.(1\text{-}Y_{o4}).0.54\text{-}C_{ao}.(1\text{-}Y_{o4}).0.31\text{-}C_{blam}.Y_{o4}$	-N _{barn} .Y ₆₄	
Uptake of Butyrate					-1		(1-Y ₌₄).0.8	(1-Y _{c4}).0.2								$C_{tar,r}C_{tac:}(1\text{-}Y_{c4}).0.8\text{-}C_{tabren},Y_{c4}$	-Norm-Ye4	
Uptake of Butyrate by bSRB					-1		(1-Y _{DSRD}).0.8			$-\frac{(1-Y_{hSRB})}{64}$		$\frac{(1-Y_{hSRB})}{64} \cdot 0,2$				$C_{bu'}C_{a_0}.((1\text{-}Y_{\text{LGH}B}),0,8)\text{-}C_{bion}.Y_{\text{LGH}B}$	-N _{biom} , Y _{bSRB}	_
Uptake of Propionate						-1	(1-Y _{pro}).0.57	(1-Y _{po}).0.43								C _{pro} -C _{ac} . (1-Y _{pro}).0.57-C _{bion} . Y _{pro}	-N _{biom} .Y _{pro}	
Uptake of Propionate by pSRB						-1	(1-Y _{pSRD}).0.57			$-\frac{\left(1-Y_{pSRB}\right)}{64}\cdot0,43$		$\frac{(1-Y_{pSRB})}{64} \cdot 0,43$				$C_{\mu ro} \cdot C_{\alpha s} \cdot ((1 - Y_{\mu S \cap B}) \cdot 0.57) \cdot C_{b \cup m} \cdot Y_{\mu S \cap D}$	-N _{tokom} , Y _{pSRB}	
Uptake of Acetate							-1		(1-Y _{ac})							$C_{ac}\text{-}C_{chc}\text{-}(1\text{-}Y_{ac})\text{-}C_{b(cm)}\text{-}Y_{ac}$	-N _{born} .Y _{ac}	
Uptake of Acetate by aSRB							-1			$-\frac{(1-Y_{aSRK})}{64}$		$\frac{(1 - Y_{asses})}{64}$				C _{ac} -C _{birn} .Y _{aSR9}	-N _{biom} .Y _{aGRB}	
Uptake of Hydrogen								-1	(1-Y _{b2})							-C _{0.04} (1-Y ₁₂)-C _{biorn} Y ₁₂	-N _{born} .Y _{h2}	
Uptake of Hydrogen by hSRB								-1		$-\frac{(1-Y_{hSRB})}{64}$		$\frac{(1-Y_{ASRB})}{64}$	Antonia anton al			-C _{Norn} , Y _{hERB}	-Noom.Yhsra	
Uptake of H ₂ S by X _{SOB}												-1	$-\frac{(16-Y_{SOB})}{32}$	1	1	-C _{stors} ,Y _{SOB}	-N _{btom} -Y _{SOB}	
Decay of X _{au}																C _{bion} -C _{xb}	Nbiom-Nxc	_
Decay of X _{aa}																C _{telom} -C _{Xe}	N _{biern} -N _{2c}	_
Decay of X _{fa}																C _{tritom} C _{Xi}	N _{blorn} -N ₃₆	_
Decay of X _{c4}																Chion-C _{Xc}	N _{biom} -N _{xc}	_
Decay of X _{bSRB}																Ctaion-Cxo	N _{biom} -N _{Xic}	_
Decay of X _{pro}																Colom-Cxc	N _{biom} -N _{Xc}	_
Decay of X _{pSRB}																C _{biom} -C _{Xe}	N _{biom} -N _{Xc}	
Decay of X _{ac}																C _{taiom} C _{Vic}	N _{bicm} -N _{Xic}	
Decay of X _{aSRB}																C _{brom} -C _{xc}	N _{brom} -N _{Xc}	
Decay of X _{h2}																C _{tritore} -C _{Xi}	N _{biern} -N ₂₆	
Decay of X _{hSRB}																C _{blom} -C _{xc}	N _{blom} -N _{Xc}	
Decay of X _{SOB}																C _{biom} -C _{Xe}	N _{biom} -N _{Xc}	
H ₂ S acid-base reaction											-1	1						
CO ₂ acid-base reaction															ч	1		
position matrix										· · · · · · · · · · · · · · · · · · ·								_
D per unit	1	1	1	1	1	1	1	1	1	0	64	64	-32	48	0	0	0	
N per unit	0	Nas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
S per unit	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	
C per unit	Csu	Cas	Cra	Cra	Cou	Cpro	Cac	0	C _{ab4}	0	0	0	0	0	1	1	0	
	charides (kg	acids (kg COD/m ³)	(g COD/m ³)	alerate (kg COD/m)	tryrate (kg COD/m ³)	nopionate (kg	cetate (kg COD/m [*])	en gas (kg	ie (kg COD/m ³)	ufate (kmol S/m [*])	en sulfide ion S/m ⁵)	en sulfide (kmole	(kmole O ₂ /m ³)	ital sulfur (kmole	onate (kmole C/m ³)	iic carbon (kmola	tic nitrogen (kmole	

Table S3.4.1: Stoichiometric matrix and composition matrix of ADM1-S/O part 1: soluble components. Based on ADM1 (Batstone et al., 2002; Fedorovich et

