Biological treatment of sulfidic spent caustics under haloalkaline conditions using soda lake bacteria

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This research was conducted under the auspices of the Netherlands Research School for Socio-Economic and Natural Sciences of the Environment (SENSE)

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Thesis

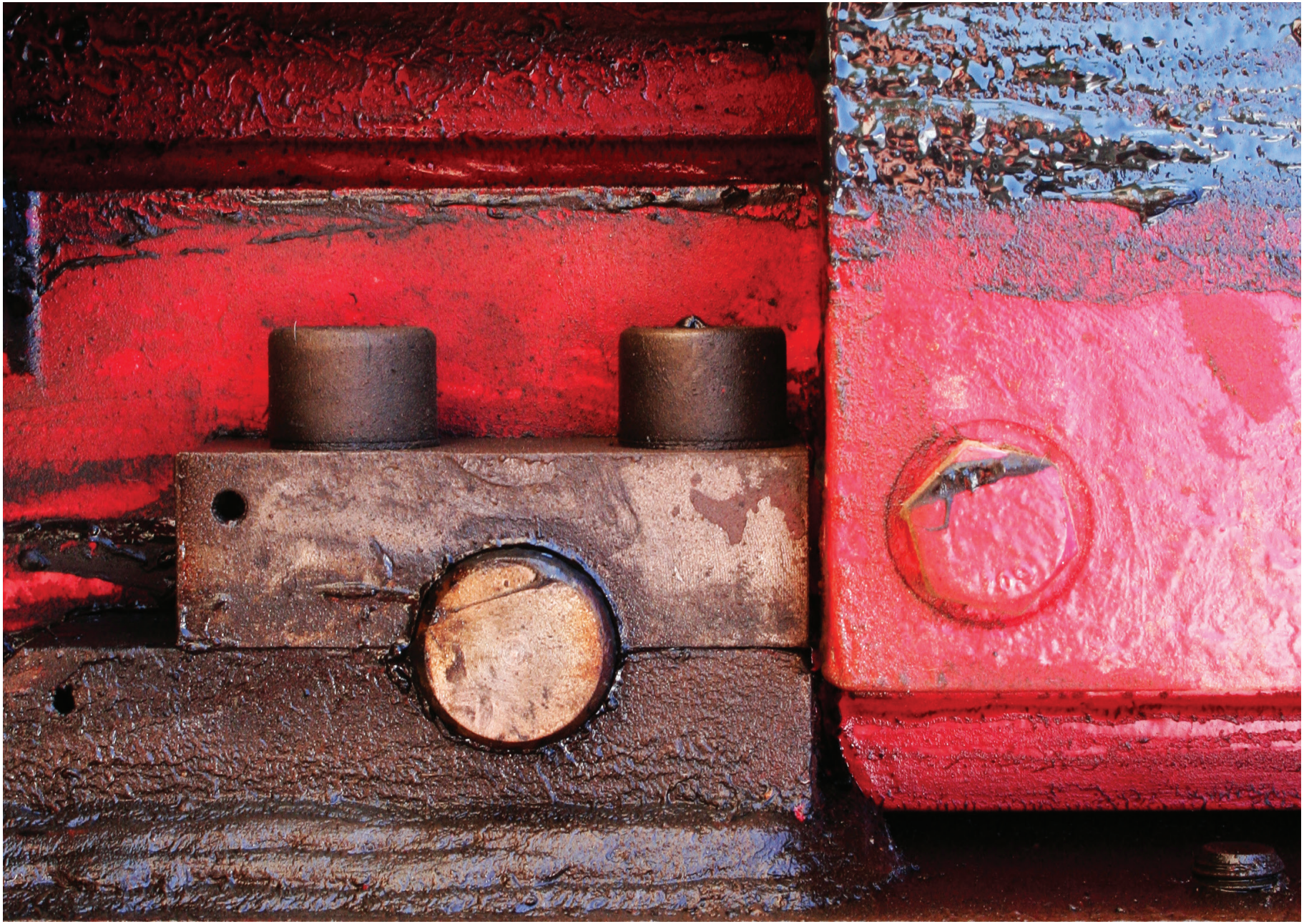
Submitted in fulfillment of the requirements for the degree of doctor
at Wageningen University
by authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
In the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 18 December 2012
at 11 a.m. in the Aula

Marco de Graaff
Biological treatment of sulfidic spent caustics under haloalkaline conditions using soda lake bacteria
132 pages

Thesis Wageningen University, Wageningen, NL (2012)
With references, with summaries in English and Dutch

ISBN 978-94-6173-445-7

Outline



Chapter 1

General introduction

Part of this chapter will be published as:

Albert J. H. Janssen, Pim L.F. van den Bosch, Robin C. van Leerdam, Marco de Graaff
Chapter 11: *Bioprocesses for the Removal of Volatile Sulphur Compounds from Gas Streams.* In: Kennes C and Veiga MC (Eds.); Air Pollution Prevention and Control: Bioreactors and Bioenergy, John Wiley, Chichester, United Kingdom (in press).

1.1 Introduction

As global fossil fuel reserves are declining, renewable energy sources, such as solar, wind and geothermal energy, are more frequently implemented and accounted up to 16% of the total global energy consumption in 2010 [1]. Despite the growing need for a society that is based on renewable energy, most countries primarily depend on fossil fuels. In 2009, for example, 81% of the total global energy consumption originated from fossil fuel consumption [1]. Traditionally, North America and Western Europe are the largest consumers of oil. In 2011, the global oil demand was approximately 14 million m^3 per day [2]. It is expected that the consumption of fossil fuels will increase rather than decrease in the following decades, as a growing population will lead to an increasing global energy demand [3, 4]. In terms of global electricity production, coal is the major energy source (41%), followed by gas (21%) and oil (6%) [5]. Especially the use of coal by fast developing economies such as China, which is responsible for more than 50% of the global annual coalconsumption, is increasing every year [6, 7].

Fossil fuels (e.g. natural gas, crude oil and coal) commonly contain sulfur compounds. Typically total sulfur levels in crude oil, may for instance vary between 0.1 and 8% (w/w) [8]. Combustion of untreated fossil fuels results in the emission of sulfur dioxide (SO_2), a corrosive and harmful gas that has a negative impact on human health. It may, for example, cause cardiovascular and serious airway injuries at 50-500 ppm [9]. Environmental problems of SO_2 are related to the formation of acid rain. In the atmosphere, SO_2 reacts with oxygen and water to form sulfuric acid (H_2SO_4) droplets. The resulting acid deposition (rain, snow, hail, dust) acidifies and damages terrestrial and aquatic ecosystems and infrastructure. Global sulfur dioxide (SO_2) emissions in 2008 amounted 97 x 10^9 kg S [10]. About 66% of the global SO_2 emission is from anthropogenic origin, such as coal-fired power plants, the petrochemical industry, and biogas. The remaining is due to natural sources, such as the natural sulfur cycling e.g. the oxidation of biogenic dimethyl sulfide (DMS) and volcanic activities [11].

To avoid sulfur-related environmental problems, industrialized countries implemented various sulfur emission control strategies. As an example, highway diesel fuel in the United States may not contain more than 15 ppm sulfur [12, 13]. In the European Union, fuels in general may not contain more than 10 ppm sulfur [14]. The fuel sulfur specifications set by governmental regulations stress the importance for desulfurization of fossil fuels. The production of high value fuels from crude oil and their (simultaneous) desulfurization occurs in the oil refining industry. Hydrogen sulfide (H_2S) is typically the most pronounced sulfur compound that is separated from the crude oil during the refining process. H_2S is a colorless, flammable, toxic and corrosive gas with a very low odor threshold [15, 16]. For H_2S (gas), the threshold limit value (TLV) in workplace conditions is 1.6 ppm in the Netherlands, and 10 ppm in the United States [13, 17]. H_2S becomes progressively more dangerous at levels above 70 ppm and is lethal at 600 ppm [17]. During the refinery process, volatile sulfur compounds (VSC's), such as H_2S, end up in refinery gases that require treatment prior to incineration. Besides H_2S, certain gaseous streams e.g. natural gas, may also contain toxic organic VSC's, such as methanethiol (MT) [18].

In this chapter a brief introduction will be given to commonly applied sulfur removal processes in the oil and gas industry. Special attention will be paid to the origin, composition and disposal of sulfidic spent caustics (SSC's), which are formed during caustic (NaOH) extraction of sulfur compounds from, amongst others, refinery gases. With regards to SSC's, the toxic properties of several (in)organic sulfur compounds and other common pollutantse.g. mono-aromatic compounds, are discussed.

1.2 Oil refining

Crude oil or petroleum is a complex mixture of hydrocarbons with varying molecular weights. The classification of "light" and "heavy" crude oil refers to the weight of its molecules and its subsequent difference in viscosity. During oil refining, the crude oil always needs to be desalted before entering a distillation column. In the crude distiller column, the different carbon fractions (C_1-C_{80}) are separated due to their differences in boiling temperature (Table 1).

Table 1: The different hydrocarbon distillation fractions

Hydrocarbon Fraction	Boiling point (°C)	Type	Application
C_1-C_4	20	gases	LPG
C_5-C_9	70	naphtha	chemicals
C_5-C_{10}	120	gasoline	car fuel
C_{10}-C_{16}	170	kerosine	jet fuel
C_{14}-C_{20}	270	diesel	general fuel
C_{20}-C_{50}	300-370	lubricating oil	waxes, motor oil
C_{20}-C_{70}	370-600	fuel oil	fuel ships/factories
>C_{70}	600	residuels	bitumen

The lightest hydrocarbon fractions will be extracted from the top of the column, while the heaviest fractions (>C_{70}) are taken from the bottom. After fractional distillation of the crude oil, the hydrocarbon fractions may undergo several conversion processes such as cracking and hydrotreating processes. During cracking processes, larger hydrocarbon fractions are cut into smaller ones to produce high value transportation products and petrochemical feedstock's [19]. The most pronounced cracking processes are thermal, catalytic and hydrocracking. During thermal cracking, high temperatures (<750 °C) and pressures (<70 bar) are applied. During steam cracking, which is a form of thermal cracking, all sulfur containing compounds in the hydrocarbon feed stream are converted to H_2S and organic sulfur compounds which end up in the steam cracker gas [20]. Catalytic cracking processes also operate at high temperatures and pressure, but use zeolite-based (luminosilicates) catalysts [19, 21]. Hydrocracking is a catalytic process in which hydrogen is added at high temperature and pressure. Hydrodesulfurization (HDS) or hydrotreating is the most widely applied process in modern petroleum refineries for the removal of VSC's [22]. In the hydrotreating process

high-pressure hydrogen is used (similar to hydrocracking) to catalytically remove VSC's from petroleum fractions [18, 22]. During hydrotreating, all organic sulfur compounds are converted to H_2S that end up in so-called "sour" refinery gas [18]. In addition to H_2S, refinery gas may also contain contaminants, such as ammonia (NH_3) and carbon dioxide (CO_2) [23].

1.3 Physico-chemical removal of VSC's

Sour gases generally require treatment. Commonly applied physico-chemical treatment processes for these gases are adsorption by activated carbon (AC), caustic scrubbing and amine treating followed by the Claus process. Most of the described processes are also used for the treatment of sour natural gas. H_2S is typically the most pronounced pollutant in natural gas varying between 0 and 5% [24]. Some gas fields, such as the ones in Alberta (Canada), even contain H_2S concentrations exceeding 35% (v/v) [25]. Common pollutants in refinery- and natural gas include mercaptans (RSH) such as MT (CH_3SH).

Activated carbon (AC)

AC is used as an adsorbent for the removal of H_2S and MT from gaseous streams [23, 26, 27]. The physical sorption capacity of AC for H_2S is about 10 kg m^{-3} [28]. AC is generally impregnated with caustic materials, such as NaOH or KOH, to increase the adsorption capacity [29]. A drawback of applying an impregnated AC treatment is that formed elemental sulfur (S^0) blocks the carbon pores leading to decreasing adsorption capacities [29, 30].

Amine-treating and Claus process

One of the most frequently applied methods for the removal of H_2S from refinery- and natural gas is the amine-treating process. Sour gas, often originating from a hydrodesulfurization (HDS) unit, is fed to the amine-treating process. First H_2S is absorbed or scrubbed by an alkanolamine solution in the amine absorber. Different amine solutions are applied depending on the quantity and ratio of H_2S and CO_2 of the gas [31]. These may include monoethanolamine (MEA), diethanolamine (DEA) and methyldiethanolamine (MDEA) [32, 33]. The amines react with the acidic compounds whereafter the so-called "rich" amine solution is led into an amine regenerator unit. The amine regenerator is heated with steam to separate the H_2S and CO_2 from the amine solution whereafter the amines are recycled [33].

The H_2S-rich gas originating from the amine regenerator is commonly treated in the Claus process. The first step of the Claus process is a simple combustion step in which approximately one third of the H_2S is oxidized to SO_2. In the second (catalytic) step, the remaining H_2S reacts with SO_2 to form S^0 and water [32]. The selectivity for S^0 formation in a two-step Claus process is around 95-98%. Higher removal efficiencies, i.e. up to 99.8%, are possible when a tail gas treatment unit (TGTU) is applied such as the SCOT process [32, 34]. In the SCOT process (Shell Claus off-gas treating process), all sulfur compounds in the tail gas (sulfur vapor and SO_2) are reduced to H_2S and are recycled into the amine- and Claus process, thereby achieving a high S^0 recovery factor of more than 99.9% [35]. The combination of the amine treating and Claus process is usually applied for H_2S loads exceeding 20 tons day^{-1} [17].

Caustic scrubbing

In the petrochemical industry, caustic (NaOH) solutions (typically < 20 wt%) are also used to be one of the primary reagents for the extraction of H_2S, mercaptans, such as MT, and organic acids from hydrocarbon streams [36, 37]. The resulting alkaline solution is referred to as a spent caustic. The sources for spent caustic generation are diverse and may include the caustic washing of straight-run light hydrocarbons, (refinery) gases originating from thermal/catalytic cracking units and the washing of middle distillates with additional mercaptans extraction operations [37]. Equations 1 and 2 show the reaction of H_2S and MT with the caustic solution.

$$H_2S + OH^- \rightarrow HS^- + H_2O \qquad \text{eq. 1}$$

$$CH_3SH + OH^- \rightarrow CH_3S^- + H_2O \qquad \text{eq.2}$$

Removal of the (in)organic sulfur compounds from the hydrocarbon streams is needed for environmental reasons, to avoid corrosion of downstream equipment, and to prevent the poisoning of catalysts [37]. The composition of spent caustics is determined by the hydrocarbon stream that is treated and the (conversion) process it is derived from [37-40]. In general, three different types of spent caustics can be distinguished (see also Table 2).

1. Sulfidic spent caustics: primarily (in)organic sulfur compounds from caustic scrubbing of straight-run gaseous hydrocarbon streams, such as LPG and gasoline-treating processes.
2. Phenolic spent caustics: phenols, cresols (*o*, *m*, *p*- methylphenols), xylenols (dimethylphenol), alkylphenols (ethylphenols, propylphenols, etc.) and thiophenols produced due to caustic scrubbing of catalytically cracked gases/gasoline.
3. Naphthenic spent caustics: naphthenic acids (cycloalkanes) from caustic scrubbing of, amongst others, diesel and kerosine fractions.

During desulfurization processes, spent caustics are produced at rates ranging from 0.5 up to 500 gram per ton of refined crude [41]. The solubility of mercaptans, also called thiols, in the caustic solution is highly dependent on the hydrocarbon portion of the mercaptan molecule, which in turn affects the total absorption capacity of the caustic solution.

Table 2: Typical compositions of the different types of spent caustics after [37] and [39]

Component	Sulfidic	Phenolic	Naphtenic
NaOH, wt%	2-10	10-15	1-4
sulfide, wt%	0.5-4	0-1	0-0.1
mercaptide, wt%	0.1-4	0-4	0-0.5
cresylic acids, wt%	-	10-25	0-3
naphthenic acids, wt%	-	-	2-15
carbonate as CO_3, wt%	0-4	0-0.5	-
pH	13-14	12-14	12-14

1.4 Composition and toxicity of sulfidic spent caustics (SSC's)

The amount of sulfur and type of sulfur compound that are present in SSC's is primarily dependent on the origin of the hydrocarbon stream that is treated [42]. In the following section, the composition of SSC's is reviewed as well as the properties of the (in)organic sulfur and mono-aromatic compounds. In addition, the toxic effects of these compounds to microorganisms are discussed.

Composition of SSC's

In general, spent caustic solutions comprise of high alkalinity (pH > 12) and salinity (Na^+ 5-12 wt%). This is simply due to the strength of the caustic solutions that are employed in the scrubbing process [39, 40]. Typically, sulfide is the most dominant sulfur compound found in SSC's with concentrations that may exceed 2-3 wt% [40]. At a pH above 9, the amount of dissolved H_2S (aq) is negligible, since the H_2S is almost entirely present as bisulfide (HS^-).

SSC's may also contain organic VSC's of which MT, ethanethiol (ET) and disulfides such as dimethyl disulfide (DMDS), are most pronounced (Table 3 and 4). Similar to H_2S, MT is a gas at ambient conditions and is notorious for its toxic and corrosive properties and low odor threshold [43]. In the Netherlands, the TLV-value for MT is lower than that of sulfide (i.e. 0.5 ppm), indicating the higher toxicity and safety risk when working with this compound [44]. MT is a weak acid (pKa = 10.3) and has a relatively high water solubility of 29 g L^{-1} (0.62 M). Previous research showed that MT can react with bio-sulfur particles resulting in polysulfides (S_x^{2-}), sulfide and mainly DMDS and DMTS [45]. MT is used for the synthesis of the amino-acid methionine and as a precursor for the synthesis of pesticides [46]. In the presence of oxygen, MT (CH_3SH) rapidly auto-oxidizes to DMDS (CH_3SSCH_3) according to [47]

$$2CH_3SH + 0.5O_2 \rightarrow CH_3SSCH_3 + H_2O \qquad \text{eq. 3}$$

DMDS has a relatively low solubility in water of approximately 2.2 g L^{-1} (23.4 mM) at 20 °C [48]. This is also indicated by the octanol/water partition coefficients (log P) of 1.77, which indicates the low water-high lipid solubility. The partition coefficient of chemicals in an octanol-water biphasic mixture (log P or Kow) is often used to predict the biosorption and

acute toxicity potential of chemicals, because it is a good indicator of a molecule's solubility in cell membranes. For this reason, the acute toxicity of structurally related chemicals is often positively correlated to their log P coefficients [49]. DMDS is a volatile toxic compound that progressively affects the respiratory system and the central nervous system leading to headaches, dizziness, nausea, and finally to a loss of consciousness. DMDS is frequently used as an additive in natural gas in which it acts as an odorant to warn for leaks and protect people, and is used in the petrochemical industry as a CO-formation inhibitor and catalyst [48]. DMDS is also used as a pre-plant soil fumigant or insecticide, which is registered under the name Paladin [50]. Paladin is an effective pesticide against nematodes parasites, weeds, and plant pathogens [51]. It was introduced as an alternative for the genotoxic soil fumigant methyl bromide (CH_3-Br) that was banned from many countries due to its greenhouse gas properties. Moreover, VSC's such as MT and DMDS, are used in the food and beverage industry as important additives that provide distinct and pleasant characteristics of certain food and beverages, such as cheese, wine and beer [52, 53].

Table 3: Literature data on the composition of sulfidic spent caustics.

Parameter ($g\ L^{-1}$)	**[39]**	**[40]**	**[47]**	**[47]**	**[47]**	**[54]**	**[55]**	**[56]**	**[57]***	**[58]**	**[58]**
COD	0.09	105	156.5	66.7	106		30	26.7	101		
TOC	0.042					1.3	1.1	4.1			
sulfide	0.5 wt%	11.2	66.8	30.6	53	16.4	16.7	5.8	33.9	7.7	5.1
MT		31.7	20	4.58	0.57			-			
DMS			0	0.63	0						
phenol	0.004		n.d.	n.d.	0	0.18		2.8			
benzene			n.d.	n.d.	0.6	0.29				0.47	0.78
toluene			n.d.	n.d.	0.36	0.003					
pH	12.6	>13	11.6	12.5	11.9	13.3	12.2	13		13.2	13
Na^+	5.9 wt%						23.7			18.4	18.4

n.d. = not detected, * = average

Mono-aromatic hydrocarbons, such as benzene and toluene, have also been found in SSC's (Table 3). Benzene is volatile and well known for its relatively high water solubility (1.79 $g\ L^{-1}$ or 22.9 mM at 25 °C) and carcinogenic properties [59, 60]. At room temperature and atmospheric pressure, benzene is a colorless flammable liquid with a boiling point of 80 °C and a log P value of 2.13 [61]. Toluene ($C_6H_5CH_3$) is a mono-substituted benzene derivative (i.e. an extra methyl group (CH_3) group instead of a hydrogen atom). Compared to benzene, toluene is also toxic and volatile, but less soluble in water (0.53 $g\ L^{-1}$ or 5.8 mM at 25 °C) and has a higher log P value (2.69) and a higher boiling temperature (110 °C) [59, 61].

Table 4: Physical and chemical properties of several VSC's (derived from [28, 46]).

Compound	Boiling point (°C)	Melting point (°C)	Odor threshold (ppbv)	Odor	$H_{25°C}$ *	pKa (25 °C)
hydrogen sulfide (H_2S)	-60.7	-85.5	8.5-1000	rotten eggs	0.41	6.90; 12.92
methanethiol (CH_3SH)	6.2	-123.0	0.9-8.5	decayed cabbage	0.10	10.30
ethanethiol (CH_3CH_2SH)	35.0	-147.9	0.01-35	skunk	0.15	10.39
dimethyl sulfide (CH_3SCH_3)	37.3	-98	0.6-40	decayed vegetables	0.073	-
dimethyl disulfide (CH_3SSCH_3)	109.7	-85	0.1-3.6	garlic, putrification, foul	0.045	-

* Dimensionless Henry coefficient at 25 °C

Toxicity

Understanding the toxic properties and the effects of (in)organic sulfur compounds on microorganisms is very important when designing a biological treatment process. Sulfide is known to inhibit cytochrome oxidase, which transfers electrons from cytochrome c to oxygen to form water [62, 63]. Cytochrome c oxidase is an important enzyme that is involved in the respiratory electron transport chain of bacteria. Sulfide is toxic for aerobic and anaerobic bacteria causing severe inhibition and eventually deterioration of biotechnological processes [64, 65].