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Components i →	13	14	15	16	17	18	19	20	20a	21	21a	22	22a	23	23a	23b	24	Browner rate (o, kg COD m ² d ⁻¹)
A∥ Processes j↓	X_c	X_ch	X_pr	X_li	X_su	X_aa	X_fa	X_c4	X_bSRB	X_pro	X_pSRB	X_ac	X_aSRB	X_h2	X_hSRB	X_SOB	X_I	Process rate (p),kg cob in 't ')
1 Disintegration	-1	6h.xc	fpr,xa	filixe													ha,xo	$\rho_1 = k_{att} \cdot X_r$
2 Hydrolisis of Carbohydrates		-1																$\rho_2 = k_{hyd,ch} \cdot X_{ch}$
3 Hydrolysis of Proteins			-1															$\rho_3 = k_{byd,pr} \cdot X_{pr}$
4 Hydrolysis of Lipids				-1														$\rho_5 = k_{krd,ii} \cdot X_{ii}$
5 Uptake of Sugars					Yna													$\rho_{\mathrm{fi}} = k_{m,\mathrm{stat}} \cdot \frac{S_{\mathrm{stat}}}{K_{5,\mathrm{stat}} + S_{\mathrm{stat}}} \cdot \mathbf{X}_{\mathrm{stat}} \cdot I_{\mathrm{B}\mathrm{B}\mathrm{A}\mathrm{S}\mathrm{stat}} \cdot I_{\mathrm{N},\mathrm{D}\mathrm{stat}} \cdot I_{\mathrm{O}_{\mathrm{Z}}}$
6 Uptake of Amino Acids						Y _{ea}												$\rho_{h} = k_{max} \cdot \frac{S_{xx}}{K_{\tau, \rho, \theta} + S_{\rho, \theta}} \cdot X_{xx} \cdot I_{p \#, blow} \cdot I_{n, blow} \cdot I_{\rho_2}$
7 Uptake of LCFA							Yfa											$\rho_{\tau} = k_{m,to} \cdot \frac{S_{to}}{K_{S,to} + S_{to}} \cdot X_{to} \cdot I_{pB,Mom} \cdot I_{RB,Mm} \cdot I_{n2,to} \cdot I_{U_2}$
8 Uptake of Valerate								Y _{oé}										$\rho_{\mathrm{B}} = k_{\mathrm{m,ret}} \cdot \frac{S_{\mathrm{ret}}}{K_{\mathrm{N},\mathrm{ret}} + S_{\mathrm{ret}}} \cdot X_{\mathrm{ct}} \cdot \frac{1}{1 + S_{\mathrm{rm}}/S_{\mathrm{ret}}} \cdot I_{\mu\mathrm{B,\mathrm{Rom}}} \cdot I_{\mathrm{N}\mathrm{B,\mathrm{rm}}} \cdot I_{\mathrm{k}\mathrm{Z}\mathrm{ct}} \cdot I_{\mathrm{N}\mathrm{2s}} \cdot I_{\mathrm{O}_2}$
9 Uptake of Butyrate								Y ₀₄										$\rho_{9} = k_{m,c4} \cdot \frac{S_{m4}}{K_{2,c4} + S_{Bn}} \cdot X_{c4} \cdot \frac{1}{1 + \frac{S_{ra}}{S_{Bn}}} \cdot I_{pn,show} \cdot I_{N,dm} \cdot I_{a2,c4} \cdot I_{h2a} \cdot I_{H2}$
9a Uptake of Butyrate by bSRB									Y _{INSRR}									$\rho_{96} = k_{m,05.66} \cdot \frac{S_{ba}}{K_{5,07.06} + S_{aa}} \cdot X_{b5.66} \cdot \frac{S_{504}}{K_{5,504,05.07}} \cdot J_{pul,bi.com} \cdot J_{n,56n} \cdot J_{h2.8}$
10 Uptake of Propionate										Ypea								$\rho_{16} = k_{m,pro} \cdot \frac{S_{pro}}{k_{S,pro} + S_{pro}} \cdot X_{pro} \cdot I_{pfl,Nom} \cdot I_{N,lon} \cdot I_{h2,pro} \cdot I_{h2s} \cdot I_{O_S}$
10a Uptake of Propionate by pSRB											Yperr							$\rho_{10u} = k_{(n,p3KB)} \cdot \frac{S_{pro}}{K_{X,p3RN} + S_{pro}} \cdot X_{p3NB} \cdot \frac{S_{504}}{K_{X,y304,p3NB} + S_{304}} \cdot I_{pi1,biors} \cdot I_{n,lon} \cdot I_{k20}$
11 Uptake of Acetate												Yac						$p_{11} = k_{m,ac} \cdot \frac{S_{ac}}{K_{s,ac} + S_{ac}} \cdot X_{ac} \cdot I_{pll,ac} \cdot I_{sllon} \cdot I_{sllon} \cdot I_{hls} \cdot I_{hls} \cdot I_{a_2}$
11a Uptake of Acetate by aSRB													Yeshb					$\mu_{11a} = k_{\text{im}, aSAR} \cdot \frac{S_{ac}}{K_{SASRB} + S_{ac}} \cdot X_{aSRR} \cdot \frac{S_{SOA}}{K_{SSOA} + S_{SOA}} \cdot I_{\text{BR,oc}} \cdot I_{\text{R,SOR}} \cdot I_{\text{RSN}}$
12 Uptake of Hydrogen														Y _{h2}				$\rho_{12} = k_{10,112} \cdot \frac{S_{b2}}{K_{5,h2} + S_{h2}} \cdot X_{h2} \cdot I_{2,16,82} \cdot I_{N,000} \cdot I_{B24} \cdot I_{O_2}$
12a Uptake of Hydrogen by hSRB															YHSRB			$\rho_{12n} = k_{mhSPB} \cdot \frac{S_{n2}}{K_{S,hSRB} + S_{k2}}, \\ K_{hSRB} \cdot \frac{S_{N04}}{K_{S,04,hSRB} + S_{504}}, \\ i_{FBLh2} \cdot i_{FAlm} \cdot i_{h2s}$
12b Uptake of H ₂ S by X _{SOB}																Y _{SOB}		$\rho_{12b} = k_{v1,SOD} \cdot \frac{S_{h2s}}{K_{Sh2s,SOB} + S_{h2s}}, X_{SOD} \cdot \frac{S_{O2}}{K_{SO2,SOB} + S_{O2}}, I_{v1l,Storn} \cdot I_{R1lm}$
13 Decay of X _{su}	1				-1												è.	$\rho_{13} = k_{stor, dsn} \cdot X_{su}$
14 Decay of X ₈₈	1				2	-1											2	$\rho_{14} = k_{\sigma \sigma c x \sigma a} \cdot X_{a \sigma}$
15 Decay of X _{fa}	1						्न											$\rho_{15} = k_{dvc,Xfu} \cdot X_{fu}$
16 Decay of X _{c4}	1							्न							1			$\rho_{16} = k_{dec,Xct} \cdot X_{cb}$
16a Decay of X _{c45RB}	1								-1									$\rho_{14a} = k_{asc.sbstr} \cdot X_{castr}$
17 Decay of X _{pro}	1					_				-1								$\rho_{17} = k_{dev,Xyro} \cdot X_{yro}$
17a Decay of X _{pSRB}	1	-					-				-1							$\rho_{1/a} = k_{dscXpSRB} \cdot X_{pSRD}$
18 Decay of X _{sc}	1											-1				-		$\rho_{18} = k_{doc,sac} \cdot X_{ac}$
18a Decay of X _{aSRB}	1				-								-1					$\rho_{20a} = k_{decXaSRD} \cdot X_{aSRD}$
19 Decay of X _{h2}	1								-					-1	54			$\rho_{19} - k_{dsc, \chi h 2} \cdot \chi_{h 2}$
19a Decay of X _{hSRB}	1				-	-	-				-		-		-1			$\rho_{19a} = k_{doc,XhSKB} \cdot X_{hSKB}$
19b Decay of X _{sos}	1					-	-		·							-1		$\rho_{1yb} = k_{dec,xsow} \cdot X_{xow}$
																		$\rho_{A1} = K_{AB,B_{J},S} \cdot \left(S_{HS} - S_{H^{-1}} - \frac{K_{B,B_{J},S}}{S_{H_{2},S}} \right)$
																		$\rho_{A2} = K_{AB,CO_2} \cdot \left(S_{HCO_4} - \cdot S_H + - \frac{K_{B,CO_2}}{S_{CO_2}}\right)$
Composition matrix			0.00	1 8 1	1 2	1 2	ř – –	1 2	1 2	1	1	1 2			1 27		2	
g COD g ⁻¹ component	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
g N g ⁻¹ component	N _{X0}	0	N _{aa}	0	Neiom	Noom	Npiom	Nbom	Ntion	Nerom	Noom	N _{biom}	Noon	Ntrom	Noiom	Noram	N _A	
g S g ⁻¹ component	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
g C g ⁻¹ component	C _{Xu}	Csu	Caa	Cii	Ctoiom	Cuiom	Cbiom	Chiom	Ction	Ction	Cularn	Chiom	Cbiom	Ctiom	Culom	Cbiam	C _N	
	Composites (kg COD/m ³)	Carbohydrates (kg	Proteins (kg COD/m ⁸)	lpids (kg COD/m ²)	sugar degradens (kg COD/m ³)	Amino acids degraders (kg	.CFA degradens (kg SOD/m²)	/alerate and butyrate legraders (kg COD/m ³)	SRB valerate and butyrate legraders (kg COD/m ³)	Propionate degraders (kg	SRB propionate degraders kg COD/m ³)	Voetate degraders (kg	Acetotrophic SRB (kg SOD/m ²)	Vurogen degraders (kg	W drogenotrophic SRB (kg	50B degraders (kg 50D/m ²)	Particulate inerts (kg	