Concentrations at which 50% of the activity is inhibited (*Ki* value) were found to range from 7 to 14, and from 4.1 to 9.2 mM sulfide for pure cultures of sulfate reducing bacteria (SRB) and methanogens, respectively [65]. *Ki* values for acetoclastic methanogenesis of 5.8 mM (pH about 7) and 1.2 mM (pH about 8) sulfide were also reported [66]. Similar *Ki* values of 5.3 and 1.8 mM sulfide were found for SRB at similar pH values [66]. Under aerobic conditions, *Thiobacilli* were found to be inhibited at concentrations well below 0.005 to more than 0.01 mg L^{-1} [67, 68].

MT was shown to inhibit the cytochrome c oxidase activity in the methionine metabolism [69]. It was suggested that inhibition by MT is caused by steric hindrance and ionic or hydrophobic interactions [67]. Due to its strong nucleophilicity, MT can also break S-S bonds in proteins and thereby inactivate enzymes [70, 71]. *Ki* values for methanogenic granular sludge are 6-8 mM (with acetate), 10 mM (with methanol), and 7 mM (with hydrogen) [72, 73]. Under aerobic conditions, *Ki* values for MT were found to be: 8 µM for *Thiobacillus thioparus* and 14 µM for *Hyphomicrobium* species [74, 75].

Table 5: *Ki* values for methanethiol (*Ki* MT) for different microorganisms

Ki MT (mM)	Microorganism	Substrate	Reference
0.004	*Hyphomicrobium* EG	methanethiol	[75]
0.008	*Thiobacillus thioparus*	sulfide	[74]
10	methanogenic sludge	methanol	[73]
8	methanogenic sludge	acetate	[73]
7	methanogenic sludge	hydrogen	[73]
<6	anaerobic sludge	acetate	[72]
>15	anaerobic sludge	methanol	[72]

Compared to MT, little is known about the effects of DMDS on the respiration of microorganisms. It was suggested that the formation of DMDS plays a role in the detoxification of MT in the aerobic treatment of SSC's [47]. However, DMDS can be toxic to microorganisms as well [76]. Strong inhibition on methanogenic activity was reported at concentrations above 0.26 mM DMDS [77].

Mono-aromatic compounds, such as benzene and toluene, interact with biological membranes resulting in (irreversible) changes in structure and function of the membranes and the activity of the cell [78]. In addition, mono-aromatic compounds possess carcinogenic and mutagenic properties [79]. Catechol ($C_6H_6O_2$), a breakdown product of benzene, has been reported to be more toxic than benzene [80]. Studies concerning the toxic effects on microorganisms are rather scarce. *Ki*-values for benzene (30, 51, 57 and 44 mM) and toluene (14, 38, 63 and 31 mM) were found for the methanogens *Methanosaeta concilii* GP6, *Methanospirillum hungatei* GP1, *Methanobacterium espanolae* GP9 and *Methanobacterium bryantii*, respectively [81]. It was also found that benzene affected cellular metabolic activity for *Pseudomonas fluorescens* and *Photobacterium phosphoreum* with *Ki values* of 0.49 mM and 0.96 mM, respectively [80]. The toxic effects of benzene, inhibiting the growth of *Pseudomonas putida,* was also shown [82]. Finally, benzene was found to inhibit the nitrifying activity of activated sludge by 57% at concentrations of 0.83 mM C [83].

1.5 Treatment of SSC's

In the United States, the disposal of SSC's by "deep well injection" is often applied. Hereby, SSC's are pumped at high-pressure into geologic formations (e.g. sandstone or sedimentary rocks with high porosities) that do not allow migration of the contaminants into potential potable water aquifers. However, deep well injection is a controversial technique with concerns related to leakage of disposal wells and subsequent contamination of water aquifers. In addition, little is known about the chemical and biological processes that may occur in the reservoir once the waste solution is injected [84]. Hence, treatment of SSC's is an attractive alternative to "deep well injection".

In the past, a lively trade was settled with specialized chemical companies in, for example, phenolic/cresylic types of spent caustics. These companies were able to recover valuable components from the spent caustic solutions or use them in their production processes (e.g.

in the pulp and paper industry) [37, 85]. However, during the last decades, spent caustics are considered a serious hazardous waste and its disposal should be in compliant with stringent environmental regulations. In addition, large fluctuations in caustic quality resulted in problems at the buyers. In the past, treating companies (e.g. Merichem) offered financial compensation for the transport cost. Nowadays, the producer is often obligated to pay to dispose the spent caustic. In Europe, costs for the transport and also the disposal fee for spent caustics may range between € 150-750 ton^{-1} if sent to a company like Merichem (in 2004) or another waste management company (personal communication A. Ooms, Shell, 2009). In the case that spent caustics are sold to and processed by specialized chemical companies, it is essential that the different types of spent caustic are delivered fully segregated. However, in practice refineries often lack the facilities to segregate spent caustics [39].

Physico-chemical treatment

Physicochemical treatment methods such as wet air oxidation (WAO), advanced oxidation and the MEROX process (Figure 1) are widely applied for the treatment of SSC's or sour gaseous streams. In the following section each of these methods will be discussed.

Wet air oxidation (WAO) is generally applied for the physico-chemical treatment of SSC's. In this process, soluble or suspended compounds are partially or completely oxidized at elevated temperatures (>320 °C) and high pressures (>210 bar) using air-oxygen as the oxidizing agent [86]. The required operating temperature depends on the treatment objectives. Higher temperatures require higher pressures to maintain a liquid phase in the system [87]. WAO detoxifies the spent caustic by oxidizing H_2S and organic sulfur compounds to sulfate (SO_4^{2-}) and breaking down naphthenics and cresylics [88]. Although dependent on the operating conditions, a part of the total organic carbon (TOC) typically remains as by-product as carboxylic acids (mainly acetate). The effluent of a WAO process is often sent to a conventional biotreater [89]. Wet air oxidation for on-site treatment of spent caustics is available as, for instance, the Zimpro® Wet Air Oxidation process (licensed by Siemens Water Technology).

SSC's may also be treated by advanced oxidation with hydrogen peroxide (H_2O_2). In the presence of H_2O_2 [90]. However, the treatment with H_2O_2 most often leads to an incomplete oxidation of the dissolved sulfide to thiosulfate ($S_2O_3^{2-}$) and hence in a residual chemical oxygen demand (COD) of the treated water. Moreover, the storage and handling of H_2O_2 is associated with considerable safety measures. In addition to H_2O_2 treatment, it was reported that treating SSC's using Fenton's agent increased the oxidation efficiency significantly [91]. By mixing ferrous iron (e.g. $FeCl_2$ or $Fe(OH)_2$) and H_2O_2 under strict regimes, highly reactive hydroxyl or peroxide radicals are formed resulting in increased oxidation power [91, 92]. Besides H_2O_2, chlorine gas (Cl_2 gas), hypochlorites (ClO^-), ozone (O_3) and potassium permanganate ($KMnO_4$) are potential sulfide oxidizing compounds [28, 93, 94].

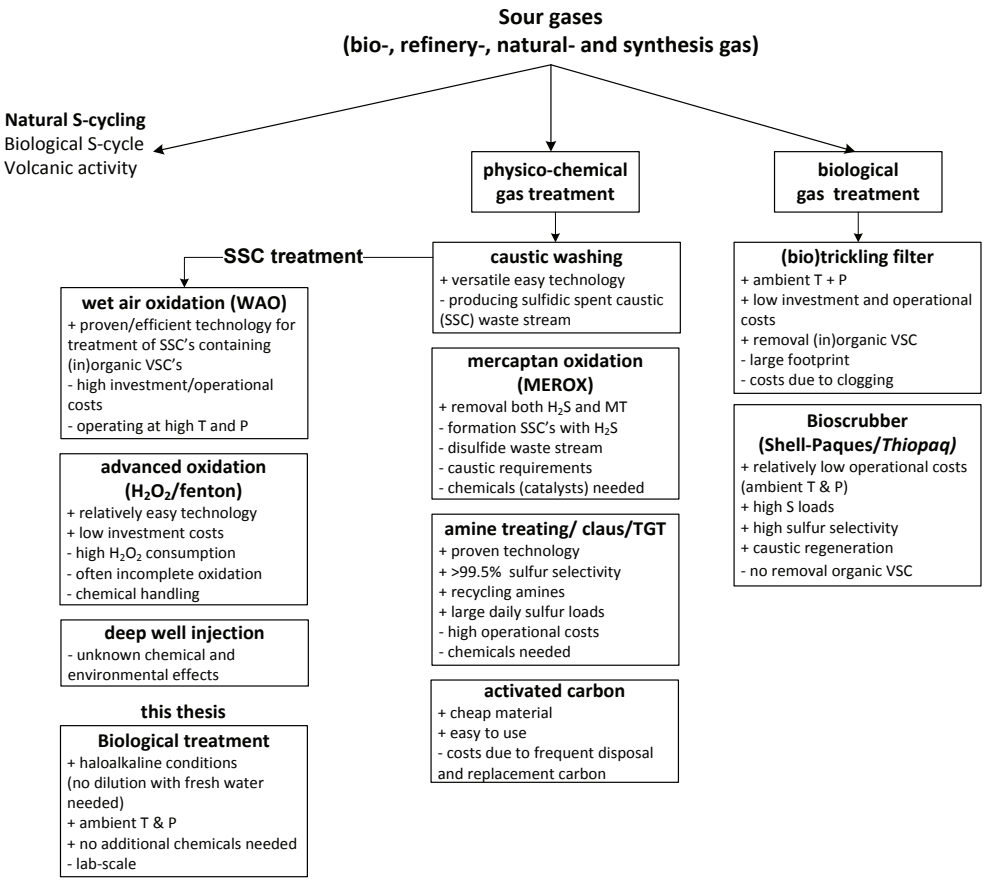


Figure 1: Selection of treatment processes for volatile sulfur compounds (VSC's) from gaseous streams and sulfidic spent caustics (SSC's). T = temperature; P = pressure. The research on the biological treatment of SSC's under haloalkaline conditions as described in this thesis is also shown

The MEROX (mercaptan oxidation) process is widely applied for the removal of low molecular weight mercaptans (e.g. MT) from gaseous streams such as LPG and natural gas [95]. In this 2-step catalytic process, the mercaptans are extracted with caustic solutions and subsequently chemically oxidized to disulfides. In the first pre-wash step, H_2S is extracted from the hydrocarbon stream by a 2 wt% caustic solution [41]. In the following process step, low molecular weight mercaptans are subsequently extracted with a strong caustic solution (20 wt%). In the third step, the mercaptans are oxidized to disulfides (eq. 4) in the presence of hot air and a specifically designed "MEROX catalysts" [41, 95, 96].

$$4NaSR + O_2 + 2H_2O \rightarrow 2RSSR + 4NaOH \qquad \text{eq. 4}$$

In the final step, the disulfides are separated from the solvent and the caustic solution is recycled to the MEROX extractor. Typical product mercaptan levels can be controlled to less than 10 ppm (U.O.P.). MEROX units may treat more than $16x10^3$ m^3 day^{-1} of propane, butane, natural gasoline or a combination of these [46].

1.6 Biological treatment

Microbial processes are considered as alternative for the currently applied physico-chemical desulfurization technologies as these can be operated at ambient temperatures and atmospheric pressures. Hence, the cost for heating, cooling and pressurization are lower compared to physico-chemical processes. Microbial processes are in general also much safer as the operators do not need to work with pressurized reactors or high temperatures. In the following section, the biological treatment of (in)organic sulfur compounds under neutrophilic and haloalkaline conditions is reviewed (Figure 1). These include (bio)trickling filters and bioscrubbing technologies like the *Shell-Paques* or *Thiopaq* process [97].

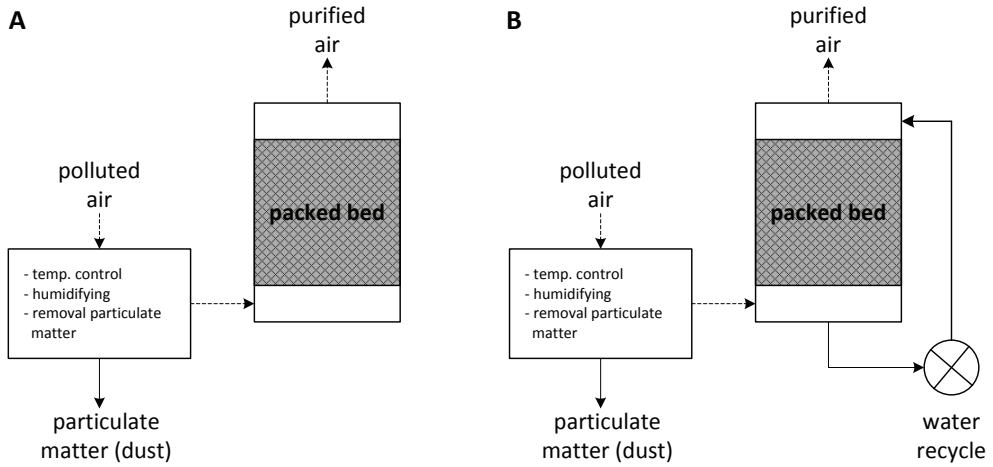


Figure 2: Flow schemes of a biofilter (A) and biotrickling filter (B).

(Bio)trickling filter

Biofilters and biotrickling filters are often applied for the treatment of sour gases. In a biofilter, the sour gas is humidified by occasional bed irrigation whereafter it is brought into contact with microorganisms that are attached to a fixed bed (Figure 2) [98]. The carrier material in the fixed bed section of the biofilter often consists of compost and peat, but may also consist of wood chips, lava rock, coconut fiber or synthetic media. In the presence of oxygen and nutrients, the microorganisms may convert and detoxify (in)organic sulfur compounds and aromatic hydrocarbons [99].

In contrast to biofilters, biotrickling filters are operated with a continuous trickling and recycling of water over the filter bed. The filter bed is filled with a chemically inert carrier

material, such as resins, ceramics, polyurethane foam, polypropylene pall rings, pumice or glass pellets (see Figure 2, and Table 6). Similar to the biofilters, the biological conversion of the gas pollutants occurs mainly on the surface of the carrier material in the so-called biofilm. Major drawbacks of biofilters and biotrickling filters are the relatively large footprint and pressure drop due to accumulation of biomass and intermediate compounds (e.g. S^0).

Operating experience from biological gas treatment systems for the removal of VSC's

The use of biofilters and biotrickling filters for the treatment of H_2S and organic sulfur compounds has been studied extensively in the last decades (Table 6). Elimination capacities (EC's) up to 125 gram H_2S m^{-3} h^{-1} were found in a 1 L lab scale biofilter packed with granular activated carbon and inoculated with SOB obtained from latex wastewater [100]. Leachate recycling was found to be an effective strategy for increasing the EC [101]. It results in uniform distribution of the biologically produced S^0 in the biofilter causing a relatively lower pressure drop compared to systems without leachate recycling. A maximum EC of 8 grams H_2S m^{-3} h^{-1} was found by Kim et al. [102] which also found that the employed biofilter quickly recovered from shock loads. Hence, it was suggested that an immobilized cell biofilter is effective in treating H_2S under steady and transient operating conditions.

Lab-scale biotrickling filters have been found to remove up to 300 grams H_2S m^{-3} h^{-1} under neutrophilic aerobic and anaerobic conditions [103, 104]. Gabriel et al. [105] studied a full-scale biotrickling filter packed with polyurethane foam able to treat 16,000 m^{-3} h^{-1} of foul air (105 gram H_2S $m^{-3}h^{-1}$) at gas residence times of 2.2 seconds. The potential of biotrickling filters for the treatment of organic VSC, such as MT, ET, DMDS, DMS and carbon disulfide (CS_2), has been shown in several studies (Table 6). Arellano-Garcia et al. [106] showed that gaseous DMS was eliminated at an EC of 4 grams DMS $m^{-3}h^{-1}$ in a biotrickling filter consisting of *Thiobacillus thioparus*. They also showed that the system supported shock loads up to 58 grams DMS m^{-3} h^{-1} while a nitrogen-enriched medium was used. Simultaneous CS_2, DMS and DMDS removal was achieved in lab-scale biotrickling filters by Pol et al. [107] employing *Thiomonas* species. *Chryseobacterium sp. JLL* and an alkaliphilic sulfur oxidizing bacterial consortium have been shown to remove MT and ET [108, 109]. In addition, ET and CS_2 were successfully co-treated under high alkaline (pH 10) conditions [108].

In contrast to conventional single-stage biotrickling filters, Ramirez et al. [110] showed the potential of a two-stage biotrickling filter for the simultaneous treatment of H_2S and organic sulfur compounds. The first biofilter was inoculated with *Acidithiobacillus thiooxidans* and the second one with *Thiobacillus thioparus*. Compared to a single-stage biotrickling filter, the simultaneous removal efficiencies of H_2S, MT, DMS and DMDS increased significantly at inlet loading rates of 5.9, 2.7, 2.3 and 5.7 grams S m^{-3} h^{-1}, respectively at an empty bed residence time (EBRT) of 59 s.

In general, biotrickling filters are operated at neutrophilic conditions (pH = 7 and 30 ^oC). However, treatment of H_2S at low pH (pH 2) has been shown in lab-scale experiments as well as full-scale biotrickling filters [105, 111]. Gonzales-Sanchez et al. [112] showed the treatment of H_2S under alkaline conditions (pH 10) by using an alkaliphilic sulfur-oxidizing bacterial (SOB) consortium in a lab-scale biotrickling filter. The also concluded that operating at high pH results in an enhancement of the mass transfer by a factor of 1,700 to 11,000.

Datta et al. [113] showed the potential of applying biotrickling filters to a wider range of H_2S-containing gaseous streams at high temperature (<70 °C). They also found that adding glucose and glutamate enhances the treatment efficiency. It was suggested that the glucose acted as carbon source, whereas glutamate is acting as a compatible solute.

Sipma et al. [72, 114] showed that MT degradation was feasible in a lab scale Upflow Anaerobic Sludge Blanket (UASB) reactor at ambient conditions (pH 7, T 30 °C) with ECs up to 2.4 gram MT L^{-1} d^{-1}. Van Leerdam et al. [17, 46, 115, 116] showed that MT could also be degraded by methanogenic archaea at a pH 8-10 and Na^+ concentrations up to 0.5 M (Table 6).

Table 6: Operating conditions for different biological gas treatment systems for the removal of VSC's. T = temperature, EC = elimination capacity, LR = loading rate, c_in = influent concentration, c_out = effluent concentration.

Process [reference]	Compound	Packing material	Bacteria	pH/ T (°C)	Efficiency (%) EC LR	Residence Time	C_in/C_out
biofilter [117]	H_2S	organic carrier material	acidophilic *Thiobacilli*	<3/30		30-60 s	
biofilter [118]	H_2S MT DMS	polypropylene pellets	*Thiobacillus thioparus* TK-m	7/25	>95% LR 17.4 mM H_2S day^{-1} 8.7 mM MT day^{-1} 3.7 mM DMS day^{-1}	SV 0.1 m/s	H_2S 35.4/1.62 MT 17.8/0.76 DMS 7.4/0.28 (µl L^{-1})
biofilter [119]	DMDS	fibrous peat	anaerobically digested night soil sludge	5-7/18	EC: 0.68 g S (kg dry peat)$^{-1}$ d^{-1}	Space velocity: 36h^{-1}	5-40/<10 (ppm)
biofilter [120]	H_2S MT	calcium chloride containing beads	activated sludge	pH H_2S: 2-3 MT: 6-8	H_2S > 98% MT > 90% EC: 6500 g H_2S m^{-3} bed d^{-1} 4 g MT m^{-3} bed d^{-1}	H_2S: 11-19 s MT: 7-18 s	H_2S: 200-1300/- MT: 3.1-30/- (mg m^{-3})

biofilter [121]	H_2S MT DMDS DMS	granular activated carbon	dominant species: *Pseudomonas* sp.	7/30	96-100% 0.48 g H_2S m^{-3} h^{-1} 2.49 g MT m^{-3} h^{-1} 5 g DMDS m^{-3} h^{-1} 0.84 g DMS m^{-3} h^{-1}	13-30 s	H_2S: 0.7/0 (ppm) MT: 2.5/0 (ppm) DMDS: 2.1/0 (ppm) DMS: 1.1/0.06 (ppm)
biofilter [102]	H_2S	biomedia, encapsulated by sodium alginate and polyvinyl alcohol	activated sludge	7/30	62% EC: 8 g m^{-3} h^{-1} LR: 13 g m^{-3} h^{-1}	51 s	-
biofilter (leachate recycling) [101]	H_2S	municipal solid waste compost and PVC	activated sludge	7/30	95% EC: 21 g m^{-3} h^{-1} LR: 22 g m^{-3} h^{-1}	60 s	20-275/- (ppm)
biofilter [100]	H_2S	granular activated carbon	sulfur-oxidizing bacteria from latex wastewater	8/30	>98% EC: 125 g m^{-3} h^{-1}	Air flow rate 5.9 L h^{-1}	200-4000/- (ppm)
2-stage biotrickling filter [110]	H_2S MT DMS DMDS	polyurethane	Stage 1 *Acidithiobacillus thiooxidans* Stage 2 *Thiobacillus. thioparus*	7/30	H_2S: 99% MT: 91% DMS: 95% DMDS: 93% LR:2.3-5.7 g S m^{-3} h^{-1}	59 s	H_2S: 75-234/- (ppmv) MT: 34/- (ppmv) DMDS: 36/- (ppmv) DMS: 29/- (ppmv)
biotrickling filter [106]	DMS	polyurethane	*T. thioparus*	7/30	77-100% EC: 2-4 g S m^{-3} h^{-1}	40 s	-
biotrickling filter [111]	H_2S	polypropylene pall rings	-	2/25	100% EC: 6.4 g H_2S m^{-3} h^{-1}	24 s	H_2S: 12/0 (ppm)
biotrickling filter [108]	CS_2 ET	polyurethane	alkaliphilic sulfoxidizing bacteria	10/30	CS_2: 50%, ET: 80% EC: 2.3 g CS_2 m^{-3} h^{-1} EC: 3.6 g ET m^{-3} h^{-1}	30-120 s	-
biotrickling filter [107]	CS_2, DMS DMDS	lava stone	*Thiomonas* sp.	7/25	±95%	Space velocity = 54-130 h^{-1}	CS_2: 2-3 µM/-

biotrickling filter [103]	H_2S	polyurethane polypropylene	sulfur-oxidizing biomass	7/30	EC: 280 g m^{-3} h^{-1} LR: 75-370 g m^{-3} h^{-1}	167 s	2500-12300/<500 (ppmv)
pilot scale biotrickling filter (anoxic) [104]	H_2S/NO_3^-	plastic fibers	*Thiobacillus denitrificans*	7/30	>99% EC: 270-300 g m^{-3} h^{-1}	Biogas flow rate: 40 L h^{-1}	1100/0 (ppmv)
full scale biotrickling filter [105]	H_2S	polyurethane	sulfur-oxidizing biomass Heterotrophs	2/30	>95% EC: 95-105 g m^{-3} h^{-1}	1.6-2.2 s	30/<1 (ppmv)
fixed film bioscrubber [122]	H_2S	polypropylene pall rings	*Thiobacillus*	7/25	99% EC: 90 g m^{-3} h^{-1}	5 s	5/- (ppm)
fixed film bioscrubber [123]	H_2S	polypropylene pall rings	*Acinetobacter* sp. *Alcaligenes faecalis*	7/30	98%	32 s	100/- (ppmv)
biotrickling filter + bioscrubber [109]	MT	pumice and activated carbon	*Chryseobacterium* sp. JLL	7/25	>98% EC: 50.8 g m^{-3} h^{-1}	60 s	0.1-1/- (mg m^{-3})
biotrickling filter [113]	H_2S	highly porous glass pellets	inoculum obtained from a hot spring	7/70	±30% EC: 35-40 g m^{-3} h^{-1} LR: 125 g m^{-3} h^{-1}	60 s	50-1650/- (ppmv)
biotrickling filter [112]	H_2S	polyurethane	alkaliphilic sulfer-oxidizing bacteria	10/30	>98% EC: 30 g m^{-3} h^{-1}	1-6 s	2.5-18/<0.1 (ppmv)
membrane bioreactor [124]	DMS	-	*Hyphomicrobium* VS	7/30	89% EC: 3.2 kg DMS g VS–1 d–1 LR: 4 kg DMS g VS–1 d–1	24 s	1.12/- (g DMS m^{-3})
UASB reactor [72, 114]	MT	-	methylotrophic methanogenic archaea from granular sludge	7/30	EC: 10-50 mM d^{-1}	-	2-14/<0.1 (mmol L^{-1})
UASB reactor [73, 115, 116]	MT	-	methanogenic archaea	8-10/30	EC: 37 mM d^{-1}	-	2-12/<0.1 (mmol L^{-1})

Shell-Paques process [125]	H_2S	raschig rings	*Thiobacilli* or HA-SOB	8/30	>99.5%	-	0.8-1.2 vol.% / <4 ppmv

***Shell-Paques*/*Thiopaq* process for H_2S removal**

The *Shell-Paques* or *Thiopaq* process is a high-rate biotechnological process for H_2S removal from gaseous streams, such as biogas, high-pressure natural gas and refinery gas. Worldwide more than 120 full-scale installations are applied, that are able to treat 50-100 tons sulfur day^{-1} [97]. The 2-step process consists of an absorber column followed by a bioreactor. In the (high pressure) absorption column, the H_2S is absorbed into the liquid phase by a reaction with hydroxyl and (bi) carbonate ions (eq. 1 and 5) [125].

$$H_2S + HCO_3^- \rightarrow HS^- + CO_2 + H_2O \quad \text{eq. 5}$$

$$HS^- + ½O_2 \rightarrow S^0 + OH^- \quad \text{eq. 6}$$

$$HS^- + 1.5O_2 + H_2O + nCO_2 \rightarrow SO_4^{2-} + H^+ + nCH_2O \quad \text{eq. 7}$$

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \quad \text{eq. 7a}$$

$$HS^- + O_2 \rightarrow ½S_2O_3^{2-} + ½H_2O \quad \text{eq. 8}$$

In the second step biological conversion of H_2S to S^0 takes place under oxygen-limited conditions at moderate pH values, i.e. about pH 8.3 (eq. 6). In addition, a minor fraction of SO_4^{2-} is formed, typically <10% (eq. 7) [63]. In the bioreactor chemolithoautotrophic sulfur oxidizing bacteria (SOB) belonging to the genus *Thiobacillus* perform the biological sulfide oxidation process [126]. Under oxygen-rich conditions, SOB oxidize sulfide completely to SO_4^{2-} (eq. 7a). SO_4^{2-} formation (ΔG^0 = -732.58 KJ mol^{-1}) yields more energy with respect to microbial respiration compared to S^0 formation (ΔG^0 = -169.35 KJ mol^{-1}). In the *Thiopaq* process commonly small amounts of SO_4^{2-} are formed due to competition with abiotic sulfide oxidation reactions (eq. 8) that produce $S_2O_3^{2-}$ as an intermediate [125, 127] or further oxidation of the meta-stable S^0 particles. The formed S^0 particles are removed from the bioreactor suspension via a gravity settler while the remaining alkaline solution is reused in the absorption column. High S^0 formation efficiencies are essential for low operation costs as the formation of other products increases caustic consumption and make up water.

Biological treatment of VSC's under haloalkaline conditions

Based on the recent developments of the *Thiopaq* process, a new biotechnological desulfurization processes for the removal of H_2S under haloalkaline conditions (2 M total salt) was developed by Van den Bosch (2008) [17]. Process operation under haloalkaline conditions (pH>9) would lead to a number of important advantages. First of all, it allows the treatment of high pressure natural gas that contains both H_2S and high levels of CO_2. In that way, the sodium ion (Na^+) serves as a counter-ion for high concentrations of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), which prevents acidification. Also the sulfide loading

capacity increases due to the high pH and (bi)carbonate concentrations. This means that the overall recirculation of the solution over the (high-pressure absorber) column can be reduced resulting in lower operational costs [17]. The new biotechnological process relies on extremophilic chemolithoautotrophic microorganisms called haloalkaliphilic sulfide oxidizing bacteria (HA-SOB) that can thrive at pH 7.5-10.6 and at Na^+ concentrations up to 4.3 M [128, 129]. Reactor isolates showed that all SOB belonged to the genus *Thioalkalivibrio* [129]. It was recently found that HA-SOB belonging to the genus *Thioalkalivibrio* also dominate a full-scale *Thiopaq* installation in Eerbeek, the Netherlands [130]. This was the result of a shift towards higher total salt content (from 0.3 to 1 M Na^+) [125].

It was shown by Van den Bosch [17] that maximum H_2S loading rates of 16.6 g L^{-1} day^{-1} could be applied with a S^0 formation rate of 13.1 g L^{-1} day^{-1}. Based on respiration experiments, it was suggested that loading rates of up to 35 g L^{-1} day^{-1} is feasible at higher biomass concentrations. It was also found that small fractions (typically >10%) of sulfide are inevitable oxidized to $S_2O_3^{2-}$ and SO_4^{2-}. Therefore, it was proposed to add a reduction step in which these sulfur compounds are reduced back to sulfide before recycling to the sulfide oxidizing bioreactor [127]. In the PhD thesis by van den Bosch, the potential for the application of HA-SOB to biotechnological desulfurization processes has been described extensively.

1.7 Biological treatment of SSC's

On-site treatment of SSC's in conventional biological wastewater treatment plants is standard practice at many refineries. In a nitrifying-denitrifying biotreatment system, ammonia (NH_3) is ultimately converted to nitrogen gas (N_2) via nitrate NO_3^- and protons (H^+) (eq. 9a). In the subsequent denitrification step (eq. 9b), the alkaline SSC's lead to pH neutralization while sulfide can be used as electron donor [131].

$$NH_4^+ + 2O_2 \rightarrow 2H^+ + NO_3^- + H_2O \qquad \text{eq. 9a}$$

$$8\ NO_3^- + 3H^+ + 5HS^- \rightarrow 4N_2 + 4H_2O + 5\ SO_4^{2-} \qquad \text{eq. 9b}$$

Although addition of small amounts of SSC's to a biotreater is feasible to some extent, these processes are not designed to handle large amounts of complex SSC waste streams. Biological processes can easily be disturbed by fluctuating pH conditions, increasing salt concentrations and the accumulation of toxic compounds [132].

Limited research has been reported on the biological treatment of SSC's. Moreover, most researchers focused on the application of neutrophilic bacteria belonging to the genus *Thiobacillus* (Table 7). In this process, the SSC solutions needs to be diluted with fresh water in order to lower the pH and Na^+ levels down to process conditions [47, 131]. Kolhaktar and Sublette [56] showed the feasibility of *Thiobacillus denitrificans* for the biological treatment of refinery SSC's [56]. Specific activities of 1.3 mM sulfide h^{-1} g suspended $solids^{-1}$ were reached. *T. denitrificans* was successfully applied by Rajganesh et al. [57], who fed refinery spent caustic (pH >12) to the bioreactor in which it was partially oxidized to H_2SO_4. The biological treatment of SSC's and simultaneous oxidation of H_2S and MT was reported by Sipma et al. [47] and Subramaniyan et al. [133]. Both studies employed aerobic bioreactors inoculated

with mixtures of *Thiobacilli* and activated sludge derived from a full-scale *Thiopaq* installation. It was observed by Subramaniyan et al. [133] that the oxidation rates for H_2S (0.64 mmol h^{-1}) and MT (1.2 mmol h^{-1}) were lower than expected. Therefore it was suggested that other, not specified, compounds in the spent caustic solution inhibited the *Thiobacilli*. Sipma et al. [47] reported simultaneous oxidation of 10 mM sulfide and 2.5 mM MT to SO_4^{2-} at a hydraulic residence time (HRT) of 6 h^{-1}. Addition of phenol, which is a common pollutant in spent caustics, did not cause inhibitory effects at concentrations of 7.5 mM. In addition, several refinery SSC's were successfully treated. Conner et al. [40] reported the complete oxidation of H_2S and MT by immobilized *Thiobacillus thioparus* in a fluidized-bed column bioreactor.

Table 7: Overview of research regarding the biological treatment of spent caustics under neutrophilic conditions using *Thiobacilli*. (T = temperature)

Process	Compound	Bacteria	pH/ T (°C)	Product	Reference
bench and pilot scale stirred tank reactors	spent caustics: H_2S	*Thiobacillus denitrificans*	7/30	SO_4^{2-}	[56, 57, 85, 134]
fluidized-bed bioreactor	spent caustics: H_2S, MT	*Thiobacillus thioparus*	7/30	SO_4^{2-}	[40]
fluidized-bed bioreactor	synthetic spent caustics: H_2S	*Thiobacillus* sp.	6/30	SO_4^{2-}	[135]
bubble column reactor	spent caustics: H_2S, MT	*Thiobacilli* from *Thiopaq* sludge	7/30	SO_4^{2-}	[47]
bioreactor	spent caustics: H_2S, MT	mixture of *Thiobacilli*	7/30	SO_4^{2-}	[133]
bubble column reactor	H_2S, MT, DMDS, DMS	*Thiobacillus novellus*	7/30	SO_4^{2-}	[136]
upflow reactor	H_2S, MT, DMDS, DMS	*Thiobacillus thioparus*	7/30	SO_4^{2-}	[137]
batch experiments	H_2S, MT, DMDS, DMS	*Thiobacillus thioparus*	7/30	SO_4^{2}	[138]
batch and continuous culture	DMDS	*Thiobacillus thioparus*	7/30	SO_4^{2}	[74, 139]

1.8 Haloalkaliphilic sulfur bacteria

Soda lakes represent highly stable alkaline habitats (pH 9.5–11) due to the high buffering capacity of sodium carbonate [140]. Soda lakes may contain Na^+ concentrations up to saturation [141]. In general, soda lakes contain cations, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), and anions including chloride (Cl^-), and SO_4^{2-} [128]. Microorganisms that live and grow under these conditions are in general classified as haloalkaliphiles indicating their preference for or dependency on high pH and high salt environments. Within soda lakes, the sulfur cycle is amongst the most active microbial processes [140]. In the anoxic zone

of the soda lake sediments SO_4^{2-}, $S_2O_3^{2-}$ and sulfite (SO_3^{2-}) are reduced to sulfide by sulfate reducing bacteria (SRB) that may belong the genera *Desulfonatronovibrio*, *Desulfonatronum*, *Desulfurivibrio* and *Desulfonatronaspira* [142]. SOB are active in the (oxic) surface sediment layers of soda lakes. The culturable species are up to now represented by four genera within the Gammaproteobacteria, including *Thioalkalivibrio*, *Thioalkalimicrobium*, *Thioalkalispira* and *Thioalkalibacter* [143].

Members of the genus *Thioalkalivibrio* are aerobic chemolithoautotrophic SOB that are the most widely distributed with including nine described species and many uncharacterized isolates [128, 140, 144]. Different *Thioalkalivibrio* species are able to oxidize a broad range of sulfur compounds including sulfide, $S_2O_3^{2-}$, S^0, SO_3^{2-}, tetrathionate ($S_4O_6^{2-}$) and thiocyanate (SCN^-) [143]. *Thioalkalivibrio* species have the availability of several enzymes for the oxidation of sulfide to S^0 and SO_4^{2-} [130].

As the HA-SOB generally are bound to high energy demands for maintenance, it is expected that HA-SOB possess energy efficient routes [145]. Sulfide enters the respiration chain via the enzyme flavocytochrome c oxidoreductase (FCC), which oxidizes sulfide to S^0 with cytochrome c as electron acceptor [130, 145]. In addition, some variants of FCC have been suggested (FQ), transferring electrons to ubiquinone [63]. Recently, it was suggested that oxidation of S^0 to SO_4^{2-} proceeds via a reversed dissimilatory sulfite reductase pathway and sulfite dehydrogenase [130]. In addition to sulfide oxidation, some bacteria can reduce nitrate (NO_3^-), nitrite (NO_2^-) or nitrous oxide (N_2O) as an energy and nitrogen source [146].

Members of the genus *Thioalkalivibrio* grow in a very broad range of salinities (0.3 - 4.3 M Na^+), have an optimum pH of 10, have a relatively low growth rate and exhibit (relative) high growth yields [128]. In contrast, members of the genus *Thioalkalimicrobium* grow in lower salinities ranges from 0.3 to 1.5 M Na^+, have an optimum pH between 9.5 and 10, have a relatively high growth rate with relatively low growth yields [128]. Furthermore, it was found that *Thioalkalimicrobium aerophilum* produced ectoine and *Thioalkalivibrio versutus* glycine betaine as their main compatible solute [147]. These compatible solutes are used for osmoregulation and the intracellular production of these compounds was found to be positively correlated to the Na^+ concentration of the medium [147]. Hence, living under hyper saline conditions requires the production of osmolites, which requires a lot of energy that cannot be used for growth.

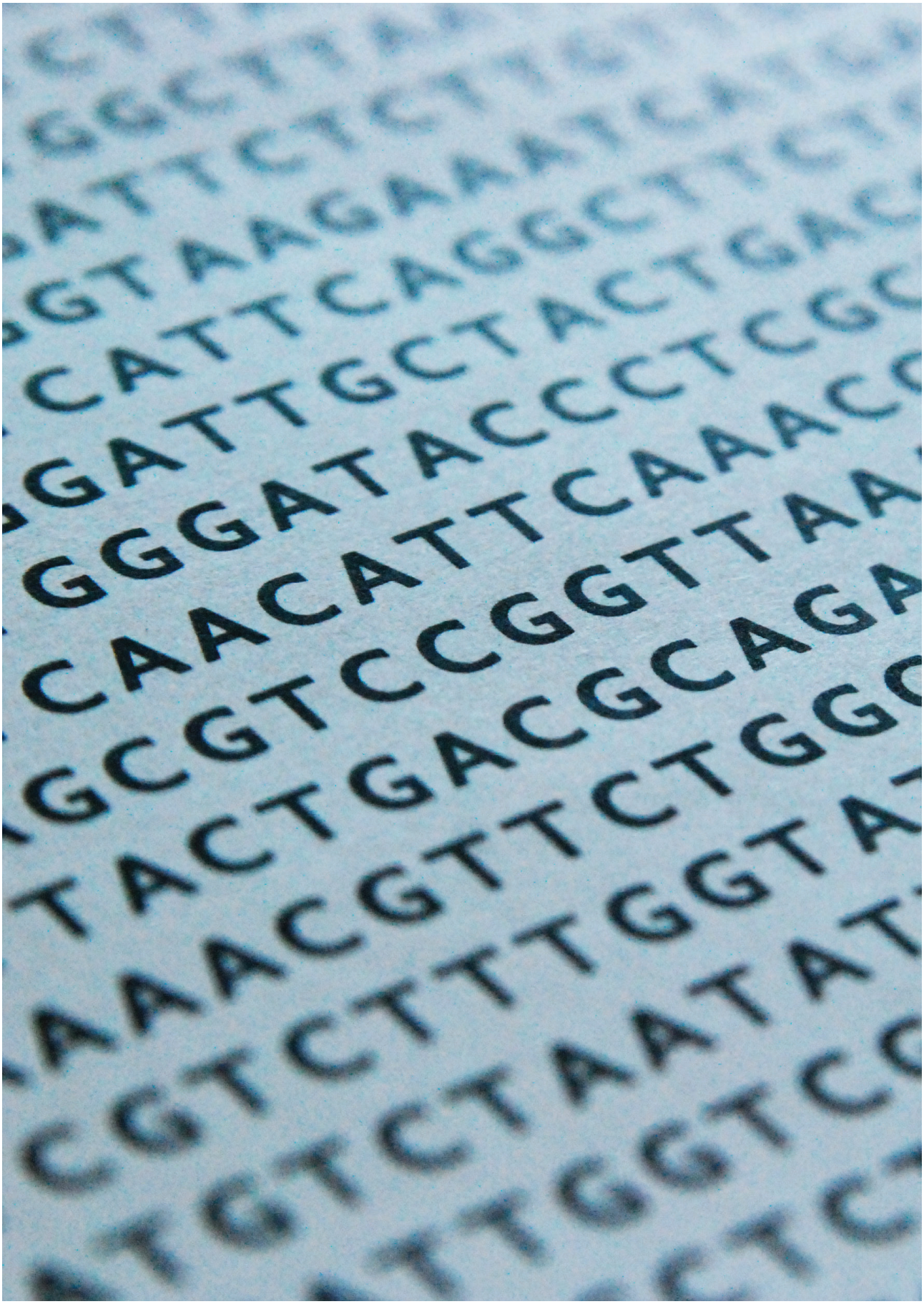
Also heterotrophic organisms, identified as members of the *Halomonas* group, have been isolated from soda lakes that are capable to partially oxidize sulfur compounds such as $S_2O_3^{2-}$ and sulfide into $S_4O_6^{2-}$. The produced $S_4O_6^{2-}$ can react with sulfide with the formation of S^0 and $S_2O_3^{2-}$ [148].

1.9 Scope and Outline

The 4-year research program of which this thesis is the result, is an initiative of Wetsus and Wageningen University in cooperation with Delft University of Technology. Within the TTIW-cooperation framework of Wetsus, the work was performed within the research Theme "Concentrates", with the following participating companies: *Shell (Amsterdam)*, *Vitens (Utrecht)*, *Aquacare* ('s-Hertogenbosch) and *PWN* (Velserbroek).

The objective of this thesis is to develop a biotechnological process for the treatment of SSC's without the need for dilution water. For this reason, haloalkaline bacteria isolated from soda lakes were used. In this process, the dissolved sulfides are oxidized to SO_4^{2-}. This offers the following advantages: (1) complete oxidation of sulfide to SO_4^{2-} results in a significant, if not complete, removal of chemical oxygen demand (COD). (2) protons (H^+) that are formed during SO_4^{2-} formation will result in a (partial) pH neutralization of the effluent.

Chapter 2 describes the biological treatment of spent caustic solutions collected at a refinery. Special attention has been paid to the microbial community analyses in the bioreactors, sulfide conversion rates and the fate of benzene. Chapter 3 describes the effects of organic VSC's on the treatment of SSC's. Bioreactor experiments are presented with synthetically prepared SSC solutions containing sulfide and DMDS in the influent. Also the effects of MT and DMDS on biological sulfide oxidation was investigated for several *Thioalkalivibrio* species and sludge taken from a full-scale *Thiopaq* unit (Industriewater Eerbeek B.V.). In chapter 4 the research on the toxicity of MT and the products from the reaction between MT and biosulfur particles is presented. The haloalkaliphilic bacterial consortium that was used in the respiration experiments originated from lab-scale fed-batch bioreactor experiments that was operated at S^0 forming conditions [127]. The application and advantages of a 2-step process for the biological treatment of SSC's is presented in chapter 5. Based on bioreactor experiments a mathematical model was developed to compare a 2-step process with a conventional 1-step treatment process. In chapter 6, the main conclusions of this thesis will be discussed. Finally, a short summary of this thesis is given.