Table S3.4.2: Stoichiometric matrix and composition matrix of ADM1-S/O part 2: particulate components. Based on ADM1 (Batstone et al., 2002; Fedorovich et al., 2003). The processes 9a, 10a, 11a, 12a, 12b, 16a, 17a, 18a, 19a and 19b concern additional processes compared to ADM1.

Symbol	Description	Value	Unit	Reference
Carbon	content of:			
C _{aa}	amino acids	0.03000	mole C g COD ⁻¹	Batstone et al. (2004)
C_{ac}	acetate	0.03125	mole C g COD ⁻¹	Batstone et al. (2004)
C_{biom}	biomass	0.03125	mole C g COD ⁻¹	Batstone et al. (2004)
C_{bu}	butyrate	0.02500	mole C g COD ⁻¹	Batstone et al. (2004)
C_{ch4}	methane	0.01563	mole C g COD ⁻¹	Batstone et al. (2004)
C_{fa}	LCFA	0.02170	mole C g COD ⁻¹	Batstone et al. (2004)
C _{li}	lipids	0.02200	mole C g COD ⁻¹	Batstone et al. (2004)
C_{pro}	propionate	0.02679	mole C g COD ⁻¹	Batstone et al. (2004)
C _{SI}	soluble inert	0.03000	mole C g COD ⁻¹	Batstone et al. (2004)
C_{su}	sugars	0.03125	mole C g COD ⁻¹	Batstone et al. (2004)
C_{va}	valerate	0.02404	mole C g COD ⁻¹	Batstone et al. (2004)
C _{xc}	complex particulate	0.02790	mole C g COD ⁻¹	Batstone et al. (2004)
C _{XI}	particulate inert	0.03000	mole C g COD ⁻¹	Batstone et al. (2004)
Nitroger	n content of:			
N_{aa}	amino acids	0.00700	mole N g COD ⁻¹	Batstone et al. (2004)
N_{biom}	biomass	0.00625	mole N g COD ⁻¹	Batstone et al. (2004)
N _{SI}	Sı	0.00200	mole N g COD ⁻¹	Batstone et al. (2004)
N_{xc}	X _c	0.00200	mole N g COD ⁻¹	Batstone et al. (2004)
N _{XI}	X	0.00200	mole N g COD^{-1}	Batstone et al. (2004)
Yield of	product from degradation of subs	trate:		
$f_{ac,aa}$	acetate from amino acid	0.40000	-	Batstone et al. (2002)
$f_{ac,su}$	acetate from sugar	0.40755	-	Batstone et al. (2002)
f _{bu,aa}	butyrate from amino acids	0.26000	-	Batstone et al. (2002)
f _{bu,su}	butyrate from sugar	0.13280	-	Batstone et al. (2002)
$f_{ch,xc}$	carbohydrates from particulates	0.15000	-	Batstone et al. (2002)
$f_{fa,li}$	LCFA from lipid	0.95000	-	Batstone et al. (2002)
f _{h2,aa}	H_2 from amino acid	0.06000	-	Batstone et al. (2002)
f _{h2,su}	H_2 from sugar	0.19055	-	Batstone et al. (2002)
f _{li,xc}	lipids from particulates	0.25000	-	Batstone et al. (2002)
f _{pr,xc}	proteins from particulates	0.15000	-	Batstone et al. (2002)
$f_{pro,aa}$	propionate from amino acid	0.05000	-	Batstone et al. (2002)
f _{pro,su}	propionate from sugar	0.26910	-	Batstone et al. (2002)
$\mathbf{f}_{SI,xc}$	S ₁ from particulates	0.10000	-	Batstone et al. (2002)
f _{va,aa}	valerate from amino acid	0.23000	-	Batstone et al. (2002)
f _{XI,xc}	X ₁ from particulates	0.35000	-	Batstone et al. (2002)

Table S3.4.3: Stoichiometric parameter values of the ADM1-S/O model. Additional parameterscompared to ADM1 are put in bold.

Symbol	Description	Value	Unit	Reference
Biomass	yield coefficient on:			
Y _{aa}	uptake of amino acids	0.0800	g COD g COD ⁻¹	Batstone et al. (2004)
\mathbf{Y}_{ac}	uptake of acetate	0.0500	g COD g COD ⁻¹	Batstone et al. (2004)
Y _{aSRB}	uptake of acetate by SRB	0.0342	g COD g COD ⁻¹	Fedorovich et al. (2003)
Y _{bSRB}	uptake of butyrate by SRB	0.0329	g COD g COD ⁻¹	Fedorovich et al. (2003)
Y _{c4}	uptake of valerate and butyrate	0.0600	g COD g COD ⁻¹	Batstone et al. (2004)
\mathbf{Y}_{fa}	uptake of LCFA	0.0600	g COD g COD ⁻¹	Batstone et al. (2004)
Y _{h2}	uptake of H ₂	0.0600	g COD g COD ⁻¹	Batstone et al. (2004)
Y _{hSRB}	uptake of H ₂ by SRB	0.0800	g COD g COD ⁻¹	Batstone (2006)
Y _{pro}	uptake of propionate	0.0400	g COD g COD ⁻¹	Batstone et al. (2004)
Y _{pSRB}	uptake of propionate by SRB	0.0329	g COD g COD ⁻¹	Fedorovich et al. (2003)
Y _{SOB}	uptake of H_2S by SOB	0.0800	g COD g COD ⁻¹	Xu et al. (2013b)
Y _{su}	uptake of monosaccharide	0.1000	g COD g COD ⁻¹	Batstone et al. (2004)