Chapter 2

Biological treatment of refinery spent caustics under haloalkaline conditions

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This chapter is published as:
Biological treatment of refinery spent caustics under haloalkaline conditions
Bioresource technology, Vol 102, 7257-7264, 2011

Abstract

The present research demonstrates the biological treatment of refinery sulfidic spent caustics (SSC's) in a continuously fed system under haloalkaline conditions (i.e. pH 9.5; Na^+ 0.8 M). Experiments were performed in identical gas-lift bioreactors operated under aerobic conditions (80-90% saturation) at 35 °C. Sulfide loading rates up to 27 mmol L^{-1} day^{-1} were successfully applied at a HRT of 3.5 days. Sulfide was completely converted to sulfate (SO_4^{2-}) by the haloalkaliphilic sulfide-oxidizing bacteria (HA-SOB) belonging to the genus *Thiolalkalivibrio*. Influent benzene concentrations ranged from 100 to 600 μM. At steady state, benzene was removed by 93% due to high stripping efficiencies and biodegradation. Microbial community analysis revealed the presence of haloalkaliphilic heterotrophic bacteria belonging to the genera *Marinobacter*, *Halomonas* and *Idiomarina* which might have been involved in the observed benzene removal. The work shows the potential of haloalkaliphilic bacteria in mitigating environmental problems caused by alkaline waste.

2.1 Introduction

Diluted caustic (NaOH) solutions are often used in the petrochemical industry for the removal of acidic compounds, e.g. volatile (organic) sulfur compounds from hydrocarbon streams, such as gaseous streams and LPG. The use of caustic leads to the formation of a waste product referred to as sulfidic spent caustic (SSC's). These spent caustic solutions originating from oil refineries are characterised by a high pH (pH >12) and elevated sodium (Na^+) concentrations up to 5-12% by weight [36]. Hydrosulfide (HS^-) and sulfide (S^{2-}) typically are the most dominant sulfur compounds found in spent caustics with concentrations that may exceed 2-3 wt% [40]. Total dissolved sulfide (i.e. the sum of H_2S, HS^- and S^{2-}) is well known for its toxic, odorous and corrosive properties. Besides total dissolved sulfide, a variety of organic sulfur compounds and aromatic hydrocarbon compounds are commonly found in spent caustics of which methanethiol (CH_3SH), benzene, toluene and phenol are most pronounced. [36, 41, 149, 150]. The actual composition of spent caustics is, however, very much dependent on the type of hydrocarbon stream that has been treated.

Due to more stringent (environmental) regulations, the transport and handling costs of spent caustics are currently very high. Disposal of spent caustics for either reuse or product recovery purposes is therefore becoming less economically attractive. In addition, the fluctuations in caustic quality, due to differences in the crude oil composition and storage policies at different refineries, causes problems for the companies that process spent caustics [36].

Wet air oxidation is generally applied for the physico-chemical treatment of spent caustics. In this process, soluble or suspended compounds are partially or completely oxidized at elevated temperatures and pressures using air-oxygen as the oxidizing agent [86]. Chemical treatment of sulfidic spent caustics, such as treatment with hydrogen-peroxide, most often leads to an incomplete oxidation of the dissolved sulfide to thiosulfate and hence in a residual chemical oxygen demand of the treated water. Moreover, the storage and handling of the hydrogen peroxide is associated with considerable safety measures.

Biological treatment of spent caustics, at atmospheric pressures and temperatures would be a cheaper and safer alternative to the currently employed physico-chemical treatment processes. On-site treatment of spent caustics in conventional biological waste water treatment plants is standard practice at many refineries. Although addition of small amounts of spent caustics to a biotreater can work to some extent, these processes are not designed to handle large amounts of complex spent caustic waste streams as the biological processes can easily be disturbed by fluctuating pH conditions, increasing salt concentrations and the accumulation of toxic compounds [132]. Typical spent caustic production rates may amount up to 15 m^3 per day [41]. This represents a significant portion of the hydraulic and COD load to the refineries biological wastewater treatment plant [151]. Moreover, the growth of filamentous bacteria, such as sulfide-oxidizing *Thiothrix* species in activated sludge systems may lead to severe operating problems as a result of the formation of bulking sludge [152]. Previous research has shown that dilution factors up to 3 had to be applied in order to lower the pH and sodium levels down to acceptable concentrations for neutrophilic sulfide-oxidizing bacteria (SOB) [150]. Particularly in arid regions this would be a serious drawback for the application of this new process. Hence, investigation of the application of haloalkaliphilic microorganisms for the treatment of complex sulfide-containing waste streams is of great interest. Recently, a new biotechnological process has been described for the removal of hydrogen sulfide from high-pressure natural gas [127]. This process relies on a specialized group of haloalkaliphilic sulfide oxidizing bacteria that is also considered for the treatment of undiluted sulfidic spent caustics [128].

In the current study, attention is also paid to the fate of benzene because it is well known for its relatively high water solubility, stability and carcinogenic properties [61]. Hence, it has to be removed from spent caustic solutions prior to discharge into the environment. Up till now a limited amount of literature is available on the biodegradation of mono-aromatics under haloalkaline conditions [153-155]. Furthermore, the effects of benzene on biological sulfide oxidation have not been investigated. Two bioreactors were inoculated with biomass obtained from soda lake sediments and continuously fed with spent caustic solutions collected from a refinery. In two long-term test runs, the oxidation of sulfide into sulfate and the removal of benzene has been evaluated over periods of 78 and 55 days, respectively. Denaturing gradient gel electrophoresis (DGGE) and cloning of PCR-amplified 16S rRNA gene fragments were used to monitor the microbial community dynamics during the experimental period to identify the community members.

2.2 Materials and Methods

2.2.1 Experimental Set-Up

Two continuously operated gas-lift reactors with a liquid volume of 2.2 L (Ø=10 cm) were used (Fig. 1). Temperature was maintained constant at 35 °C using a water-jacket and a thermostat bath (Haake, Germany). Influent was added to the reactor using peristaltic pumps (Masterflex® L/S®, Cole-Parmer instruments, USA). The influent was added to the downer section of the reactor to prevent short-circuiting. pH was monitored using a pH sensor

(Endress+Hauser orbisint CPS12D, Naarden, The Netherlands).

The percentage of oxygen saturation (% sat) was monitored (Mettler Toledo Inpro 6050 oxygen sensor) and controlled at 80-90 % by supplying pure oxygen via mass flow controllers (Bronkhorst, The Netherlands). The gas phase was continuously recycled using a small compressor (N820 (20 L min^{-1}), KNF pumps, Germany) (Fig. 1, A). The recirculation gas phase first passes a condenser (10 °C) to recover volatile compounds that are stripped from the bioreactor suspension (Fig. 1, B).

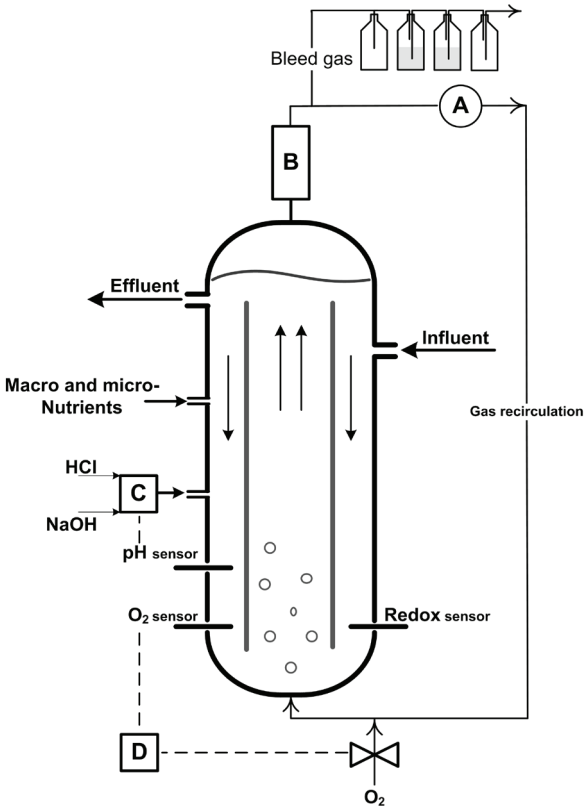


Figure 1: Schematic overview of the continuously Run gas-lift reactors. Sensoring and controlling units for oxygen, pH and redox are shown as well as the gas recirculation pump (A), condenser (B), and pH (C) and oxygen (D) controlling unit. Bleed gas left the reactor via a series of washing bottles.

2.2.2 Inoculum and influent

The inoculum consisted of Russian soda lakes sediments (Kulunda Steppe, Altai) that were kindly provided by Delft University of Technology [156].

Two spent caustic solutions (Solution A and B), taken from the same refinery at different moments in time, were used as influent solutions (Table 1). Because the sulfide concentrations

in Solution A and B were very high, the solutions were mixed with sodium carbonate (Na_2CO_3) solutions that were prepared at the same salinity (0.8 M) and pH (pH 9.5). This improved the stability of the system by decreasing the hydraulic retention time (HRT) whilst keeping the sulfide load and sodium concentration in the influent constant. During the experimental runs, the ratio of spent caustic solution over Na_2CO_3 solution was changed to meet the desired HRT. 1 g L^{-1} NaCl was added to the Na_2CO_3 solution to meet the chloride requirements for growth of haloalkaliphilic sulfide oxidizing bacteria [128]. Macro-nutrients (range of 2.5^{-4} to 2^{-3} mL min^{-1} for HRT 30 to 3.5 days) were continuously added to the reactor liquid in the following amounts: 1 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgCl_2.6\ H_2O$ and 0.6 g L^{-1} urea. 0.5 ml L^{-1} of trace element solution was added as described elsewhere [157].

Table 1: Characterization of raw refinery spent caustic solutions, sampled at two different moments in time.

	Solution A	Solution B
sulfide (mM)	240	160
benzene (mM)	0.6	10
sodium (M)	0.8	0.8
conductivity (mS cm^{-1})	92	94
pH	13.2	13

Table 2: Influent sulfide and benzene concentrations during different periods of Run 1 and 2. Sulfide concentrations for Run 2 were increased to a concentration of 61 mM. In addition, the specific spent caustic (SC) solution that was used for each Run and the mixing ratio of SC with the sodium carbonate solution (0.8 M; pH 9.5) are given.

	Run 1			Run 2		
Period	I	II	III	I	II	III
time (days)	0-34	34-55	55-78	0-24	24-36	36-58
spent caustic		solution A			solution B	
SC / soda mixing ratio	1	6.2	2.5		100	
sulfide (mM)	240±2	39±7	93±3		61±2	
benzene (µM)	600±6	100±9	240±6		107±7	

2.2.3 Experimental design

Run 1 and 2 were performed in separate reactor systems. Operating conditions of both runs are given in Table 2. During start-up (period I) of Run 1, the pure spent caustic (Solution A) was added to the system. After start-up period the spent caustic solution A was mixed with the Na_2CO_3 solution to obtain the desired HRT and sulfide load. At day 34, the pH was decreased from 10.4 to 9.8 by addition of a 0.1 M HCl solution. The biomass used to inoculate Run 1 originated from a batch experiment (pH 9.5; Na^+ 0.8 M) that was inoculated with 5 grams of soda lake sediment.

Biomass extracted from Run 1 was used as inoculum for Run 2. For Run 2, spent caustic Solution B was mixed with Na_2CO_3. Because the benzene concentration in spent caustic Solution B was higher than in Solution A (Table 1) the ratio of spent caustic solution over Na_2CO_3 solution differed between the experiments. The final influent for Run 2 was supplemented with $Na_2S.9H_2O$ (Sigma-Aldrich, The Netherlands) to obtain a sulfide concentration of 61±2 mM.

In addition, an experiment without biomass (abiotic control) was performed for a period of 21 days to assess the chemical distribution of benzene over the gas and liquid phases. The experiment was operated at the same conditions as Run 1 and 2 (pH 9.5; Na^+ 0.8 M; temperature 35 °C; HRT 3.5 days) except that benzene concentrations were increased (from 240 µM in Run 1 up to 583 µM) to better study the abiotic removal from the system. Benzene concentrations were measured in both the reactor liquid and in the stripping bottle liquid.

2.2.4 Analytical procedures

Biomass samples were washed 3 times with a Na_2CO_3 solution (pH 9.5; Na^+ 0.8 M) to remove any dissolved nitrogen compounds before the biomass content was measured as total-Nitrogen, thereby using the Hach Lange cuvette test LCK238 (Hach Lange, Düsseldorf, Germany).

Total sulfide was analysed using Hach Lange cuvette test LCK653 (Hach Lange, Düsseldorf, Germany) and sodium concentrations were determined using ICP-OES (Perkin Elmer Optima 5300 DV).

Sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$) were determined by ion chromatography (761 compact IC with a 762 IC interface, Metrohm, Switserland) equipped with a conductivity detector. A metrosep A supp 5 column was used at ambient temperature and a flow rate of 0.7 mL min^{-1}. A pre-column (metrosep A supp 4/5 guard) was used. The injection volume was 20 µL. The eluent comprised of 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$ and 1% acetone. In addition, suppressors for eluent conductivity and CO_2 were used (Metrohm, Switzerland).

Benzene concentrations were determined by headspace GC-MS analyses using an Agilent 6890N GC and an Agilent 5975 Inert MSD. A capillary GC column, Agilent HP5 5% Phenyl Methyl Siloxane (30.0 m x 250 µm i.d. and 1.00 µm film thickness) was used in a constant flow mode with helium as a carrier gas (1.0 ml min^{-1}). The oven temperatures were operated at 40 °C for 2 min, 15 °C min^{-1} ramp to 100 °C, 50 °C min^{-1} ramp to 200 °C. The MS (source temperature = 230 °C and quadrupole temperature = 150 °C) was operated in Select Ion Mode (SIM). Headspace vials (10 ml) were filled with 5 ml of liquid sample and an excess (3 g) of sodium chloride. External standards were prepared in ethanol and diluted in water. Benzene-d6) was used as internal standard and was obtained from Sigma-Aldrich (The Netherlands). The headspace vials were equilibrated for 15 minutes at a temperature of 70 °C. The probe temperature was set to 80 °C and the transfer line temperature to 90 °C. The headspace of the vial was pressurised to 1 bar overpressure prior to injection. A 2.5 ml gas sample was injected into the GC-MS.

2.2.5 Microbial community analysis

Genomic DNA was extracted from reactor samples of Run 1 using the FastDNA®SPIN for soil kit (MP Biomedicals, USA).

For denaturing gradient gel electrophoresis (DGGE) analysis, partial 16S rDNA was amplified using the bacterial primers GC341f and 907rM. DGGE was performed as described by [158] using a denaturing gradient of 30 to 60% denaturants (urea and formamide; UF) in 8% polyacrylamide gel. Individual bands were excised, placed in 10mM Tris buffer, re-amplified and sequenced.

The nearly complete 16S rDNA of samples taken on day 7, 39 and 75 of Run 1 were amplified using bacterial primers GM3f and GM4r [159]. The PCR products were ligated into pCR4-TOPO and transformed into competent cells of *Escherichia coli* according to the TA Cloning® kit (Invitrogen, USA). Transformed cells were plated on Luria–Bertani medium plates containing 50 μg ml^{-1} kanamycin. After overnight incubation at 35 °C, clones were randomly selected for sequencing. PCR products for sequencing were purified using the Qiaquick PCR purification kit (QIAGEN) and sequenced by a commercial company (Macrogen, South Korea).

The obtained 16S rRNA gene sequences were first compared to sequences stored in GenBank using the BLASTN algorithm [160]. Subsequently, the sequences were aligned using the SILVA website, imported into ARB and added to a neighbor-joining tree made of complete sequences. The sequences have been stored in GenBank under accession numbers: HQ413781-HQ414030.

2.3 Results and Discussion

2.3.1 Biological sulfide oxidation

From figure 2 A and C it can be seen that sulfide was (almost) completely removed during Run 1 and 2; sulfide effluent concentrations were below the detection limit of 3 μg L^{-1} (data not shown). Hence, sulfide removal from spent caustics has been proven to be successful for influent sulfide concentrations up to 90 mM and loading rates up to 27 mmol sulfide L^{-1} day^{-1} (Run 1, period III). Sulfide was converted into sulfuric acid (H_2SO_4) resulting in a small pH decrease. When using a raw refinery spent caustic as a feedstock, it is likely that the produced H_2SO_4 can replace chemicals for pH control resulting in lower overall operating costs.

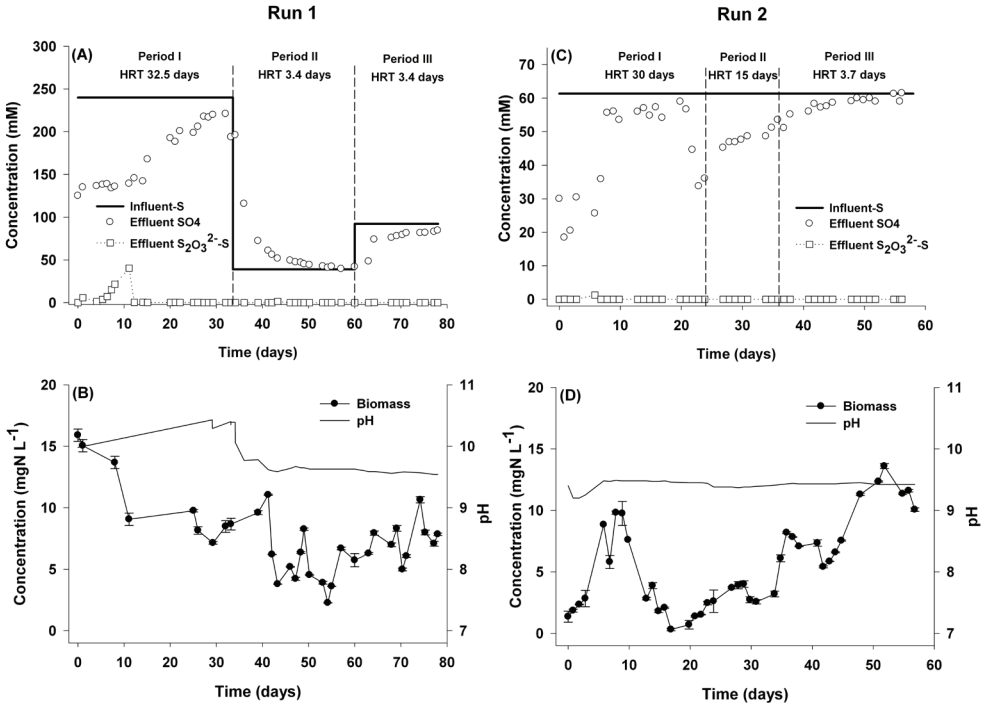


Figure 2: Reactor performance of Run 1 and 2. A,C: Total influent-S concentration (sum of sulfate (SO_4^{2-}), thiosulfate-S ($S_2O_3^{2-}$-S) and sulfide (S^{2-}) in the influent) and concentrations of SO_4^{2-} and $S_2O_3^{2-}$-S in the effluent; B,D: Biomass concentration in mg Nitrogen per liter and pH.

Sulfide conversion into sulfate (sulfate selectivity) amounted up to 88 mol% during steady state (day 68-78) of Run 1 (Table 3). Little sulfur formation was observed as no whitish colloidal particles could be seen in the bioreactor. Therefore, quantification of elemental sulfur was not attempted. Moreover, in the event that small amounts of elemental sulfur would be formed this is difficult to quantify due to attachment of the sulfur particles onto the reactor wall. No thiosulfate was formed during this period of Run 1, meaning that any abiotic sulfide oxidation did not occur [161].

From figure 2 C, it follows that more than 90% of the sulfide was converted into sulfate during period I of Run 2 (day 8-20) at a HRT of 30 days. During steady state of Run 2 (day 41-57), sulfate selectivity amounted up to 96 mol% (Table 3). Hence, it can be concluded that during this run an almost complete oxidation to sulfate was achieved (Fig. 2 C). The small difference in the sulfur balance can be attributed to losses as a result of assimilation processes and perhaps some analytical inaccuracies. In Run 2, the thiosulfate concentration was always below the detection limit of 2 μM (Fig. 2 C).

At day 22 (Run 2), the sulfate concentration in the reactor was decreased because half of

the reactor fluid was replaced with fresh buffer solution (Fig. 2 C).

Table 3: Average influent concentrations, sulfide load and sulfate selectivity during steady state in period III of Run 1 and 2.

	Run 1	**Run 2**
time (days)	68-78	41-57
HRT (days)	3.4	3.7
influent sulfide (mM)	93±3	61±2
sulfide load (mmol L^{-1} day^{-1})	27±1	16±1
SO_4^{2-} selectivity (mol%)	88±2	96±4

2.3.2 Biomass concentration

Biomass concentrations roughly fluctuated around 6-8 mgN L^{-1} for Run 1 (Fig. 2 B) while showing an increasing trend up to day 52 (13 mgN L^{-1}) for Run 2 (Fig. 2 D). The biomass was able to grow and thrive at the employed operating conditions without the use of a biomass retention system such as a settler, carrier material or a membrane. In case of biomass retention, the number of organisms in the reactor will increase, resulting in higher treatment capacities. This could be beneficial to a full-scale industrial application since this leads to smaller reactor sizes and thus lower investment costs.

The observed decrease in biomass during period I (Run 1, day 0-34) may be attributed to the wash out of non-sulfide oxidizing microorganisms that were present in the initial inoculum (Fig. 2B). At day 34 of Run 1, a sulfate selectivity of 80 mol% was achieved (Fig. 2 B). In period I, a pure (i.e. non-mixed) spent caustic (Solution A) was used as influent with a sulfide concentration of 0.24 M at a HRT of 32.5 days (Fig. 2 A). From the whitish appearance of the reactor suspension during period I, it can be concluded that a fraction of the sulfide was oxidized to elemental sulfur. This process is accompanied by the formation of hydroxide which caused the pH to increase from 9.8 to 10.4 between day 0 and 34. Thiosulfate concentrations up to 40 mM were recorded from day 0-11 (Fig. 2 A). As a result of O_2-limiting conditions due to failure of the O_2-sensor, the accumulation of thiosulfate can be attributed to the chemical, i.e. non-biological, oxidation of sulfide [161]. Immediately after replacement of the O_2-sensor and adjustment of the O_2 supply, the sulfide oxidation capacity was fully restored. Moreover, the formed thiosulfate was converted to sulfate reaching concentrations less than 2 μM.