Symbol	Description	Value	Unit	Reference
Threshold	I value of pH inhibition: (UL =	no inhibition,	LL = full inhibition)
I _{pH,ac,LL}	LL for ac ⁻ degradation	6	-	Batstone et al. (2004)
I _{pH,ac,UL}	UL for ac ⁻ degradation	7	-	Romli et al. (1995)
I _{pH,biom,LL}	LL for biomass	4	-	Batstone et al. (2004)
I _{pH,biom,UL}	UL for biomass	5.5	-	Batstone et al. (2004)
I _{pH,h2,LL}	LL for H ₂ degradation	5	-	Romli et al. (1995)
I _{pH,h2,UL}	UL for H ₂ degradation	6	-	Romli et al. (1995)
Decay rat	e of:			
k _{dec,Xaa}	X _{aa}	0.050	d ⁻¹	Batstone et al. (2004)
$k_{dec,Xac}$	X _{ac}	0.100	d ⁻¹	Batstone et al. (2004)
k _{dec,XaSRB}	X _{aSRB}	0.015	d ⁻¹	Fedorovich et al. (2003)
k _{dec,XbSRB}	X _{bSRB}	0.010	d ⁻¹	Fedorovich et al. (2003)
k _{dec,Xc4}	X _{c4}	0.100	d ⁻¹	Batstone et al. (2004)
$k_{dec,Xfa}$	X _{fa}	0.100	d ⁻¹	Batstone et al. (2004)
k _{dec,Xh2}	X _{h2}	0.100	d ⁻¹	Batstone et al. (2004)
k _{dec,XhSRB}	X _{hSRB}	0.010	d ⁻¹	Fedorovich et al. (2003)
k _{dec,Xpro}	X _{pro}	0.100	d ⁻¹	Batstone et al. (2004)
k _{dec,XpSRB}	X _{pSRB}	0.010	d ⁻¹	Fedorovich et al. (2003)
k _{dec,XSOB}	X _{SOB}	0.010	d ⁻¹	Same as hSRB
k _{dec,Xsu}	X _{su}	0.100	d ⁻¹	Batstone et al. (2004)
Disintegra	ation and hydrolysis first rate	constant of:		
k _{dis}	particulate disintegration	0.5	d ⁻¹	Batstone et al. (2004)
$k_{hyd,ch}$	carbohydrate hydrolysis	106	d ⁻¹	Gavala and Lyberatos (2001)
k _{hyd,li}	lipid hydrolysis	0.4	d ⁻¹	Gujer and Zehnder (1983)
k _{hyd,pr}	protein hydrolysis	2.7	d ⁻¹	Gavala and Lyberatos (2001)
Inhibitory	concentration of:			
k _{I,h2,c4}	H ₂ for X _{c4}	1.0 10 ⁻⁵	kg COD.m⁻³	Batstone et al. (2004)
k _{I,h2,fa}	H ₂ for X _{fa}	5.0 10 ⁻⁶	kg COD.m⁻³	Batstone et al. (2004)
k _{I,h2,pro}	H_2 for X_{pro}	3.5 10 ⁻⁶	kg COD.m⁻³	Batstone et al. (2004)
k _{I,h2s}	H_2S for all X_{SRB} , X_{ac} and X_{h2}	1.6 10 ⁻²	kmole S.m⁻³	Reis et al. (1992)
k _{I,NH3,ac}	NH_3 for X_{ac}	1.8 10 ⁻³	kmole N.m⁻³	Batstone et al. (2004)
k _{1,02}	O ₂ for X _{ac} and X _{h2}	2.5 10 ⁻¹	kmole O₂.m⁻³	Shen and Guiot (1996)
Maximum	n uptake rate of:			
k _{m,aa}	X _{aa}	250	g g ⁻¹ d ⁻¹ (COD)	Batstone et al. (2004)
k _{m.ac}	X _{ac}	40	g g ⁻¹ d ⁻¹ (COD)	Batstone et al. (2004)
k _{m,aSRB}	X _{aSRB}	7.1	g g ⁻¹ d ⁻¹ (COD)	Fedorovich et al. (2003)
k _{m,bSRB}	X _{bSRB}	13.7	g g ⁻¹ d ⁻¹ (COD)	Fedorovich et al. (2003)
k _{m,c4}	X _{c4}	100	g g ⁻¹ d ⁻¹ (COD)	Batstone et al. (2004)
k _{m,fa}	X _{fa}	30	$g g^{-1} d^{-1}$ (COD)	Batstone et al. (2004)
k _{m,h2}	X _{h2}	175	g g ⁻¹ d ⁻¹ (COD)	Batstone et al. (2004)
k _{m,hSRB}	X _{hSRB}	50	g g ⁻¹ d ⁻¹ (COD)	Batstone (2006)
k _{m,pro}	X _{pro}	65	g g ⁻¹ d ⁻¹ (COD)	Batstone et al. (2004)
k _{m,pSRB}	X _{pSRB}	12.6	g g ⁻¹ d ⁻¹ (COD)	Fedorovich et al. (2003)
k _{m,SOB}	Х _{ѕов}	82.3	g g ⁻¹ d ⁻¹ (COD)	Nishimura and Yoda (1997)
k _{m,su}	X _{su}	150	g g ⁻¹ d ⁻¹ (COD)	Batstone et al. (2004)

Table S3.4.4: Kinetic parameters of ADM1-S/O. Additional parameters compared to ADM1 are put in bold.

Symbol	Description	Value	Unit	Reference
Half-satu	ration constant for:			
K _{S,aa}	amino acid degradation	3.0 10 ⁻¹	kg COD m⁻³	Batstone et al. (2004)
K _{S,ac}	acetate degradation	2.1 10 ⁻¹	kg COD m⁻³	Fedorovich et al. (2003)
K _{S,aSRB}	acetate degradation by SRB	2.2 10 ⁻¹	kg COD m ⁻³	Fedorovich et al. (2003)
K _{S,bSRB}	butyrate degradation by SRB	1.0 10 ⁻¹	kg COD m ⁻³	Fedorovich et al. (2003)
K _{S,c4}	C4 degradation	$1.0 \ 10^{-1}$	kg COD m⁻³	Fedorovich et al. (2003)
K _{S,fa}	LCFA degradation	4.0 10 ⁻¹	kg COD m⁻³	Batstone et al. (2004)
K _{S,h2}	hydrogen degradation	$1.0 \ 10^{-4}$	kg COD m⁻³	Fedorovich et al. (2003)
K _{s,h2s,sob}	sulfide degradation by SOB	1.0 10 ⁻⁴	kmole S m ⁻³	Same as hSRB
K _{s,hSRB}	H ₂ degradation by SRB	1.0 10 ⁻⁴	kg COD m ⁻³	Batstone (2006)
K _{s,o2,sob}	oxygen consumption by SOB	1.0 10 ⁻⁴	kmole O₂ m ⁻³	Xu et al. (2013b)
K _{S,pro}	propionate degradation	1.0 10 ⁻¹	kg COD m⁻³	Batstone et al. (2004)
K _{S,pSRB}	propionate degradation by SRB	1.1 10 ⁻¹	kg COD m ⁻³	Fedorovich et al. (2003)
K _{S,SO4,aSRB}	sulfate degradation by aSRB	1.0 10 ⁻⁴	kmole S m⁻³	Fedorovich et al. (2003)
K _{s,so4,bsrb}	sulfate degradation by bSRB	2.1 10 ⁻⁴	kmole S m⁻³	Fedorovich et al. (2003)
K _{s,so4,hsrb}	sulfate degradation by hSRB	1.0 10 ⁻⁴	kmole S m ⁻³	Batstone (2006)
K _{s,so4,psrb}	sulfate degradation by pSRB	2.0 10 ⁻⁴	kmole S m⁻³	Fedorovich et al. (2003)
K _{S,su}	monosaccharide degradation	5.0 10 ⁻¹	kg COD m⁻³	Batstone et al. (2004)

Symbol	Description	Value	Unit	Reference
Acidity co	onstants (K _a) and a	cid-Base reaction constants (K _{AB})		
K _{a,ac}	acetic acid	10 ^{-4.76}	М	Lide (2003)
K _{a,bu}	butyric acid	10 ^{-4.83}	Μ	Lide (2003)
V	<u> </u>	$10-6.35_{\text{outp}}\left(7646_{1}\left(1 \ 1\right)\right)$	Μ	Lide (2003)
K _{a,co2}	CO_2	$10^{-100} exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}} - \frac{1}{T}\right)\right)$		Batstone et al. (2002)
V		10-13.995 $(55900 (1 1))$	Μ	Lide (2003)
κ _{a,h2o}	H ₂ U	$10^{-1000} exp\left(\frac{1}{100 \cdot R} \cdot \left(\frac{1}{T_{std}} - \frac{1}{T}\right)\right)$		Batstone et al. (2002)
14		(21670 (1 1))	Μ	Lide (2003)
K _{a,h2s}	H ₂ S	$10^{7.5}exp\left(\frac{1}{100 \cdot R} \cdot \left(\frac{1}{T_{std}} - \frac{1}{T}\right)\right)$		Batstone et al. (2002)
.,	···· +	(51965 (1 1))	М	Lide (2003)
K _{a,nh4}	NH ₄ '	$10^{-9.23} exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}} - \frac{1}{T}\right)\right)$		Batstone et al. (2002)
K _{a pro}	propionic acid	10 ^{-4.87}	М	Lide (2003)
K _{a va}	valeric acid	10 ^{-4.80}	М	Lide (2003)
K _{AB-H2S}	H₂S	10 ¹⁴	d ⁻¹	Batstone (2006)
KAB CO2	CO ₂	10 ¹⁴	d ⁻¹	Batstone (2006)
Henry's l	aw constants			
		(-14134)	1))	Sander (1999)
K _{H,ch4}	CH ₄	$0.00140 \cdot R \cdot T \cdot exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}}\right)\right)$	$\left(\frac{1}{T}\right) - \frac{1}{T}$	Wilhelm et al. (1977)
.,		(-19954)	1)	Sander (1999)
K _{H,co2}	CO_2	$0.03400 \cdot R \cdot T \cdot exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}}\right)\right)$	$\left(\frac{1}{T}\right) - \left(\frac{1}{T}\right)$	Wilhelm et al. (1977)
		(-4074)	1)	Sander (1999)
K _{H,h2}	H ₂	$0.00078 \cdot R \cdot T \cdot exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}}\right)\right)$	$\left(\frac{1}{T}\right)$ -	Wilhelm et al. (1977)
		Q 10000 D 7 (−17459 (1	1)	Sander (1999)
K _{H,h2s}	H ₂ S	$0.10000 \cdot R \cdot T \cdot exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}}\right)\right)$	$-\frac{1}{T}$) -	Wilhelm et al. (1977)
14		-10808 (1	1)	Sander (1999)
К_{Н,о2}	N ₂	$0.00065 \cdot R \cdot T \cdot exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}}\right)\right)$	$-\frac{1}{T}$) -	Wilhelm et al. (1977)
14	•	0 00120 P T $(1)^{-12471}$	1)	Sander (1999)
K _{H,n2}	02	$0.00130 \cdot R \cdot I \cdot exp\left(\frac{100 \cdot R}{100 \cdot R} \cdot \left(\frac{1}{T_{std}}\right)\right)$	$-\overline{T}$) -	Wilhelm et al. (1977)
Diffusivit	y in water of:			
D_{aa}	amino acids	8.62 ⁻ 10 ⁻⁶	$m^2 d^{-1}$	Batstone et al. (2004)
D_{ac}	acetate	6.48 ⁻ 10 ⁻⁶	$m^2 d^{-1}$	Batstone et al. (2004)
D_bu	butyrate	5.04 ⁻ 10 ⁻⁶	$m^2 d^{-1}$	Batstone et al. (2004)
D_{ch4}	methane	1.29 ⁻ 10 ⁻⁴	$m^2 d^{-1}$	Lide (2003)
D_{co2}	carbon dioxide	1.53.10-4	$m^2 d^{-1}$	Reid et al. (1977)
D_{fa}	LCFA	5.33 ⁻ 10 ⁻⁶	$m^2 d^{-1}$	Batstone et al. (2004)
D _{h2}	hydrogen	4.75 ⁻ 10 ⁻³	$m^2 d^{-1}$	Verhallen et al. (1984)
D _{h2s}	hydrogen sulfide	1.38 ⁻ 10 ⁻³	m² d⁻¹	Cunningham et al. (2011)
D _{ion}	cations and anion	s 1.17 ⁻ 10 ⁻⁴	$m^2 d^{-1}$	Batstone et al. (2004)
D _{n2}	nitrogen	1.73 ^{-10⁻⁴}	m² d⁻¹	Lide (2003)
D _{nh3}	ammonia	1.53.10-4	$m^2 d^{-1}$	Reid et al. (1977)
D _{o2}	oxygen	2.09'10 ⁻⁴	$m^2 d^{-1}$	Lide (2003)
D_{pro}	propionate	6.00.10-6	$m^2 d^{-1}$	Batstone et al. (2004)
D _{SI}	soluble inerts	8.62 10 ⁻⁶	$m^2 d^{-1}$	Batstone et al. (2004)
D_{so4}	ionic sulfate	9.16 10-5	m² d⁻¹	Cunningham et al. (2011)
D_{su}	monosaccharide	4.56 10 ⁻⁶	$m^2 d^{-1}$	Batstone et al. (2004)
D_{va}	valerate	5.00 ^{-10⁻⁶}	$m^2 d^{-1}$	Batstone et al. (2004)

Table S3.4.5: Physicochemical parameters of ADM1-S/O. Additional parameters compared to ADM1 are put in bold.