The increasing biomass concentrations observed in Run 2 (day 41-52) resulted from increased sulfide loading rates (Fig. 2 D).

2.3.3 Benzene removal efficiency

Table 4 shows the benzene removal at steady state conditions of the biological experiments (Run 1 and 2) and the abiotic control experiment. Average benzene removal efficiencies of 93% were found during final steady state of Run 1 and 2 (Table 4).

The control experiment shows that in the absence of microorganisms 67% of the benzene was stripped from the gas-lift reactor liquid (Table 4). The benzene was continuously removed from the reactor liquid to the gaseous phase and then left the system via the bleed gas stream (Fig. 1). The ability to remove benzene by stripping or air-sparging techniques is well known and commonly used for the remediation of benzene contaminated soils and benzene polluted water [162, 163]. It was already noticed that although several bioreactor studies on the removal of petroleum hydrocarbons from (waste)water have been carried out and high removal efficiencies are described, the possibility of pollutant removal by means of physical removal such as gas-stripping is rarely discussed [162, 163] .

A non-biological control experiment has been carried out to assess the effluent benzene concentrations at steady state conditions in the absence of biomass (Table 4). The results were compared to the measured values in the presence of biomass. It has been assumed that the lower influent benzene concentrations in the biological experiments had no effect on the stripping efficiency.

It was found that actual effluent concentrations in the biological experiments were significantly lower than in the control experiments (Table 4). These results indicate that the high removal efficiency of benzene in the biological experiments is not only due to stripping but that biodegradation plays a role as well.

Table 4: HRT, benzene load, influent, effluent and total removal percentage of benzene during steady state conditions of the biological experiments (Run 1 and 2) and the control experiment are shown. The theoretical effluent concentrations (Influent-stripping) were calculated and based on the stripping percentages found in the control experiments.

	Run 1	**Run 2**	**Control**
time (days)	55-78	41-57	15-21
HRT (days)	3.4	3.7	3.5
influent (μM)	240±6	107±7	583±3
influent - stripping (μM)	79	35	192
effluent (μM)	15±7	9±3	192±3
removal efficiency (%)	93±7	93± 7	67±1
load (μmol L^{-1} day^{-1})	71±3	29±2	157±2

2.3.4 DGGE and clone library analysis

To investigate microbial population dynamics and overall diversity in time, DGGE analysis was used of which 19 bands were excised and sequenced (Fig. 3). Clone libraries of reactor samples taken at day 1, 39 and 75 of Run 1 provided a more detailed view of the microbial community (Fig. 4 and 5).

The DGGE gel showed different profiles over time with a changing degree of diversity in the reactor samples with the presence of up to 4 dominant bands (Fig. 3). In general, the DGGE profile stayed similar after day 20 indicating a stable community.

From the DGGE gel and the clone libraries it appeared that the bands and clones

clustered within the α- and γ-Proteobacteria, Bacteriodetes, Clostridia and Bacillales (Fig. 3-5).

Among the sulfur bacteria, members of the genus *Thioalkalivibrio* were found in DGGE bands d1-4, d11, d13-14 and d19 (Fig. 3). 99-100% similarity was found for *Thioalkalivibrio* sp. ALBR_X3 (d1-3, d16 and d19), *Thioalkalivibrio* sp. ALR20 (d4) or *Thioalkalivibrio* sp. K90mix (d11,d13-14). These strains represent a core group of the genus *Thioalkalivibrio* for which extreme tolerance to sodium and potassium carbonates and high pH (up to 10.6) has been demonstrated [164]. The DGGE also shows that different phylotypes of *Thioalkalivibrio* were present at different times during the reactor run (Fig. 3). A shift in dominance between different phylotypes of *Thioalkalivibrio* could be the result of small changes in the operating conditions and might indicate subtle differentiations within the same niche (genetic microdiversity). It might also indicate differences in tolerance towards benzene. The DGGE band representing *Thioalkalivibrio* sp. K90 mix (d11, d13 and d14) becomes, for instance, dominant between day 33 and 47. During this time the effluent benzene concentrations were on average 2 times higher (26 ±3 μM) compared to the rest of the experiment (data not shown).

All clone libraries confirmed the dominant presence of haloalkaliphilic sulfide-oxidizing *Thioalkalivibrio* (Fig. 4). The presence of *Thioalkalivibrio* was also demonstrated in fed batch reactors operated under haloalkaline conditions fed with solely H_2S gas [127, 164].

Research on the biological treatment of spent caustics is limited and up till now focused on the conversion of (in)organic sulfur compounds by chemolithoautotrophic neutrophilic *Thiobacilli* [40, 135, 150]. These *Thiobacilli* grow at pH 7-8 and low sodium concentrations. In order to decrease the salt concentration and the pH, dilution of the spent caustics with water is needed to operate this process, e.g. 3 times the influent flow [150].

Sequences related to the genus *Idiomarina* were found for bands d9-10 and within all clone libraries (Fig. 4, 5). The sequences were closely related to Gram-negative isolates (10B1 and 11C1) obtained from soda lakes in the Kenyan-Tanzanian Rift Valley [141]. The halophilic *Idiomarina loihiensis*, isolated from hydrothermal vents in the Hawaiian deep sea, was used as a reference strain [165].

Members of the genus *Marinobacter* were dominant in all clone libraries especially at day 1 and 75, but were not retrieved from the DGGE gel (Fig. 3, 5).

The DGGE profile of day 11 showed a different pattern compared to the profiles at other days. Band d5 as well as clones at day 1, 39 and 75 showed to contain sequences closely related to the genus *Halomonas* (Fig. 3).

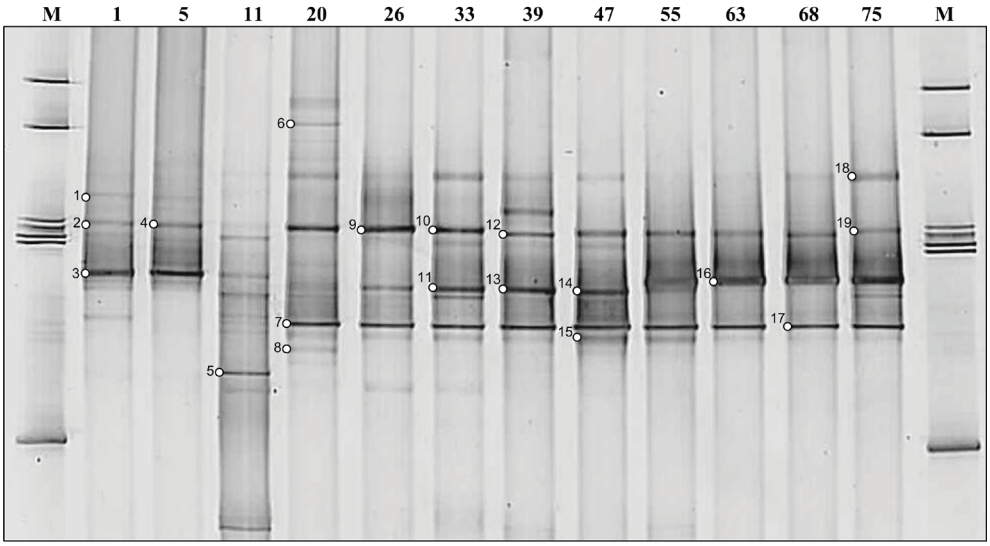


Figure 3: DGGE analysis of bacteria from Run 1. The numbers above the lanes refer to the marker lanes (M) or to the days of sampling (day 1-75). Bands d1-d19 were excised, reamplified and sequenced.

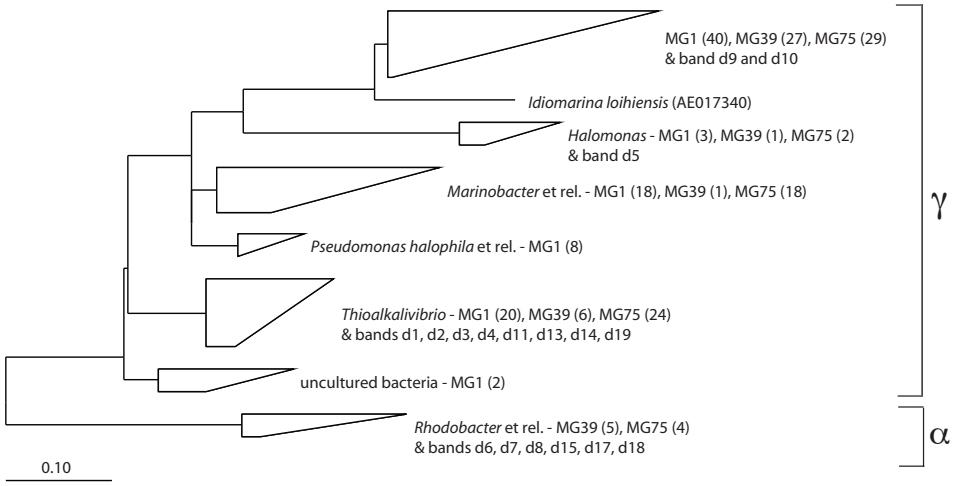


Figure 4: Phylogenetic analysis of the bacterial 16S rRNA gene sequences representing the genera α- and γ proteobacteria. Sequences were obtained from clone libraries made from samples taken at day 1 (MG1), day 39 (MG39) and day 75 (MG75) in Run 1. Sequences are clustered and the number of sequences of every time point is given in brackets. Sequences obtained from DGGE bands d1-11 and d13-19 (Fig. 3) are also included. The bar indicates 10% sequence difference.

The (halo)alkaliphilic heterotrophic bacteria, closely related to the genera *Idiomarina, Marinobacter* and *Halomonas* may well have contributed to the removal of benzene in Run 1 and 2 by its biodegradation. Members of the genus *Marinobacter*, for instance, are a well known and metabolically versatile group of marine facultative hydrocarbon degraders that are often found in oil contaminated saline environments [154, 166, 167]. In addition, it was shown that pure cultures of *Marinobacter* were able to degrade BTEX at moderately (halo) alkaliphilic conditions [168, 169]. Members of the genera *Halomonas* and *Idiomarina* have also been related to degradation of crude oil and diesel fuel at halophilic conditions [169, 170]. One of the few studies concerning mono-aromatic degradation under haloalkaline conditions showed the biodegradation of catechol, which is a common intermediate in benzene biodegradation, by *Halomonas campisalis* [153].

A distinct cluster within the α-Proteobacteria comprised of members related to the genera *Rhodobacter* and *Roseinatronobacter* (Fig. 4). Within these genera several bands (d6-8, d15, d17 and d18) in the DGGE and several clones from day 39 and 75 were closely related to *Rhodobaca barguzinensis* [171], *Roseinatronobacter thiooxidans* [144] and *Roseinatronobacter monicus* [172]. Overall these bacteriochlorophyll α containing bacteria thrive in alkaline and/or (hyper) saline environments.

R. Thiooxidans and *R. monicus* are known to oxidize sulfur compounds such as sulfide, thiosulfate and elemental sulfur into sulfate using it as an additional energy source. In addition, several members of the *Halomonas* group are capable to partially oxidize sulfur compounds such as thiosulfate and sulfide into tetrathionate under haloalkaline conditions. The produced tetrathionate can react with sulfide with the formation of sulfur and thiosulfate [164]. Therefore, species related to the genus *Rhodobacter* and *Halomonas* present during the reactor experiments may have contributed directly or indirectly (through tetrathionate catalysis) to the complete sulfide conversion into sulfate as observed in the reactor runs.

Sequences closely related to the halophilic bacteria *Pseudomonas halophila* = *Halomonas variabilis* [173] were only found in the clone library derived from day 1 (Fig. 4).

Within the *Bacteriodetes* band d12 and 19 clones from day 39 were found to be closely related to an uncultured *Lewinella*-like organism (CFB group bacterium; AF45299) from haloalkaline Mono Lake in the USA (Fig. 3, 5).

Some sequences from clone libraries day 1, 39 and 75 were found to be closely related to an alkaliphilic dissimilatory iron-reducing bacterium from the haloalkaline Soap Lake (US) within the Clostridia (unpublished, Fig. 5).

Within the Bacillales (Fig. 5), several clones from day 39 and 75 could be divided in three distinct groups: (1) associated with *Alkalibacterium olivapovliticus* [174],(2) related to *Bacillus selenitireducens* [175] and (3) related to uncultured bacteria isolated from Mono Lake (AF454301) and from a hypersaline lake in Chili [176].

All heterotrophic microorganisms detected in the reactor biomass by molecular analysis were related to alkaliphilic and/or halophilic bacteria. However, the presence of heterotrophic organisms belonging to the *Clostridiales* and anaerobic members of *Bacillales* indicates that also (facultative) anaerobic microorganisms were present in the bioreactor. Apparently small anaerobic niches are formed within the bioreactor possibly related to small areas (<0.1 % of

total volume) where solids were able to settle.

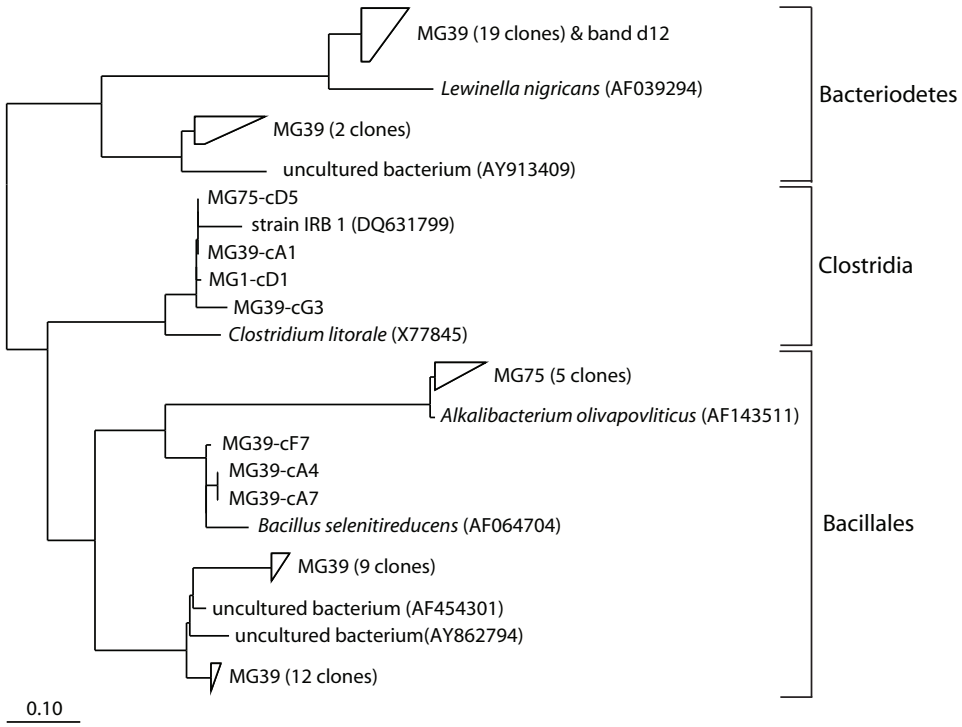


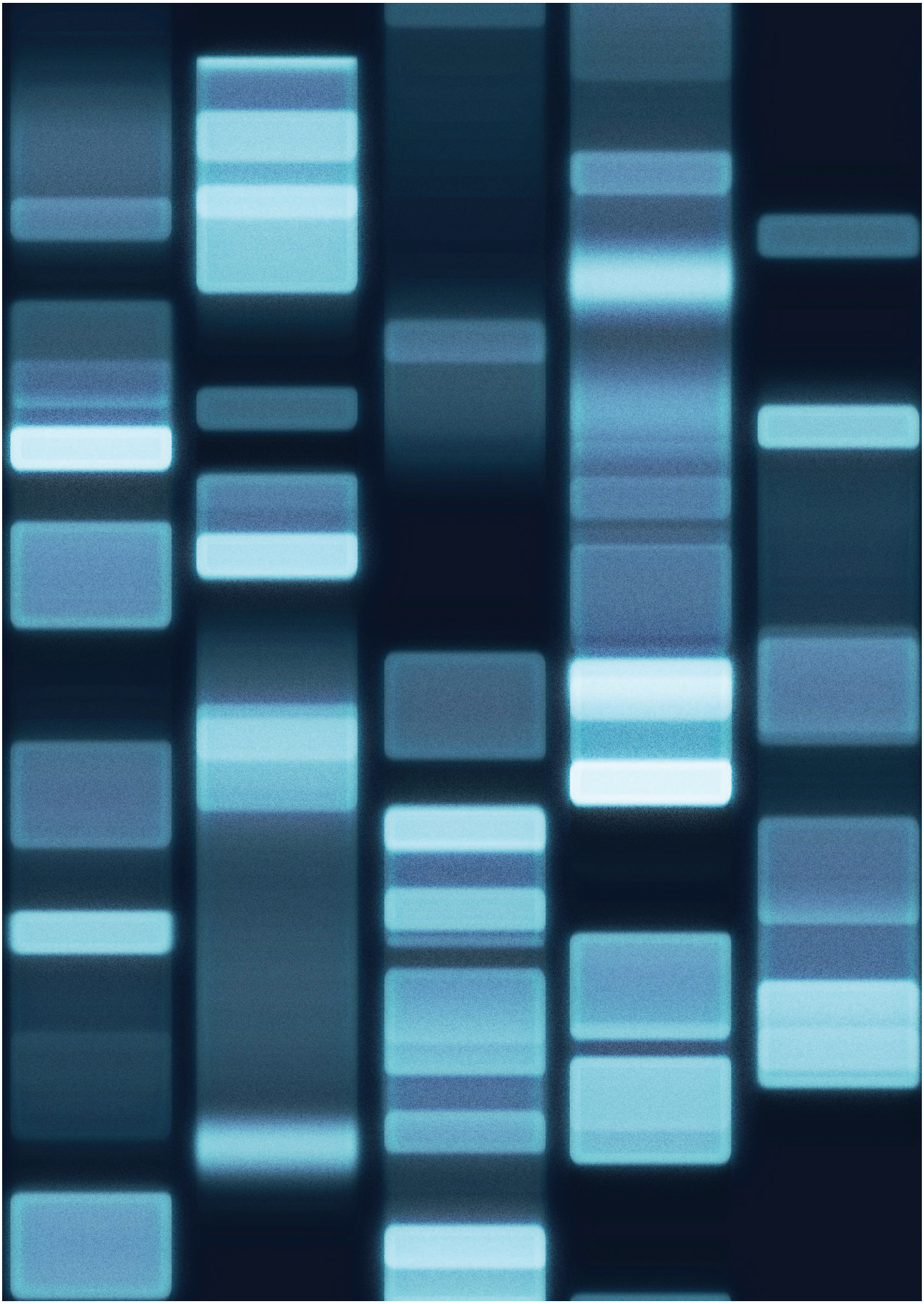
Figure 5: Phylogenetic analysis of the bacterial 16S rRNA gene sequences representing the genera Bacteriodetes, Clostridia and Bacillales. Sequences were obtained from clone libraries made from samples taken at day 1 (MG1), day 39 (MG39) and day 75 (MG75) in Run 1. Sequences are clustered and the number of sequences of every time point is given in brackets. Sequences obtained from DGGE band d12 (Fig. 3) is also included. The bar indicates 10% sequence difference.

2.4 Conclusion

This study shows that it is possible to biologically treat sulfidic spent caustic solutions, originating from a refinery, under haloalkaline conditions. Sulfide removal was complete up to 27 mmol L^{-1} day^{-1} by its conversion into sulfate. The sulfide conversion was accomplished by soda lake bacteria belonging to the genus *Thioalkalivibrio*. Benzene was removed by 93% in the biological reactor experiments. Calculations indicate that besides a high stripping efficiency also biodegradation of benzene took place. Heterotrophic organisms related to the genera *Marinobacter*, *Halomonas* and *Idiomarina* were shown to be present in the reactor and might have been involved in benzene biodegradation.

Acknowledgments

This work was performed in the TTIW-cooperation framework of Wetsus, Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs, the European Union Regional Development Fund, the Province of Fryslân, the City of Leeuwarden and the EZ/Kompas program of the "Samenwerkingsverband Noord-Nederland". The authors like to thank the participants of the research theme "Concentrates" for the discussions and their financial support. The authors also want to acknowledge Dr. D.Y. Sorokin (Delft University of Technology, The Netherlands and Winogradsky Institute of Microbiology, RAS, Moscow, Russia) for his support and discussions regarding this manuscript.



Chapter 3

Inhibition of microbiological sulfide oxidation by methanethiol and dimethyl polysulfides

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This chapter is published as:
Inhibition of microbiological sulfide oxidation by methanethiol and dimethyl polysulfides
Applied Microbiology and Biotechnology Vol 83, 579-587, 2009

Abstract

To avoid problems related to the discharge of sulfidic spent caustics to a wastewater treatment plant, a three-step biotechnological process is developed for the treatment of gasses containing both H_2S and methanethiol (MT). In the process, which operates at natron-alkaline conditions (>1 M of sodium- and potassium carbonates and a pH of 8.5-10), MT reacts with biologically produced sulfur particles to form a complex mixture predominantly consisting of inorganic polysulfides, dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). The inhibitory effect of MT, DMDS and DMTS was studied on bacterial oxidation of sulfide and polysulfide. Results show that biological oxidation of sulfide to elemental sulfur is reduced by 50% at 0.05 mM MT, while for DMDS and DMTS, 50% reduction was estimated at 1.5 and 1.0 mM, respectively. Results obtained with a reaction mixture of MT and biologically produced sulfur particles show that MT is detoxified by its reaction with biological sulfur. Adaptation of haloalkaliphilic biomass to MT was shown after prolonged exposure to (di)methyl sulfur compounds. The results indicate that application of the biotechnological process for the treatment of gasses containing both H_2S and MT is feasible as long as MT, DMDS and DMTS do not accumulate in the bioreactor.