Symbol	Description	Value	Unit	Reference		
Other physicochemical parameters:						
k _p	pipe resistance coefficient	1.6	m ³ d ⁻¹ bar ⁻¹	adjusted		
\mathbf{P}_{atm}	atmosphere pressure	1.013	bar	standard		
\mathbf{p}_{h2o}	pressure of water	$0.0313 \cdot exp\left(5290 \cdot \left(\frac{1}{T_{std}} - \frac{1}{T}\right)\right)$	bar	Rosen and Jeppsson (2006)		
R	gas law constant	0.08314	bar M ⁻¹ K ⁻¹	standard		
Т	temperature (35°C)	308.15	К	measured		
T_{std}	standard temperature (25°C)	298.15	К	standard		

S.3.4.2 Reactor configuration

The biofilm surface area A [m²] was calculated as:

$$A = 4 \cdot \pi \cdot \left(r_p + z\right)^2 \cdot n_{sp}$$
 Eq. S3.4.3

where r_p [m] represents biofilm support thickness, which is zero for granular sludge (no support) but it is set to a negligibly small value to avoid numerical problems in Aquasim, *z* is the biofilm depth [m] and n_{sp} is the number of granules in UASB reactor. The growth of granules was defined such that they reach a steady state (maximum) radius (i.e. biofilm thickness, LF_{ss} [m]) of 3.5 mm.

$$u_{de} = u_F \cdot \left(\frac{LF}{LF_{SS}}\right)^{10} \text{ for } u_F > 0$$
Eq. S3.4.4a
$$u_{de} = 0 \qquad \text{for } u_F \le 0$$
Eq. S3.4.4b

where u_{de} is the detachment velocity [m d⁻¹], u_F is the biofilm growth velocity [m d⁻¹] and LF is the biofilm thickness [m].

S.3.4.3 Calculation of pH

pH is calculated by means of the charge balance method (electro-neutrality equation) in the reactor, stating that the sum of all charges [kmole m^{-3}] in the reactor equals to zero:

$$\Delta_{charge \ balance} = S_{H^+} + S_{Z^+} + S_{NH_4^+} - \frac{K_{a,H_2O}}{S_{H^+}} - S_{HCO_3^-} - \frac{S_{ac^-}}{64} - \frac{S_{pro^-}}{112} - \frac{S_{bu^-}}{160} - \frac{S_{va^-}}{208} - 2 \cdot S_{SO_4^{2^-}} - S_{HS^-} = 0$$
Eq. S3.4.6

 S_{Z^+} are the net positive charges which don't take part in chemical equilibria. If S_{Z^+} is negative, more anions than cations are present.

By substituting the steady state expressions for ammonium, acetate, propionate, butyrate and valerate into the charge balance, (**Table 3.4.1**), the latter is rewritten as:

$$\Delta_{charge\ balance} = S_{H^+} + S_{Z^+} + \frac{S_{TIN} \cdot S_{H^+}}{K_{a,NH_4^+} + S_{H^+}} - \frac{K_{a,H_2O}}{S_{H^+}} - S_{HCO_3^-} - \frac{\frac{S_{Tac}}{64} \cdot K_{a,ac}}{K_{a,ac} + S_{H^+}} - \frac{\frac{S_{Tpo}}{112} \cdot K_{a,pro}}{K_{a,pro} + S_{H^+}} - \frac{\frac{S_{Tbu}}{160} \cdot K_{a,bu}}{K_{a,bu} + S_{H^+}} - \frac{\frac{S_{Tva}}{160} \cdot K_{a,bu}}{K_{a,bu} + S_{H^+}} - \frac{\frac{S_{Tva}}{160} \cdot K_{a,bu}}{K_{a,bu} + S_{H^+}} - \frac{S_{Tva}}{K_{a,bu} + S_{H^+}} - \frac{S_{$$

The concentration of each component (including Z^+) is calculated every time step from the corresponding mass balances. In the mass balance for the net positive charges (Z^+), the influent concentration $S_{Z^+}^{in}$ is calculated from the charge balance over the influent:

$$S_{Z^{+}}^{in} = -S_{H^{+}}^{in} - \frac{S_{TIN}^{in} \cdot S_{H^{+}}^{in}}{K_{a,NH_{+}^{4}} + S_{H^{+}}^{in}} + \frac{K_{a,H_{2}O}}{S_{H^{+}}^{in}} + \frac{S_{CO_{2}}^{in} \cdot K_{a,CO_{2}}}{K_{a,CO_{2}} + S_{H^{+}}^{in}} + \frac{S_{Tac}^{in} \cdot K_{a,ac}}{K_{a,ac} + S_{H^{+}}^{in}} + \frac{S_{Tpro}^{in} \cdot K_{a,bro}}{K_{a,pro} + S_{H^{+}}^{in}} + \frac{S_{Tbu}^{in} \cdot K_{a,bu}}{K_{a,bu} + S_{H^{+}}^{in}} + \frac{S_{Tbu}^{in} \cdot K_{a,bu}}{K_{a,bu} + S_{H^{+}}^{in}} + \frac{S_{Ts}^{in} \cdot K_{a,bu}}{K_{a,bu} + S_{H^{+}}^{in}} + \frac{S_{Tbu}^{in} \cdot K_{a,bu}}{K_{a,bu} + S_{H^{+}}^{in}} + \frac{S_{Tbu}^{in} \cdot K_{a,bu}}{K_{a,bu} + S_{H^{+}}^{in}} + \frac{S_{Ts}^{in} \cdot K_{a,bu}}{K_{a,bu} + S_{H^{+}}^{in}} + \frac{S_{Ts}^{in}$$

Components involved in a chemical equilibrium and present only in the liquid phase, i.e. acetate, propionate, butyrate, valerate, and inorganic nitrogen were assumed to reach their chemical equilibrium instantaneously. The total concentrations $S_{T,i}$ [kmole m⁻³ or kg COD m⁻³] of these components i were taken as a state variables and were calculated from their corresponding liquid phase mass balances (Eq. S3.4.9):

$$\frac{dS_{T,i}}{dt} = Q_{in}S_{T,in,i} - Q_{out}S_{T,i} + \sum_{j=1-19b}S_{T,in,i} \cdot \rho_j$$
 Eq. S3.4.9

The concentrations of the individual chemical equilibrium (ionized and non-ionized) forms were subsequently calculated from the total concentrations and the prevailing pH through the equilibrium equation (**Table S3.4.6**).

Table S3.4.6: Overview of species involved in a chemical equilibrium and only present in the liquid phase, for which instantaneous equilibrium is assumed

Total concentration	Equilibrium equation
$S_{Tac} = S_{Hac} + S_{ac}$	$K_{a,ac} = \frac{S_{ac} - S_{H^+}}{S_{Hac}}$
$S_{Tpro} = S_{Hpro} + S_{pro}$	$K_{a,pro} = \frac{S_{pro} - S_{H^+}}{S_{Hpro}}$
$S_{Tbu} = S_{Hbu} + S_{bu^-}$	$K_{a,bu} = \frac{S_{bu} - S_{H^+}}{S_{Hbu}}$
$S_{Tva} = S_{Hva} + S_{va^-}$	$K_{a,va} = \frac{S_{va} - S_{H^+}}{S_{Hva}}$
$S_{TIN} = S_{NH_3} + S_{NH_4^+}$	$K_{a,NH_4^+} = \frac{S_{NH_3} \cdot S_{H^+}}{S_{NH_4^+}}$

For components involved in a chemical equilibrium and taking part in gas-liquid transfer, i.e. inorganic carbon and sulfide, the equilibria reactions were not considered to be in steady state as this would overly slow down the calculation. Instead, the uncharged form, which is exchanged with the gas phase, was taken as a state variable and its concentration was calculated dynamically.

The corresponding liquid phase mass balances of these components are given by Eqs. S3.4.10-13.

in which ρ_{A1} and ρ_{A2} refer to the rate of H₂S and CO₂ acid-base reaction (in kg COD m⁻³ d⁻¹).

S.3.4.4 Gas-liquid transfer

Gas-liquid transfer of the gases CH_4 , CO_2 , H_2S , and H_2 was implemented through the diffusive links between the liquid phase of the UASB reactor and its gas phase (**Figure S3.4.1**) according to Batstone et al. (2004). The gases added through microaeration, O_2 and N_2 , were implemented through its own diffusive link (gas-liquid transfer 2). Two transfers were made in ADM1-S/O to distinguish between new added components and to be able to turn on and off the whole transfer if possible.



Figure S3.4.1: Schematic representation of microaeration implementation into ADM1-S/O

The mass transport coefficients $k_L a_i$ [d⁻¹] are related to the superficial gas velocity, v_{Gs} [m d⁻¹]. For relatively low but commonly used gas flow rates $v_{Gs} < 0.1$ m s⁻¹, as in the present study), the following linear relationship applies for the mass transport coefficient for O₂ (van der Lans, 2000):

$$k_L a_{O_2} = 0.6 \cdot v_{Gs}$$

Eq. S3.4.14

where v_{Gs} is the superficial gas velocity, i.e. the gas flow rate ($Q_{gas,in}$) divided by the reactor cross section area, A_r [m²].

The temperature dependency of the mass transfer coefficient was taken into account through the relationship:

$$k_L a_{O_2,T} = k_L a_{O_2} \cdot \theta_{k_L a}^{T-293.15}$$
 Eq. S3.4.15

in which a value of $\theta_{k_L a}$ = 1.024 is typical for both diffuse and mechanical aeration devices (Tchobanoglous et al., 1991).

The mass transport coefficient for N_2 is related to the mass transport coefficient for O_2 through diffusion coefficients, according to the relationship (de Heyder et al., 1997):

$$k_L a_{N_2,T} = k_L a_{O_2,T} \cdot \sqrt{\frac{D_{N_2}}{D_{O_2}}}$$
 Eq. S3.4.16

S.3.4.5 Results



Figure S3.4.1: Model validation concerning the methane and carbon dioxide composition of biogas.

8.3. Supplementary material of Section 4.2.



S.4.2.1 Lab-scale biomembrane unit

Figure S4.2.1: Composition of gasses in air side and biogas side: $A - CH_4$ in air side; $B - CH_4$ in biogas side; $C - CO_2$ in air side; $D - CO_2$ in biogas side; $E - O_2$ in air side; $F - O_2$ in biogas side; $G - N_2$ in air side; $F - N_2$ in biogas side.

9. Curriculum Vitae

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10/2015 – present	Maternity leave					
	Son Tobias Pokorný, born 7.12.2015					
09/2011 – present	Joint doctorate (Ph.D. Studies)					
University of Chemistry	Specialization: Water Technology & Biosystems Control					
and Technology Prague	Ph.D. Topic: "Microaeration for biogas desulfurization – experimental					
(UCT Prague) & Ghent	and simulation study of various reactor types"					
University						
01/2013 - 08/2013	Internship					
University of South Elerida	Department of Civil and Environmental Engineering					
Tampa 1154	Fopic: "A Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process					
rumpu, osa	for decentralized wastewater treatment"					
09/2008 - 06/2011	Master's Degree					
University of Chemistry	Specialization: Water Technology					
and Technology Prague	M.Sc. Thesis: "Separation of fluoride from water sample"					
03/2009 - 07/2009	Erasmus					
Politecnico di Milano,	Faculty of Environmental Protection, Civic and Territorial					
Milano, Italy						
09/2005 - 07/2008	Bachelor's Degree					
University of Chamistry	Specialisation: Environmental Chemistry and Technology					
oniversity of Chemistry	B.Sc. Thesis: "Presence of free fluoride anion in water and its					
und rechnology ridgae	determination"					
Work experience						
08/2016 - present	Administrator and coordinator of European project "SuPER-W"					
University of Chemistry	Duties: administration and coordination of the project					
and Technology Prague						
08/2005 - 12/2015	Assistant in private company					
"Advisory and consultancy	Duties: Feasibility studies (in environmental protection), paper work,					
in environmental	material collection,					
protection"						
Stredočeské vodárny a.s.	Summer intern (sample collection and measurement)					

07/2003-08/2004	Laboratory Technician
Barvy & Laky Hostivař	Summer brigade (07-08/2003, 08/2004)
08/2002	Worker in Claims department
Barvy & Laky Hostivař	Summer brigade, duties: paper work, reclamation
Other information	
Courses	Italian language
	ELLCI – Ente Lombardo Lingua e Cultura Italiana (03/2009 – 06/2009)
	Italian Culture Institute in Prague (09/2008 – 03/2009)
	Pollution in Europe (Erasmus Intensive Program)
	Sections of course: an overview of pollution in Europe; risk assessment;
	polluted area management; emerging topics and field visit
Language skills	English (fluently), Italian (basics), French (basics)
Other skills	PC: MS office, Adobe Photoshop, Adobe Reader
	Driving licence type B
Awards	
2016	2 nd place in the presentation of Full-scale operational results
Conference "Wastewater"	Conference Wastewater 2016, section: Full-scale operational results
	for young scientists (under 33)
2016	Special Price of Czech Chemical Society
French Embassy in Prague	Jean-Marie Lehn Price in Chemistry, 22. 6. 2016
2015	Josef Hlávka´s Award
	Award for the best students of public universities in Prague and Brno and
ΗΙάνκα Foundation	talented young workers of Czech Academy of Sciences.
2014	Dean's award
LICT Drague	The first place in the publication activity of the Faculty of Environmental
oci Plugue	Technology for 2014.
2010	1 st place in Student Scientific Conference
UCT Prague	Faculty of Environmental Technology, section: Water Technology

10. Publications

10.1. Papers in international journals

10.1.1. Published

Pokorná-Krayzelová, L., Mampaey, K.E., Vannecke, T.P.W., Bartáček, J., Jeníček, P., Volcke, E.I.P. "Model-based optimization of microaeration for biogas desulfurization in UASB reactors." *Accepted in Biochemical Engineering Journal (June 2017)*. <u>IF 2015: 2.463</u>

Jeníček, P., Horejš, J., **Pokorná-Krayzelová, L.**, Bindzar, J., Bartáček, J. (2017). "Simple biogas desulfurization by microaeration - full scale experience." *Anaerobe (published 2017, January 5th)*. <u>IF</u> 2015/2016: 2.424

Krayzelová, L., Bartáček, J., Díaz, I., Jeison, D., Volcke, E.I.P., Jeníček, P. (2015). "Microaeration for hydrogen sulfide removal during anaerobic treatment - a review." *Reviews in Environmental Science and Bio/Technology* 14 (4): 703-725. <u>IF 2015/2016: 4.352</u>