3.1 Introduction

Biogas and gasses produced in the refining and petrochemical industry often contain volatile sulfur compounds, of which hydrogen sulfide (H_2S) is the most common. To prevent emission of sulfur dioxide and problems related to odour, toxicity and corrosivity, H_2S needs to be removed from the gas before combustion. For the removal of H_2S from sour gasses, caustic scrubbing is often applied. In this process, H_2S is absorbed into an aqueous alkaline solution in an absorber, under the formation of bisulfide (HS^-, referred to as "sulfide"):

$$H_2S(aq) + OH^- \rightleftarrows HS^- + H_2O \qquad (pK_a = 7.04) \qquad \text{(eq. 1)}$$

In conjunction with H_2S, sour gasses often contain carbon dioxide (CO_2). Presence of high CO_2 partial pressures leads to the formation of (bi)carbonate in the alkaline scrubber solution:

$$H_2CO_3 + OH^- \rightleftarrows HCO_3^- + H_2O \qquad (pK_a = 6.36) \qquad \text{(eq. 2)}$$
$$HCO_3^- + OH^- \rightleftarrows CO_3^{2-} + H_2O \qquad (pK_a = 10.25) \qquad \text{(eq. 3)}$$

Besides H_2S and CO_2, sour gasses may also contain volatile organic sulfur compounds (VOSCs) such as methanethiol (CH_3SH, or MT), ethanethiol (ET), propanethiol (PT), carbonyl sulfide (COS) and dimethyl disulfide (DMDS) [177].

Most of these VOSCs are even more toxic and malodorous than H_2S. Methanethiol is one of the most common VOSCs in sour gasses [178-180], and can also be adsorbed in the alkaline scrubbing liquid:

$CH_3SH + OH^- \rightleftarrows CH_3S^- + H_2O$ ($pK_a = 9.7$) (eq. 4)

The sulfide-loaded alkaline solution, also referred to as "sulfidic spent caustics", is often directed to a wastewater treatment plant (WWTP). Several problems are related to the discharge of sulfide to a WWTP, including corrosion, release of odors and safety hazards [86, 181]. Negative impacts of sulfide on the performance of wastewater treatment facilities have also been observed, e.g. problems related to nitrification [182] and the formation of bulking sludge due to the growth of filamentous sulfur-oxidizing bacteria such as *Thiothrix* and *Beggiota* species [183].

To overcome these problems, a three-step biotechnological process has been recently developed for the removal of H_2S from sour gasses with high CO_2 partial pressures [184]. The first step consists of absorption of H_2S and, if present also MT, into an alkaline scrubbing solution as described above. In the second step, the sulfide-loaded alkaline solution is sent to a bioreactor where it is biologically oxidized to elemental sulfur (S^0, referred to as "biosulfur"). The overall reaction for the conversion of H_2S to biosulfur is shown in eq. 5. To maximize the recovery of biosulfur and to reduce the consumption of caustic and make-up water, complete oxidation of H_2S to sulfate (SO_4^{2-}, eq. 6) is unwanted. Sulfate formation can be controlled by operating the bioreactor at oxygen limiting conditions [127].

$H_2S + ½\ O_2 \rightarrow S^0 + H_2O$ (eq. 5)

$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2\ H^+$ (eq. 6)

In a third step, biosulfur particles are separated from the reactor liquid by sedimentation. To treat sour gasses with a high partial CO_2 pressure and to maximize the H_2S loading capacity of the alkaline solution, the process has to be operated at high concentrations of (bi)carbonate [127]. With sodium (Na^+) and potassium (K^+) as counter ions of the (bi)carbonate, the process operates at natron-alkaline conditions (e.g. 2 M Na^+/K^+, pH 9). Therefore, specialized natron-alkaliphilic sulfur oxidizing bacteria (SOB) are applied in the process [185].

To avoid the discharge of sulfidic spent caustics in a WWTP, the abovementioned biotechnological process further developed for the treatment of gasses containing both H_2S and MT. Previous research on sulfidic spent caustics suggests that biological oxidation of MT to sulfate at neutral pH conditions occurs via intermediary DMDS [47]. The effect of MT on the biological conversion of H_2S to elemental sulfur at natron-alkaline conditions is however not yet known. Being a strong nucleophile, MT can react with sulfur particles in an aqueous solution, initially forming methyl polysulfide by opening the sulfur ring (eq. 7) [186]. In subsequent spontaneous reaction steps, shorter dimethyl polysulfides are formed along with inorganic (poly)sulfides (eq. 8).

$CH_3SH + S_8 \rightarrow CH_3S_9^- + H^+$ (eq. 7)

$CH_3SH + CH_3S_9^- \rightarrow CH_3S_nCH_3 + S_x^{2-} + H^+$ ($n + x = 10$) (eq. 8)

In previous research we have shown that these reactions indeed take place between MT and biosulfur particles [187]. The main end-products when MT is added to an excess of biosulfur at pH 8.7 are polysulfide, sulfide and (di)methyl polysulfides, consisting predominantly of DMDS and DMTS. In the presence of oxygen, DMDS also can be formed by rapid auto-oxidation of MT (eq. 9) [188].

$$2\ CH_3SH + ½\ O_2 \rightarrow CH_3SSCH_3 + H_2O \qquad \text{(eq. 9)}$$

This paper focuses on the inhibitory effects of MT and the formed reaction products, on oxidation of sulfide and polysulfide by haloalkaliphilic SOB. Knowledge on the potential toxic effects of these compounds is required to further develop a biotechnological process for removal of H_2S and MT from sour gasses as alternative to treatment of sulfidic spent caustics in a WWTP.

3.2 Material and Methods

3.2.1 Respiration tests

Respiration tests were performed in a thermostated 7.5 mL glass chamber mounted on a magnetic stirrer and fitted with a piston holding a dissolved oxygen (DO) electrode (Yellow Springs Instr., OH, USA). This piston contained a small opening to allow air to be removed from the chamber and to add reactants. A schematic representation of the setup is shown elsewhere [189]. Cell suspensions (70-100 µL) were added to a carbonate buffer solution (pH 9.0), to a final concentration of 14-21 mg N L^{-1}, while the solution was saturated with oxygen by bubbling with air for at least 5 minutes. Experiments were started by injection of 20 – 320 µL of sulfur substrate stock solutions (sulfide, polysulfide, a mixture of MT and biosulfur, MT, DMDS and/or DMTS). The decrease of the DO concentration was measured in time and the initial slope $(d[O_2]/dt)_0$ was used as a measure of the oxidation rate. Oxidation rates were measured in the absence (chemical controls) and presence of cells. Biological oxidation rates were calculated by subtracting the rates in the absence of cells (chemical oxidation) from the rates in the presence of cells (combined chemical and biological oxidation). At the end of each respiration experiment, samples were taken for analysis of the residual total sulfide (S^{2-}_{tot}) concentration, to enable the calculation of the molar O_2/S^{2-}_{tot} consumption ratio. All experiments were performed in duplicate. Controls were performed with autoclaved cells (20 min, 121 °C).

3.2.2 Biomass source

Haloalkaliphilic SOB were obtained from a lab-scale gas-lift bioreactor inoculated with a mixture of hypersaline soda lake sediments from Mongolia, south-western Siberia and Kenya [128], operating at natron-alkaline, sulfur producing conditions (2M Na^+/K^+, pH 9.0±0.2, $[S^{2-}_{tot}]$ = 0.2-0.3 mM, DO <0.1% sat., 2.2 mM h^{-1} H_2S supply, 35 °C). A detailed description of the reactor setup and a composition of the microbiological population is given in [127, 185].

Bacterial cells were separated from extracellular sulfur by several successive steps of low-speed centrifugation (500 rpm), washed and resuspended in a (bi)carbonate buffer (pH 9.0, 1.67 M K^+, 0.33 M Na^+, see section *Chemicals used*), resulting in cell suspensions with a final concentration of 1100-1700 mg N L^{-1}.

In a dedicated experiment, MT (1.5-3.3 µM h^{-1}) and H_2S (2.2 mM h^{-1}) were continuously supplied to the bioreactor (pH 9.1±0.1). This way, natronophilic biomass was exposed to (di) methyl sulfur compounds for a prolonged period. The DO concentration was controlled at >5% sat. and the S^{2-}_{tot} concentration was kept below 0.01 mM, to prevent limitation. After 27 days of operation, cells were harvested from the bioreactor as described above and used in respiration tests.

3.2.3 Analytical procedures

The maximum oxygen solubility of the alkaline buffer solution (see section *Chemicals used*) was determined by placing buffer (pH 9.0) in an air-tight thermostated vessel (400 mL). After oxygen saturation by bubbling with air (DO = 100% sat.), the vessel was equipped with a DO electrode. A 50 mM sodium sulfite solution ($NaSO_3$, Merck, Darmstadt, Germany) was stepwise added to the vessel in the presence of copper sulfate to act as a catalyst. Based on the stoichiometry of the oxidation of sulfite to sulfate, combined with the decrease of the DO concentration, the maximum oxygen solubility of the buffer was found to be 0.15 mmol L^{-1} at 35 °C (data not shown).

Total sulfide (S^{2-}_{tot}) concentrations were measured on the basis of a modified methylene blue method as described previously in [127]. At the experimental pH of 9.0, the main sulfide species are HS^- and S_x^{2-}. Therefore, the total sulfide concentration can be described as:

$$[S^{2-}_{tot}] = [HS^-] + [S_x^{2-}] \qquad \text{(eq. 10)}$$

Polysulfide anion concentrations were determined spectrophotometrically as described elsewhere [127, 190], at a wavelength of 285nm (Perkin-Elmer, Lambda 2, Norwalk, CT, USA). With this method, which can only be used in the absence of VOSCs, the total concentration of zerovalent sulfur atoms in polysulfide (S_x^{2-}-S^0) is determined. Biomass concentrations were measured as the amount of total nitrogen, as described in [127].

3.2.4 Chemicals used

Carbonate buffer was prepared by mixing bicarbonate (pH 8.3) and carbonate (pH 12.3) buffer to a final pH of 9.0. Both buffers contained 0.67 M Na^+ and 1.33 M K^+ as (bi)carbonate (Merck, Darmstadt, Germany).

Sodium sulfide stock solutions (20-30 mM) were freshly prepared by dissolution of $Na_2S{\cdot}9H_2O$ crystals (Merck, Darmstadt, Germany) in de-aerated Milli-Q water. Before dissolution, the oxidized surface of the crystals was removed by flushing with de-aerated water. The exact sulfide concentration of the stock solutions was determined afterwards.

Polysulfide stock solutions were prepared by reaction of excess biosulfur (250 mM) with

a 30 mM sulfide solution at 50 °C, as described elsewhere [189] (eq. 11). Polysulfide solutions as prepared in this way consist of a mixture of polysulfide (S_x^{2-}, with an average value of x = 4.9 [190] and sulfide (HS^-), of which the equilibrium is defined by Equation 12 [190, 191]. The pH of the polysulfide stock solutions was 10.3. According to the equilibrium (eq. 8) at this pH, 93% of the S^{2-}_{tot} concentration is present as polysulfide. This was confirmed by analysis of S^{2-}_{tot} and polysulfide concentrations.

$$HS^- + (x\text{-}1)\ S^0 \rightleftarrows S_x^{2-} + H^+ \qquad \text{with } x = 4.9 \text{ (average)} \qquad \text{(eq. 11)}$$

$$K_x = \frac{[S_x^{2-}][H^+]}{[HS^-]} \qquad \text{with } pK_x = 9.17 \qquad \text{(eq. 12)}$$

A sodium methylmercaptide ($NaCH_3S$) solution (2.5 M) was supplied by Arkema Group (Rotterdam, the Netherlands). Stock solutions of 20 mM MT were prepared by dilution with oxygen-free ultrapure (MilliQ) water and kept at a slight nitrogen gas overpressure to prevent oxidation by air.

Biosulfur was obtained from a full-scale biogas treatment facility (Eerbeek, the Netherlands) and dialyzed in demineralised water to remove salts to a conductivity below 40 μS cm^{-1}. The mixture of MT and biosulfur (further referred to as "MT-S^0 mixture") was prepared by addition of 20 mM MT and 400 mM biosulfur to oxygen-free carbonate buffer (pH 9.0) as described above. The MT-S^0 mixture was incubated overnight at 30 °C. After incubation, the S^{2-}_{tot} concentration in the MT-S^0 mixture was 9.6 mM, which is close to the expected value of 10 mM, based on the reaction stoichiometry according to equations 7 and 8. According to this stoichiometry, the total concentration of dimethyl polysulfides should also be 10 mM. The exact composition of the MT-S^0 mixtures was not determined, but a more detailed description of the composition of similar MT-S^0 mixtures (30 °C, pH 8.7) is given elsewhere [187]. Before use, remaining biosulfur particles were allowed to settle so that no biosulfur was introduced to the respiration chamber.

Stock solutions of DMDS and DMTS (20 mM) were prepared from pure solutions (Merck, Darmstadt, Germany) by dilution in water (DMDS) or HPLC-grade methanol (DMTS).

3.3 Results

3.3.1 Sulfide and polysulfide oxidation rates in the absence of methyl sulfur compounds

Oxidation rates of sulfide and polysulfide ions were initially determined in the absence of (di)methyl sulfur compounds. Upon addition of biomass (15 mg N L^{-1}) to oxygen saturated buffer, the DO concentration decreased up to a rate of 4.8 mM O_2 h^{-1} (data not shown), even without addition of substrate. This phenomenon was also previously observed and is

assumed to be attributed to the oxidation of membrane-bound polysulfur compounds, such as polysulfide [192, 193]. After re-aeration for at least 5 minutes, only endogenic oxygen consumption was observed and experiments were started. Oxidation rates were determined with sulfide (Fig. 1 A) and polysulfide (Fig. 1 B) as substrates (0.05 to 0.5 mM S^{2-}_{tot}), in the absence and presence of cells. In the absence of cells (chemical controls), oxidation rates increased slightly with increasing sulfide concentrations. For polysulfide, chemical oxidation rates increased proportionally to the concentration, indicating first-order reaction kinetics, as was also reported by Kleinjan et al. [189]. The rate of chemical polysulfide oxidation in the buffer was approximately twice as high as that of sulfide.

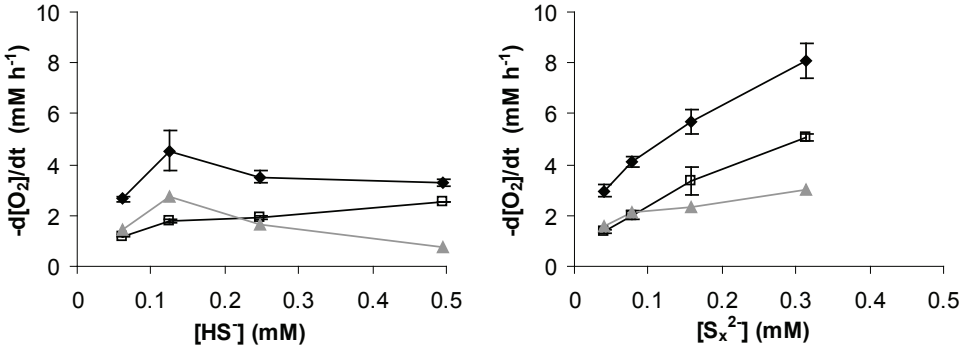

Figure 1: A-B Oxidation rates with different concentrations of HS^- (A) and S_x^{2-} (B). Both Figures show rates in the presence of cells, in the absence of cells and rates as a result of biological oxidation only. Biomass concentration = 15 mg N L^{-1}; pH=9.0; total salt = 2 M Na^+/K^+ as carbonates.

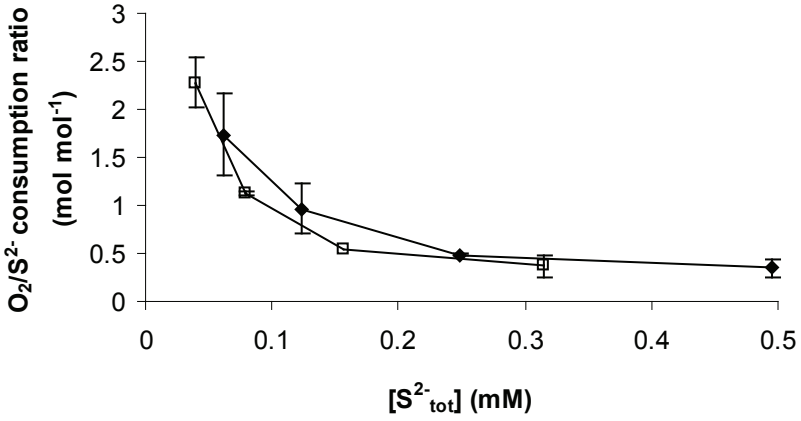

Figure 2: Molar O_2/S^{2-}_{tot} consumption ratio as a result of biological oxidation of sulfide and polysulfide. Biomass concentration = 15 mg N L^{-1}; pH=9.0; total salt = 2 M Na^+/K^+ as carbonates.

The biological oxidation of sulfide showed a maximum rate of 2.7±0.3 mM O_2 h^{-1} (0.18±0.2 mM O_2 mg N^{-1} h^{-1}) at a sulfide concentration of 0.12 mM, whereas at higher sulfide concentrations, the oxidation rate decreased. For biological polysulfide oxidation, a similar maximum oxidation rate of 3.0±0.1 mM O_2 h^{-1} (0.20±0.1 mM O_2 mg N^{-1} h^{-1}) was observed at the highest polysulfide concentration tested (0.31 mM S^{2-}_{tot}). By measurement of the molar O_2/S^{2-}_{tot} consumption ratio both in the absence and presence of cells, the stoichiometry of biological substrate oxidation could be calculated (Fig. 2). For both the sulfide and polysulfide bio-oxidation, the molar O_2/S^{2-}_{tot} consumption ratio was around 2 at a S^{2-}_{tot} concentration of around 0.05 mM. At increasing S^{2-}_{tot} concentrations, the molar O_2/S^{2-}_{tot} consumption ratio gradually decreased to a final value of around 0.5 at a S^{2-}_{tot} concentration of 0.20-0.25 mM and above.

3.3.2 Inhibition by methanethiol and (di)methyl polysulfides

The effect of MT, DMDS and DMTS on sulfide oxidation was studied at various concentrations of these VOSCs. As elemental sulfur is the preferred end-product [127, 161], a S^{2-}_{tot} concentration of 0.25 mM was applied. At this S^{2-}_{tot} concentration, sulfide is oxidized to elemental sulfur, as described above. Oxygen consumption rates with only MT, DMDS and DMTS were negligible both in the absence and presence of cells (<0.1 mM h^{-1} at 0.4 mM). Chemical oxidation of sulfide was only slightly affected by these (di)methyl sulfur compounds (data not shown). Biological sulfide oxidation rates were strongly affected by MT (Fig. 3). A 50% decrease of the oxidation rate (*Ki*) was already observed at a MT concentration of 0.05 mM, while at concentrations above 0.65 mM, biological oxidation was completely inhibited. Inhibition of biological sulfide oxidation by DMDS and DMTS was less severe. At the highest concentration applied (0.85 mM), the oxidation rate of sulfide in the presence of DMDS or DMTS decreased to 55-60%. Based on a log-linear fit, it was estimated that for DMDS and DMTS, the *Ki* value was 1.5 and 1.0 mM, respectively.

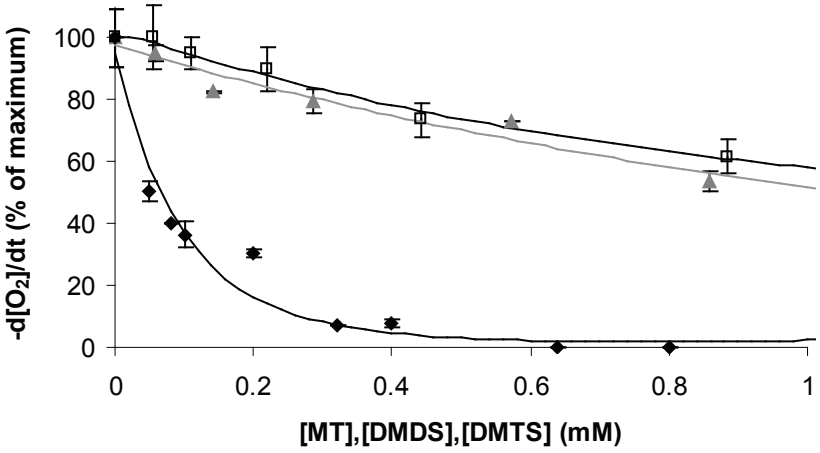

Figure 3: Relative biological oxidation rates during sulfide oxidation (0.25 mM HS^-) in the presence of varying concentrations of MT, DMDS and DMTS. Biomass concentration: 20.5 mg N L^{-1}, specific biological oxidation rate with sulfide only (100%): 0.18±0.02 mmol O_2 mg N^{-1} h^{-1}. pH=9.0; total salt = 2 M Na^+/K^+ as carbonates. Solid lines represent the results of a log-linear regression model fitted to the results.

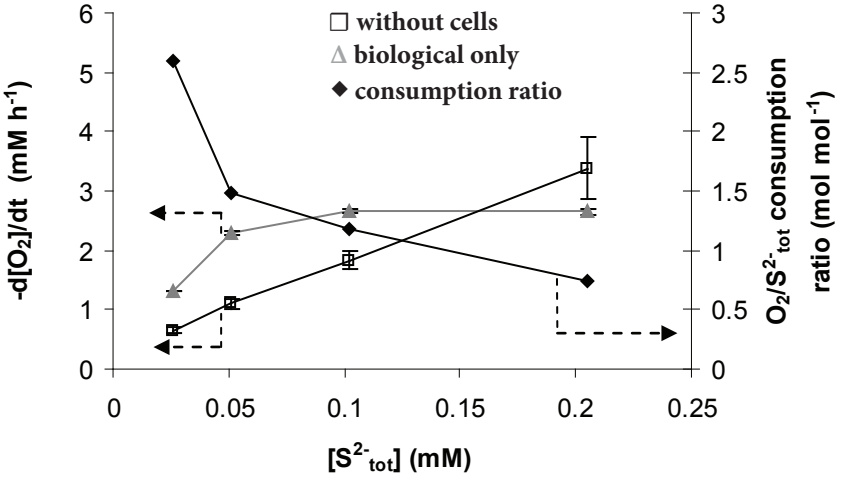

Figure 4: Oxidation rates with the MT-S^0 mixture. The x-axis shows the initial S^{2-}_{tot} concentration after addition of the mixture, being equal to the sum of concentrations of (di) methyl polysulfides, according to the stoichiometry of the reaction between MT and biosulfur (Eq. 3 and 4). The primary y-axis shows oxidation rates in the absence of cells and as a result of biological oxidation only. The secondary y-axis shows the molar O_2/S^{2-}_{tot} consumption ratio as a result of biological oxidation only. Biomass concentration = 18 mg N L^{-1}; pH=9.0; total salt = 2 M Na^+/K^+ as carbonates.

Oxidation rates of the MT-S^0 mixture were also tested in the absence and presence of cells. Chemical oxidation rates increased proportionally to the amount of MT-S^0 mixture added (Fig. 4) and the relation between oxidation rate and S^{2-}_{tot} concentration was comparable to that of polysulfide (Fig. 1 B). Biological oxidation rates obtained with the MT-S^0 mixture increased with increasing S^{2-}_{tot} concentrations, thereby reaching a maximum rate of 2.7±0.3 mM O_2 h^{-1} (0.18±0.2 mM O_2 mg N^{-1} h^{-1}) at 0.1 mM S^{2-}_{tot}. The measured rates were comparable to those of biological polysulfide oxidation (Fig. 1 B). The relation between the S^{2-}_{tot} concentration and the molar O_2/S^{2-}_{tot} consumption ratio as a result of biological oxidation of the MT-S^0 mixture shows a similar pattern as observed with only sulfide and polysulfide as substrates (Fig. 2). According to the stoichiometry of the reaction between MT and biosulfur (eq. 7 and 8), the formed dimethyl polysulfide concentration is equal to the formed inorganic polysulfide concentration. As the maximum (inorganic) polysulfide concentration applied in the respiration tests was 0.2 mM, this implies that at least up to the same concentration of 0.2 mM, dimethyl polysulfides (DMDS and DMTS) do not inhibit biological sulfide oxidation.

3.3.3 Respiration rates after exposure to MT

In the respiration experiments discussed so far, the biomass had not been exposed to MT, DMDS or DMTS prior to the respiration experiments. To test whether adaptation of biological sulfide oxidation to MT occurs after long-term exposure to (di)methyl sulfur compounds, biomass was pre-exposed to MT and the products formed from its reaction with biosulfur in a H_2S-oxidizing bioreactor operating without O_2 limitation. At a continuous MT supply of 1.5-3.3 μM h^{-1}, all H_2S supplied to the reactor (2.2 mM h^{-1}) was converted to sulfur, whilst little, if any, accumulation of sulfate, sulfide or thiosulfate was found. This indicates that the biological activity was not severely inhibited by MT. After 27 days of adaptation, a comparison was made between pre-exposed and not pre-exposed biomass. For both type of cells, comparable sulfide oxidation rates were found at a sulfide concentration of 0.16 mM (Fig. 5).

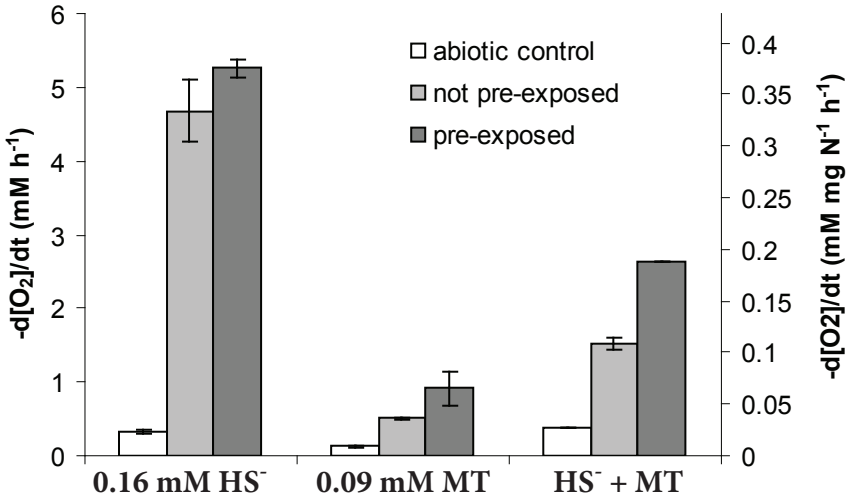

Figure 5: Oxidation rates with HS^- (0.16 mM), MT (0.09 mM) and a mixture of HS^- (0.16 mM) and MT (0.09 mM), for respiration experiments without biomass (abiotic control), biomass that has not been pre-exposed to MT (light gray) and biomass that was pre-exposed to MT (dark gray) in a H_2S oxidizing bioreactor. The primary y-axis shows volumetric oxygen consumption rates. The secondary y-axis shows the specific biological oxygen consumption rates, corrected for abiotic oxidation. Biomass concentration = 14 mg N L^{-1}; pH=9.0; total salt = 2 M Na^+/K^+ as carbonates.

Oxidation rates with only MT (0.09 mM) were slightly higher for pre-exposed cells (0.07±0.02 mM mg N^{-1} h^{-1}) compared to the not pre-exposed cells (0.04±0.00) mM h^{-1}). Although the oxidation rate with MT was somewhat higher in the presence of cells compared to abiotic control experiments, it was not clear if any biological MT degradation occurred. The reason for this is that concentrations of MT and DMDS could not be measured. When both sulfide (0.16 mM) and MT (0.09 mM) were added, respiration rates with pre-exposed cells (0.19±0.01 mM mg N^{-1} h^{-1}) were significantly higher (73±6 %) compared to rates with not pre-exposed cells (0.11±0.01 mM mg N^{-1} h^{-1}).

3.4 Discussion

3.4.1 Inhibition by methylated sulfur compounds

This study shows that sulfide oxidation to elemental sulfur by natron-alkaliphilic SOB is severely inhibited in the presence of MT, already at low concentrations (*Ki* = 0.05 mM MT). As an intermediate in the methionine metabolism, MT was reported to be responsible for inhibition of cytochrome *c* oxidase activity [194]. It was suggested that inhibition by MT is

caused by steric hindrance and ionic or hydrophobic interactions. As alkaliphilic SOB have a high cytochrome *c* content and a very high cytochrome *c* oxidase activity [195, 196], it is likely that inhibition of the oxidation rate by MT is caused by inhibition of cytochrome *c* oxidase activity. Being a strong nucleophile, MT can also break S-S bonds in proteins and thereby rendering enzymes inactive [197]. If the nucleophilicity of MT indeed plays a role in the inhibiting effect, a high pH is expected to result in more severe inhibition compared to low pH values, as the deprotonated form of MT (CH_3S^-) is a stronger nucleophile than molecular MT. At the experimental pH of 9.0, 2% of MT is present in the deprotonated form (pK = 10.4). Most information about the inhibiting effects of MT on microorganisms originates from experiments in anaerobic environments. Reported *Ki* values for methanogenic granular sludge are 6-8 mM (with acetate), 10 mM (with methanol) and 7 mM (with hydrogen) [72, 73]. Under aerobic conditions, much lower substrate inhibition values for MT are reported: 8 µM for *Thiobacillus thioparus* [198] and 14 µM for *Hyphomicrobium* species [199]. The *Ki* values for MT on sulfide oxidation at natron-alkaline conditions found in our tests are thus comparable to values reported for aerobic neutrophilic SOB.

It was shown that compared to MT, DMDS and DMTS exhibit a less severe inhibiting effect on sulfide oxidation by natron-alkaliphilic SOB. This may be explained by the lower nucleophilicy of DMDS and DMTS compared to MT. Apparently, hydrophobic interactions do not play a major role in the inhibition of sulfide oxidation by VOSCs, as DMDS and DMTS are more hydrophobic compared to MT. As polysulfide, DMDS and DMTS are the main products from the reaction between MT and biosulfur, this reaction effectively results in a partial detoxification of MT. This was confirmed by bio-oxidation experiments performed with MT-S^0 mixtures. It was found that the results of these tests were similar to those using only polysulfide as a substrate (Fig. 5.1 B and Fig. 5.4). Formation of DMDS was also proposed to play a role in detoxification of MT in the aerobic treatment of sulfidic spent caustics [47]. It was hypothesized that biological oxidation of MT proceeds in 2 steps. First, MT is chemically oxidized to DMDS, where-after DMDS is biologically oxidized to sulfate. Any biological oxidation of DMDS was not observed during the short duration of our respiration experiments (max. 15 min.), but may take place after prolonged incubation periods. Although DMDS seems to play an important role in detoxification of MT, it can also be toxic to microorganisms [200]. Kiene et al. [201] for example, showed that while DMDS and MT could stimulate methanogenesis in different sediments when added at low concentrations (26-56 µM DMDS and 20-52 µM MT), strong inhibition on methanogenesis occurred at concentrations above 0.26 mM DMDS. Little is known about the effect of DMTS on microorganisms. Rappert et al. [202] found that *Pseudonocardia asacharolytica* was able to oxidize 0.5 mM DMTS as sole carbon and energy source. It was hypothesized that DMTS was first converted into DMDS and subsequently to sulfate and CO_2. Our results indicate that adaptation of the haloalkaliphilic biomass to MT takes place after prolonged exposure to low concentrations of MT. It is not known if this was the result of adaptation of the cells, or a change in the composition of the mixed bacterial population.

3.4.2 Influence of the S^{2-}_{tot} concentration

This study shows that the biological molar O_2/S^{2-}_{tot} consumption ratio varies with the S^{2-}_{tot} concentration. At S^{2-}_{tot} concentrations around 0.05 mM, the molar O_2/S^{2-}_{tot} consumption ratio was around 2, while at [S^{2-}_{tot}] >0.2-0.25 mM, the molar O_2/S^{2-}_{tot} consumption ratio was around 0.5 (Fig. 2). The explanation for these results is that at S^{2-}_{tot} concentrations around 0.05 mM, (poly)sulfide is completely oxidized to sulfate, with a theoretical molar O_2/S^{2-}_{tot} consumption ratio of 2 (eq. 6). At [S^{2-}_{tot}] >0.2-0.25 mM, (poly)sulfide is biologically converted to elemental sulfur, with a theoretical molar O_2/S^{2-}_{tot} consumption ratio of 0.5 (eq. 5). Intermediate molar O_2/S^{2-}_{tot} consumption ratios can be explained by a combined sulfate and sulfur formation, with the selectivity shifting towards sulfur formation with increasing S^{2-}_{tot} concentrations. This relation was found for all substrates used (sulfide, polysulfide and the MT-S^0 mixture, Fig. 4). The reaction stoichiometry could not be confirmed by analysis of the oxidation products (sulfate, thiosulfate and biosulfur), as the concentrations were too low to be detected in the carbonate buffer. The same relation between S^{2-}_{tot} concentration and the molar O_2/S^{2-}_{tot} consumption ratio was also observed in bioreactor studies described in [127]. In these bioreactor studies also the products of (poly)sulfide oxidation could be analysed, confirming the stoichiometry of sulfate and sulfur formation according to Equations 5 and 6. While the DO concentration in the bioreactor study was always below the detection limit of 0.1% sat., the respiration experiments were performed at saturated DO conditions. This indicates that the S^{2-}_{tot} concentration and not the DO concentration determines the selectivity for the various products (i.e. sulfur or sulfate) from biological oxidation of (poly)sulfide.

Although chemical and biological oxidation rates of sulfide and polysulfide observed in the respiration experiments were comparable (Fig. 1 A and B and Fig. 4), biological oxidation of these substrates can outcompete chemical oxidation in a bioreactor. Chemical oxidation rates of sulfide and polysulfide are higher at increased DO concentrations [189, 203]. Consequently, at the low DO concentration (<0.1% sat.) prevailing in a sulfur-producing bioreactor [127, 161], chemical oxidation rates are much lower compared to the rates found in the respiration experiments, which were performed at saturated DO concentrations.

3.4.3 Gas treatment considerations

Application of the newly developed process for treatment of gasses containing both H_2S and MT at natron-alkaline conditions will mainly depend on the concentrations of MT, DMDS and DMTS prevailing in the bioreactor. Usually, the MT concentration in sour gasses is much lower than the H_2S concentration [178, 179]. Moreover, as a result of the reaction between MT and biosulfur particles, MT is converted in the absorber column into the far less toxic DMDS and DMTS. Also auto-oxidation of MT to DMDS may contribute to this apparent detoxification of MT. The rate of these reactions determines if the MT concentration in the bioreactor remains below values that severely inhibit biological sulfide oxidation (<0.05 mM). Another prerequisite for biotechnological treatment of H_2S and MT containing gasses, is the degradation of the dimethyl polysulfides (mainly DMDS and DMTS) that are produced from the reaction between MT and biosulfur. If these compounds are degraded at a sufficient rate, no accumulation will occur, preventing inhibitory concentrations (1-1.5 mM). Degradation of DMDS and DMTS may proceed by biological oxidation, although this was not observed

in the respiration tests presented in this study. Further study on MT, DMDS and DMTS in a H_2S oxidizing bioreactor operating at natron-alkaline conditions is therefore essential to give more insight in the feasibility of treatment of gasses containing both H_2S and MT.

In our experiments, only MT and its derived compounds produced from the reaction with biosulfur particles were studied for their inhibitory effects. However, besides MT also higher organic sulfur compounds like ethanethiol and propanethiol may be present in sour gasses [177]. Like MT, also these higher thiols can react with biosulfur particles [187]. Possibly, this reaction has the same detoxifying effect as observed with MT, so that treatment of sour gasses containing these higher thiols may also be feasible.

3.5 Conclusions

The main results of this work may be summarized as follows:

- Microbiological oxidation of sulfide to elemental sulfur by natron-alkaliphilic SOB is severely inhibited in the presence of MT. A 50% decrease of the oxidation rate (*Ki*) occurs at a concentration of 0.05 mM MT.
- Compared to MT, DMDS and DMTS exhibit a less severe inhibiting effect on sulfide oxidation at sulfur-forming conditions ($[S^{2-}_{tot}]$ = 0.25 mM). For these the *Ki* value was estimated to be 1.5 (DMDS) and 1.0 mM (DMTS).
- As DMDS and DMTS are products from the reaction between MT and biosulfur particles, this reaction effectively results in a partial detoxification of MT in a sulfur-producing bioreactor.
- At S^{2-}_{tot} concentrations of 0.20-0.25 mM and above, the product of microbiological (poly)sulfide oxidation is elemental sulfur, while at S^{2-}_{tot} concentrations around 0.05 mM and below, (poly)sulfide is completely oxidized to sulfate. At intermediary S^{2-}_{tot} concentrations, both biosulfur and sulfate are formed. This relation is found for sulfide and polysulfide, as well as for a reaction mixture of MT and biosulfur, containing polysulfide, sulfide, DMDS and DMTS.
- Adaptation of haloalkaliphilic biomass to MT takes place after prolonged exposure to (di)methyl sulfur compounds in a H_2S oxidizing bioreactor.
- Application of a biotechnological process for the treatment of gasses containing both H_2S and MT at natron-alkaline conditions is feasible as long as MT, DMDS and DMTS do not accumulate in the bioreactor. Accumulation of MT can be prevented by auto-oxidation or the reaction between MT and biosulfur particles. Further research is required to study microbiological degradation of MT, DMDS and DMTS at natron-alkaline conditions.


300

Chapter 4

Effect of organic sulfur compounds on the biological treatment of sulfidic spent caustics

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This chapter has been submitted for publication

Abstract

The present research demonstrates that biological sulfide oxidation is possible in the presence of low concentrations of dimethyl disulfide (DMDS) under haloalkaline conditions (i.e. pH 9.5; Na^+ 0.8 M). Experiments with synthetically prepared sulfidic spent caustic (SSC) solutions were performed in continuously fed gas-lift bioreactors. Sulfide was completely oxidized to sulfate (SO_4^{2-}) by haloalkaliphilic sulfur oxidizing bacteria (HA-SOB) belonging to the genus *Thioalkalivibrio*. Severe inhibition of the biological sulfide oxidation capacity resulting in a subsequent process deterioration occurred at DMDS effluent concentrations between 0.1 and 0.9 mM. The total measured DMDS removal efficiency amounted to 40-70%, of which 25% can be attributed to air stripping. It is yet unclear what other processes contributed to the total DMDS removal and it can only be speculated that the remainder is removed by biological conversion and/or adsorption. Respiration experiments revealed that pure cultures of HA-SOB (*Thioalkalivibrio* sp. K90-mix, *Thioalkalivibrio sulfidophilus*) as well as biosludge taken from a full-scale installation for hydrogen sulfide (H_2S) removal (*Thiopaq*) were more severely inhibited by methanethiol (MT) than by DMDS. From bioreactor- and respiration experiments it follows that MT and DMDS concentrations need to be below 0.02 and 0.1 mM, respectively to ensure stable process conditions.

4.1 Introduction

H_2S-rich sour gas (e.g. high pressure natural gas and biogas) and products from crude oil processing such as liquefied petroleum gas (LPG) are commonly washed with diluted caustic (NaOH) solutions to remove (in)organic sulfur compounds before further processing [41, 135]. This results in highly alkaline (pH > 12) and saline (Na^+ < 12% wt) waste solutions that are commonly known as 'sulfidic spent caustics' (SSC's). Besides elevated levels of sulfide (HS^- and S^{2-}), high concentrations of organic sulfur compounds such as MT (CH_3-S^-) have been reported to be present in SSC's [36, 40, 47]. MT is well known for its toxic properties and low odor threshold value. Hence, SSC solutions need treatment prior to discharge in the environment [43]. Physico-chemical processes, such as wet air oxidation (WAO) and the Merox process, are frequently applied to treat SSC's and hydrocarbon streams such as LPG [204]. During WAO, the oxidation of sulfides and organic sulfur compounds (also known as mercaptans) to thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}) and SO_4^{2-} is achieved at high pressures and elevated temperatures [86]. The Merox process is specifically designed for the extraction of mercaptans in a caustic solution, followed by an oxidation step to form the corresponding disulfides. In the Merox process, DMDS (CH_3-S-S-CH_3) for example, is the final product of the catalytic oxidation of MT [205].

Biotechnological treatment of SSC's would be a less expensive and safer alternative to these physico-chemical treatment processes as no additional chemicals are needed and ambient pressures and temperatures can be applied. So far, it has been shown that under neutrophilic conditions the aerobic biodegradation of sulfide, MT and DMDS is feasible [47, 133, 137, 138]. It has also been suggested that biological oxidation of MT to SO_4^{2-} by *Thiobacilli* mainly occurs via intermediary DMDS [47]. This intermediary DMDS is rapidly formed by the auto-oxidation reaction of MT with oxygen.

Compared to neutrophilic conditions, haloalkaline process conditions offer major advantages since no dilution of the SSC solution with fresh process water is required. Hence, little if any make-up water is needed and significantly less effluent is produced. Discharge of this moderately saline effluent may be problematic in areas where discharge of salty water is not allowed. For the treatment of SSC's at elevated pH levels and salinities, a new biotechnological process has been developed that relies on the activity of soda lake bacteria (chapter 2, [58]). It has also been shown that detoxification of sulfide in a non-biological pre-treatment step allows higher sulfide loads (chapter 5, [206]). In order to expand the operating window of this biotechnological process for the treatment of SSC's, the simultaneous treatment of sulfide and mercaptans has been studied. In our previous work we studied the inhibition of MT and DMDS on biological sulfide oxidation (chapter 3, [207]). It was shown that both MT and DMDS inhibit sulfur oxidizing biomass. It was also shown that MT was 30 times more toxic to biological sulfide oxidation than DMDS (chapter 3, [207]). The present study focuses on the biological treatment of synthetically prepared SSC's consisting of sulfide and DMDS under SO_4^{2-} forming conditions. Long-term experiments were performed in continuously fed gas-lift bioreactors. In addition, respiration experiments have been performed to investigate the effect of DMDS and MT on the sulfide oxidation capacity, using pure strains of HA-SOB (*Thioalkalivibrio* sp. K90-mix, *Thioalkalivibrio sulfidophilus*) and sulfur oxidizing biosludge taken from a full-scale installation for H_2S removal from biogas (*Thiopaq*) [97].

4.2 Materials and Methods

4.2.1 Experimental Set-Up

Results in this study were obtained using two identical continuously operated gas-lift reactors. The liquid volume of the reactors was 2 L (Ø=10 cm). The temperature and pH were controlled at 35 °C and 9.5, respectively. The percentage of oxygen saturation in the reactor liquid was between 80-90% sat. (100% ~ 5.6 mmol L^{-1}). The gas phase was continuously recycled using a small compressor (N820 (20 L min^{-1}), KNF pumps, Germany). Detailed information about the experimental set-up can be found in chapter 2.

4.2.2 Inoculum and influent

The inoculum consisted of concentrated biomass, that was taken from a continuously fed bioreactor, operated at pH 9.5, a hydraulic residence time (HRT) of 30 days. The sodium (Na^+) concentration was 0.8 M. The bioreactor was inoculated with 5 grams of soda lake sediment that originated from the Kulunda Steppe (Altai, Russia) [156].

The mineral medium consisted of a sodium carbonate/bicarbonate buffer solution (pH 9.5; Na^+ 0.8M). 1 g L^{-1} sodium chloride (NaCl) was added to the mineral medium to reach the chloride requirements for the growth of HA-SOB [142]. Influent was supplied with sulfide ($Na_2S.9H_2O$) and DMDS (Sigma-Aldrich) as shown in Table 1 and 2. Macro-nutrients were continuously added in the following amounts: 1 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgCl_2.6\ H_2O$ and 0.2 g L^{-1} urea. 0.5 ml L^{-1} of trace element solution was added as described elsewhere [157].