Krayzelová, L., Bartáček, J., Kolesarová, N., Jeníček, P. (2014). "Microaeration for hydrogen sulfide removal in UASB reactor." *Bioresource Technology* 172(0): 297-302. <u>IF 2014: 5.039</u>

Krayzelová, L., Lynn, T.J., Banihani, Q., Bartáček, J., Jeníček, P., Ergas, S.J. (2014). "A Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) process for decentralized wastewater treatment." *Water Research* 61: 191-199. <u>IF 2014: 5.323</u>

Jeníček, P., Celis, C. A., **Krayzelová, L.**, Anferova, N., Pokorná, D. (2014). "Improving products of anaerobic sludge digestion by microaeration." *Water Science and Technology* 69(4): 803-809. <u>IF 2014:</u> <u>1.212</u>

10.1.2. Submitted

Pokorná-Krayzelová, L., Bartáček, J., Vejmelkova, D., Alvarez, A.A., Slukova, P., Prochazka, J., Volcke, E.I.P., Jeníček, P. "The use of a silicone-based biomembrane for microaerobic H₂S removal from biogas." *Submitted in Separation and Purification Technology (March 2017)*

Pokorná-Krayzelová, L., Bartáček, J., Theuri, S., Segura Gonzalez, C.A., Procházka, J., Volcke, E.I.P., Jeníček, P. "Microaeration through biomembrane for biogas desulfurization: lab-scale and pilot-scale experiences" *Submitted in Applied Microbiology and Biotechnology (June 2017)*

Pokorná-Krayzelová, L., Selan, L., Jeníček, P., Volcke, E.I.P., Bartáček, J. "Kinetics of (bio) chemical sulfide and thiosulfate oxidation under microaerobic conditions." *Prepared for submission*.

10.2. Papers in conference proceedings

Pokorná-Krayzelová L., Bartáček J., Theuri S.N., Segura Gonzalez C.A., Procházka J., Jeníček P. (2016): *Biogas desulfurization using biomembranes: Pilot-scale experiments.* Wastewater 2016. Štrbské pleso. Slovakia. Oral presentation (in Czech) **Krayzelová, L.**, Mampaey, K.E., Wannecke, T.P.W., Bartáček, J., Jeníček, P., Volcke, E.I.P. (2015). *Modelling microaeration for biogas desulfurization in a UASB reactor*. The 14th World Congress on Anaerobic Digestion. Viña del Mar, Chile. Poster presentation (in English)

Alvarez da Costa, A.A., Bartáček, J., **Krayzelová, L.**, Pícha, A., Prochazka, J., Jeníček, P. (2015). *Use of a biomembrane module for biogas desulfurization*. The 14th World Congress on Anaerobic Digestion. Viña del Mar, Chile. Poster presentation (in English)

Payne, K., **Krayzelová, L.**, Rodriguez-Gonzalez, L.C., Cunningham, J., Trotz, M., Ergas, S. (2015). *Mathematical Modeling of a Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) bioreactor*. 24th American Water Resources Association Conference. Fort Myers, Florida, USA. Poster presentation (in English)

Jeníček, P., **Krayzelová, L.**, Stará, H., Horejš, J., Bartáček, J. (2015). *Efficient Biogas Desulfurization by Microaeration - Full Scale Experience*. 12th IWA Specialised Conference on Design, Operation and Economics of Large Wastewater Treatment Plants. Prague, Czech Republic. Oral presentation (in English)

Ergas, S.J., **Krayzelová, L.**, Rodriguez-Gonzalez, L., Payne, K., Trotz, M. (2015). *Hybrid Adsorption Biological Treatment Systems (HABITS) for Improved Nitrogen Removal in Onsite Wastewater Treatment*. WEF Nutrient Symposium 2015. San Jose, California, USA. Oral presentation (in English)

Jeníček, P., **Krayzelová, L.,** Stará, H., Bartáček, J. (2015). *Efficient Biogas Desulfurization by Microaeration - Full Scale Experience*. Water and Energy 2015: Opportunities for Energy and Resource Recovery in the Changing World. Washington D.C., USA. Poster presentation (in English)

Krayzelová, L., Bartáček, J., Volcke. E.I.P., Jeníček, P. (2015). *Microaeration for H*₂*S removal in UASB reactor*. The 20th National Symposium for Applied Biological Sciences (NSABS 2015). Louvain-la-Neuve, Belgie. Poster presentation (in English)

Veloz, A., Anferova, N., **Krayzelová, L.**, Jeison, D., Procházka, J., Bartáček, J., Jeníček, P. (2014). Desulfurization of biogas in microaerobic bio-membrane module. Wastewater 2014 (ISBN 978-80-263-0506-4, pp. 111-118). Full paper. Strbske Pleso, Slovakia. Oral presentation (in Czech)

Krayzelová, L., Anferova, N., Kolesárová, N., Bartáček, J., Jeníček, P. (2013). The efficiency of hydrogen sulfide removal from biogas using microaeration during the anaerobic treatment of brewery wastewater in UASB reactor. Water 2013 (ISBN 978-80-263-0506-4, pp. 111-118). Full paper. Podebrady, Czech Republic. Oral presentation (in Czech)

Krayzelová, L., Banihani, Q., Bartáček, J., Ergas, S.J., Jeníček, P. (2013). The application of used tires for the removal of nitrates from wastewater. Water 2013 (ISBN 978-80-263-0506-4, pp. 363-366). Full paper. Podebrady, Czech Republic. Poster presentation (in Czech)

Bartáček, J., **Krayzelová, L.**, Kolesárová, N., Jeníček, P. (2013). Removal of hydrogen sulphide from biogas by microaeration in UASB reactor. 13th World Congress on Anaerobic Digestion (ISBN 978-84-695-7756-1). Santiago de Compostela, Spain. Oral presentation (in English)

Jeníček, P., Celis, C.A., **Krayzelová, L.**, Anferova, N., Pokorná, D. (2013). Microaerobic for improving products of anaerobic sludge digestion. Holistic Sludge Management 2013. Västerås, Sweden. Oral presentation (in English)

Krayzelová, L., Bartáček, J., Jeníček, P. (2012). Microaeration as a way of biogas desulphurization in UASB reactor. Wastewater 2012 (ISBN 978-80-970896-2-7, pp. 135-139). Full paper. Strbske Pleso, Slovakia. Oral presentation (in Czech)

Krayzelová, L., Bartáček, J., Jeníček, P. (2012). Removal of hydrogen sulfide from biogas by microaeration. Biogas 2012. Ceske Budejovice, Czech Republic. Poster presentation (in Czech)

Krayzelová, L., Kolesárová, N., Bartáček, J., Jeníček, P. (2012). Use of microaerobic conditions for the removal of hydrogen sulfide from UASB reactor. Sludge and Waste 2012 (ISBN 978-80-970896-0-3; pp. 127-131). Full paper. Banska Bystrica, Slovakia. Oral presentation (in Czech)