4.2.3 Reactor conditions

Two experimental runs were performed in two identical bioreactor systems. Operating conditions for Run 1 and 2 are shown in Table 1 and 2, respectively. Until day 56, operating conditions for both runs were similar. During day 56-59, the influent flow of Run 2 was interrupted to allow the system to recover from a process upset. After recovery of the process at day 59 (i.e. conversion of sulfide and $S_2O_3^{2-}$ to SO_4^{2-} and recovery of the biomass concentration), Run 2 was continued at a HRT of 3.7 days.

An abiotic control experiment was performed for a period of 21 days to assess the molar distribution of DMDS over the gas and liquid phase. The control experiment was operated at similar conditions as Run 1 and 2 (i.e. temperature 35 °C, pH 9.5, Na^+ 0.8M and a HRT of 3.7 days). An influent DMDS concentration of 4.9 mM was applied (Table 1).

Table 1: Overview of the operating conditions of Run 1: Hydraulic residence times (HRT), Influent concentrations for total-S, sulfide and DMDS.

Time (days)	0-17	17-24	24-28	28-35	35-47	47-64
HRT (days)	30	15	15	7.5	7.5	3.7
influent-S total (mM-S)	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5
influent sulfide (mM-S)	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5
influent DMDS (mM-S)	0.06	0.06	0.12	0.12	0.2±0.02	0.2±0.02

Time (days)	64-73	73-95	95-102	102-112
HRT (days)	3.7	7.5	7.5	7.5
influent-S total (mM-S)	38±0.5	34±1	32±1	33±1
influent sulfide (mM-S)	37±0.5	33.6±1	30.5 ±2	30.5 ±2
influent DMDS (mM-S)	0.8±0.1	0.8±0.1	1.8±0.5	2.8±0.4

Table 2: Overview of the operating periods of Run 2: the hydraulic residence times (HRT) and influent concentrations for total-S, sulfide and DMDS. Also included are the HRT and the influent DMDS concentration for the abiotic control experiment (c).

Time (days)	0-17	17-24	24-28	28-35	35-47	47-56
HRT (days)	30	15	15	7.5	7.5	3.7
influent-S total (mM-S)	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5
influent sulfide (mM-S)	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5	37±0.5
influent DMDS (mM-S)	0.06	0.06	0.12	0.12	0.2±0.02	0.2±0.02

Time (days)	56-59	59-64	64-73	73-88	***C*** 14-21
HRT (days)	-	3.7	3.7	7.5	3.7
influent-S total (mM-S)	-	37±0.5	38±0.5	34±1	-
influent sulfide (mM-S)	-	37±0.5	37±0.5	33.6±1	-
influent DMDS (mM-S)	-	0.2±0.02	0.8±0.1	0.8±0.1	9.8±0.4

4.2.4 Analytical procedures

Biomass concentrations (as total-nitrogen) were determined using the Hach Lange cuvette test LCK238 (Hach Lange, Düsseldorf, Germany). This method was calibrated by standard addition of ureum and nitrate to reactor samples as well as fresh medium. Total sulfide was analysed using Hach Lange cuvette test LCK653 (Hach Lange, Düsseldorf, Germany).

SO_4^{2-} and $S_2O_3^{2-}$ were determined by ion chromatography (761 compact IC with a 762 IC interface, Metrohm, Switzerland) equipped with a conductivity detector. A metrosep A supp 5 column was used at ambient temperature and a flow rate of 0.7 mL min^{-1}. A pre-column (metrosep A sup 4/5 guard) was used. The injection volume was 20 µL. The eluent comprised of 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$ and 1% acetone. In addition, suppressors for eluent conductivity and CO_2 were used (Metrohm, Switzerland). Na^+ concentrations were determined using ICP-OES (Perkin Elmer Optima 5300 DV).

DMDS and MT were measured using high pressure liquid chromatography (Waters corporation, Milford, Massachusetts, USA) using a Phenomenex Synergi Hydro-RP 80Å 4 µm column (Utrecht, The Netherlands) with a length of 25 cm and a diameter of 4.6 mm. The oven temperature was 30 °C. Within a single HPLC run different gradients of eluent (acetonitrile and water) were used (0'-8' 45% acetonitril/55% water; 8'-22' gradual change to 75% acetonitril/25% water; 22'-23' 75% acetonitril/25% water; 23'-34' gradual change to 45% acetonitril/55% water). The flow rate was 0.8 ml min-1. Injection volume of the samples was 20 µl. An UV detector (type 486 Tunable Absorbance detector; Gynotek Germering, Germany) was used to monitor MT and DMDS at a wavelength of 210 nm. Proper quality control of this method was ensured by applying a standard addition of the organic sulphur compounds to samples taken from the bioreactor samples as well as fresh medium.

4.2.5 Microbial community analysis

Genomic DNA was extracted from reactor samples taken at day 1 and 100 from Run 1 using the FastDNA SPIN for soil kit (MP Biomedicals, USA). DNA amplification of the 16S rRNA gene fragments and denaturing gradient gel electrophoresis (DGGE) analysis was performed as described elsewhere [158]. Excised bands were sequenced by the commercial company Marcogen (Korea). The sequences were first compared to sequences stored in the GenBank database using BLAST. Subsequently, a phylogenetic tree was made using ARB [208]. The sequences have been submitted to GenBank under accession numbers: JQ917204-JQ917211.

4.2.6 Respiration tests

Respiration tests were performed as described in chapter 3 and 4. A 4 mL reaction chamber was used. Active cell suspensions (10 mg N L^{-1}) were added to a carbonate buffer solution (pH 9.5; Na^+ 0.8 M). The temperature was controlled at 35 °C. Two pure cultures of HA-SOB (Thioalkalivibrio sp. strain K90-mix and Thioalkalivibrio sulfidophilus strain HL-EbGr7) were used. Sludge from a full-scale hydrogen sulfide (H_2S) removal system (*Thiopaq* bioreactor) was kindly provided by Industriewater Eerbeek B.V. [125]. Biomass was washed

three times with mineral medium (pH 9.5; Na^+ 0.8 M).

The biological sulfide oxidation rate ($mMO_2\ h^{-1}$) at 0.25 mM sulfide was determined and defined as the maximum oxidation rate. In addition, the inhibition of the biological sulfide oxidation by varying MT and DMDS concentrations was determined. Stock solution of MT and DMDS were either dissolved in buffer solution (pH 9.5; Na^+ 0.8 M) or in methanol. Control experiments were performed with both autoclaved dead cells (20 min; 121°C) and viable cells to assess the fate (chemical and biological) of MT or DMDS in the reaction chamber. All respiration experiments were performed in duplicate.

The respiration experiments were modelled according to equation 1 [209]. The outcome of the respiration experiments were used to assess the inhibition coefficients (Ki) for MT and DMDS by deriving a linear regression model (eq. 2) from eq. 1.

$$R_I = R_{max} \bullet \left(\frac{Ki}{Ki + Ci} \right) \qquad \text{(eq. 1)}$$

$$\frac{R_{max} - R_I}{R_I} = \left(\frac{1}{Ki} \right) \bullet Ci \qquad \text{(eq. 2)}$$

in which

R_I reaction rate at concentration of inhibitor I ($mMO_2\ h^{-1}$)

R_{max} maximum reaction rate ($mMO_2\ h^{-1}$)

K_i inhibition coefficient (mM)

C_i concentration inhibitor (mM)

4.3 Results and Discussion

In the presence of DMDS, complete sulfide to SO_4^{2-} conversion was observed during different periods of Run 1 (until day 112) and Run 2 (periods I, III and IV) (Figs. 1A and D). This was emphasized by the absence of any detectable sulfide species (i.e. < 0.003 mM) in the effluent during these periods (data not shown). Critical DMDS effluent concentrations for Run 1 appeared to be between 1.3 and 1.8 mM-S (day 112 to 114, Fig. 1A and B). Lower critical DMDS effluent concentrations of 0.22 and 0.42 mM-S (day 49 and 74) were observed for Run 2 (Fig. 1D and E). At these concentrations, biological sulfide oxidation was inhibited as indicated by a sudden accumulation of sulfide for Run 1 (less than 0.003 up to 0.2 mM; period VI) and Run 2 (from 0.01 to 0.6 mM; period II and 0.7 to 0.2 mM; period V) and a sharp decline in the selectivity for SO_4^{2-} formation (Figs. 1 A and D). As a consequence of the increased sulfide levels, sulfide oxidized chemically to $S_2O_3^{2-}$ [68], whilst the biomass concentration started to decrease (Figs. 1A, C, D and F; Tables 3 and 4). Previous research showed that at least 3 times higher sulfide loading rates could be applied without inhibiting *Thioalkalivibrio* sp. K90 mix (chapter 2, [58]). Hence, it is assumed that the observed inhibition was caused by the presence of DMDS and not due to any sulfide toxicity.

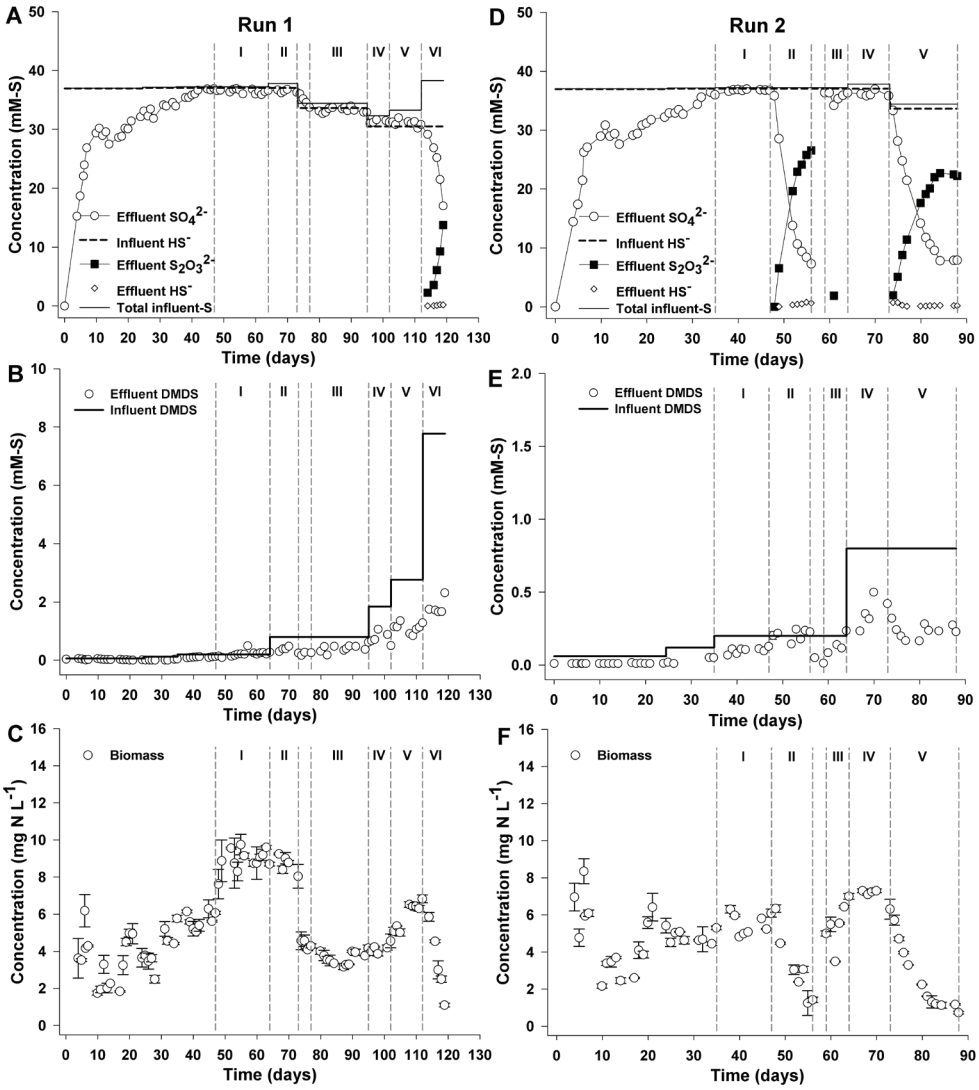


Figure 1: Reactor performance of Run 1 (A-C) and Run 2 (D-E). A, D Total influent-S concentration (sum of thiosulfate-S ($S_2O_3^{2-}$-S) and sulfide (HS^-) in the influent) and concentrations of sulfate (SO_4^{2-}), $S_2O_3^{2-}$-S and HS^- in the effluent; B, E Influent and effluent dimethydisulfide (DMDS) concentrations; C, F Biomass concentration in mg nitrogen per liter.

Critical DMDS effluent concentrations for Run 1 were 3-4 times higher compared to Run 2. This indicates different levels of tolerance to DMDS, which can be caused by small differences

in biomass concentration or differences in the microbial population.

From day 73 onwards, the HRT was increased from 3.7 to 7.5 days to mitigate any process instabilities that may result from increasing effluent DMDS concentrations. (Tables 1 and 2). Consequently, the total-S load decreased by 50%, resulting in a rapid decrease (±55%) of the biomass concentration (Fig. 1C).

Table 3: Total-S, sulfide and DMDS loading rates and effluent concentrations of the total-S, SO_4^{2-} and DMDS of Run 1 during steady state periods as well as the sulfate selectivity and DMDS removal percentages.

Period	I	II	III	IV	V	VI
time (days)	47-64	64-73	77-95	95-102	102-112	112-119
total-S load (mmol L^{-1} day^{-1})	10.1	10.4	4.5	4.3	4.4	5.1
sulfide load (mmol L^{-1} day^{-1})	9.8	9.8	4.5	4	4	4
DMDS-S load (mmol L^{-1} day^{-1})	0.05	0.22	0.11	0.24	0.37	1
effluent S-total (mM-S)	36.7±0.3	36.8±0.3	33.7±0.4	32.1±0.2	32.3±0.5	32.3±0.5
effluent sulfate (mM-S)	36.6±0.3	36.5±0.3	33.3±0.4	31.3±0.3	31.1±0.5	decrease
effluent DMDS (mM-S)	0.22±0.1	0.32±0.1	0.38±0.1	0.74±0.2	1.1±0.2	-
sulfate selectivity (mol %)	99	99	99	97	96	-
DMDS removal (%)	0	60	53	59	61	-

Table 4: Total-S, sulfide and DMDS loading rates and effluent concentrations of the total S, sulfate (SO_4^{2-}) and DMDS of Run 2 during steady state periods as well as the SO_4^{2-} selectivity and DMDS removal percentages. Also included are DMDS load, effluent DMDS-S concentration and the DMDS removal for the abiotic control experiment (c).

Period	I	II	III	IV	V	*C*
time (days)	35-47	47-56	59-64	64-73	73-88	14-21
total-S load (mmol L^{-1} day^{-1})	5	10.1	10.1	10.4	4.5	1.3
sulfide load (mmol L^{-1} day^{-1})	4.9	9.8	9.8	9.8	4.5	-
DMDS-S load (mmol L^{-1} day^{-1})	0.03	0.05	0.05	0.22	0.11	1.3
effluent S-total (mM-S)	36.8±0.5	35±1	36.2±0.5	36.6±0.5	33±2	-
effluent sulfate (mM-S)	36.6±0.4	decrease	36±0.5	36.3±0.5	decrease	-
effluent DMDS (mM-S)	0.10±0.03	0.21±0.04	0.12±0.08	0.37±0.1	0.24±0.05	7.3±0.3
sulfate selectivity (mol %)	99	-	99	99	-	-
DMDS removal (%)	50	0	40	54	70	25

DMDS removal efficiencies of 53-61% (Run 1) and 40-70% (Run 2) were observed during steady state operation (Figs. 1B and E; Tables 3 and 4). This was significantly higher compared to the abiotic control experiment in which only 25% of the DMDS was removed from the reactor liquid, most likely due to air-stripping (Tables 2 and 4). Stripping of DMDS has been, amongst others, reported in aerated membrane bioreactor systems at elevated temperatures (55 °C) [210]. In the present study, part of the incoming DMDS was continuously removed from the system via the bleed gas stream where it condensed at lower temperatures in the wash flasks. From the low effluent DMDS concentrations in both Runs, it may be assumed that DMDS stripping rates were significantly lower (i.e. less than 25%) as compared to the abiotic control experiment.

DGGE analysis showed that different phylotypes of HA-SOB closely related to *Thioalkalivibrio* sp. strain K90-mix (MdG_B2 and B5) were present in Run 1 (Fig. 2). Phylotype MdG_B2 was found to represent the most dominant band in the DGGE which indicates its dominant presence in Run 1. *Thioalkalivibrio* species oxidize sulfide either to SO_4^{2-} or elemental sulfur (S^0) depending on the dissolved oxygen concentration and are able to grow at high pH (> 10) and Na^+ concentrations [142]. *Thioalkalivibrio* sp. strain K90-mix, could be regarded as the main sulfide-oxidizing bacteria in Run 1.

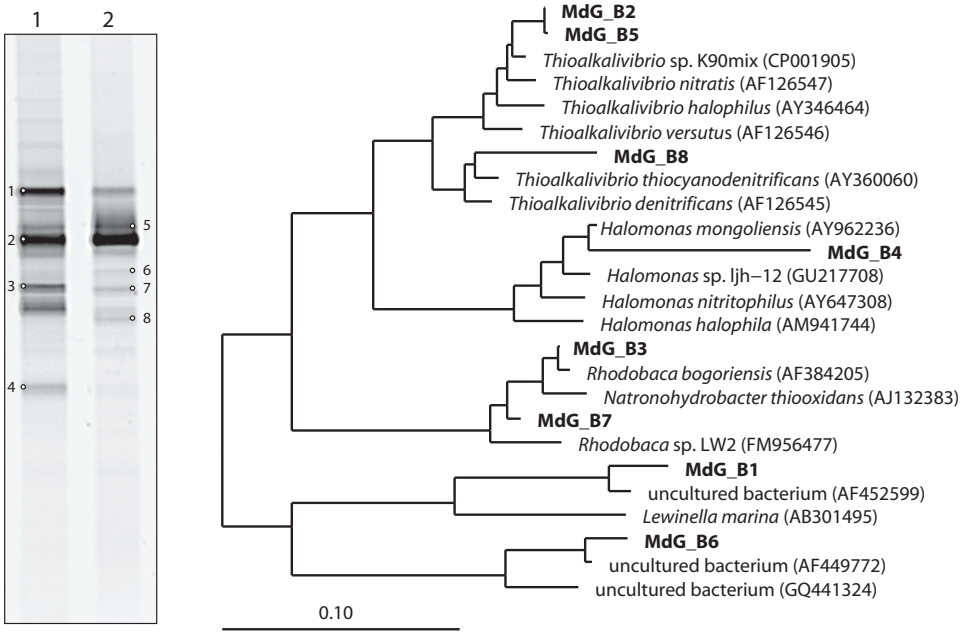


Figure 2: DGGE analysis of bacteria from Run 1 on day 1 (lane 1) and day 100 (lane 2). Bands MdG_B1 to B8 were excised, reamplified and sequenced. The sequences obtained from denaturing gel (bacterial 16S rRNA gene sequences) were sequenced and used to determine a phylogenetic tree. The bar indicates 10% sequence difference.

Besides *Thioalkalivibrio* sp. strain K90-mix, bacteria closely related to *Halomonas mongoliensis* (MdG_B4) and *Thioalkalivibrio thiocyanodenitrificans* (MdG_B8) were present in Run 1 at day 1 and 100, respectively. The denitrifying soda lake bacteria *Halomonas mongoliensis* has also been shown to be capable of oxidizing sulfide [211]. *Thioalkalivibrio thiocyanodenitrificans* is able to grow under aerobic and anaerobic conditions with thiocyanate (SCN^-) or $S_2O_3^{2-}$ as electron donor [212]. Therefore, phylotypes MdG_B4 and B8 as found in Run 1 possibly played a role in the oxidation of sulfide to SO_4^{2-} (Fig. 2).

Sequences (MdG_B3 and B7) closely related to the genus *Rhodobaca* (*Rhodobaca* sp. and *Rhodobaca bogoriensis*) were found on day 1 and 100 (Fig. 3). Members of the genus *Rhodobaca* are bacteriochlorophyll α containing purple non-sulfur bacteria that are commonly found in soda lakes [213]. The sequences MdG_B1 and B6 found on day 1 and 100 are closely related to uncultured CFB group bacteria isolated from the haloalkaline Mono Lake (USA) (Fig. 2).

Previous studies show that DMDS degradation under neutrophilic conditions is feasible for several members of the genera *Thiobacillus*, *Hyphomicrobium*, *Pseudomonas* and *Pseudonocardia* [47, 76, 119, 137, 214, 215]. However, it is not clear if the bacteria as identified by DGGE in this study were involved in the removal of DMDS. In addition, short term (7 days) batch experiments (T = 35 °C; pH 9.5; Na^+ 0.8 M) with bioreactor biomass (10 mgN L^{-1}) did not reveal biodegradation of DMDS at influent concentrations ranging from 0.1 to 1 mM (data not shown). Moreover, the final product of complete biological oxidation of sulfide and DMDS would be SO_4^{2-}. From the total S-balance for Run 1, it becomes apparent that SO_4^{2-} was predominantly formed from biological sulfide conversion (Fig. 1A; Table 3). Hence, the current dataset does not provide evidence for the simultaneous biological oxidation of sulfide and DMDS. The gap in the total S-balance, especially during period V in Run 1, and the observed removal of DMDS can therefore not be explained yet (Fig. 1A and B; Table 3). It may be that intermediate organic sulfur compounds were formed that were not detected by the employed analytical methods. In a previous study, for instance, it is shown that intermediate elemental S^0 particles react with dissolved organic sulfur compounds to form polymercaptides [45]. It might also be that the hydrophobic DMDS molecules are absorbed in the amphiphilic lipid membrane layer of the bacteria.

Respiration experiments with a pure culture of *Thioalkalivibrio* sp. K90-mix showed that DMDS seriously inhibits biological sulfide oxidation (Fig. 3A, Table 5). A 50% decrease of the sulfide oxidation rate (*Ki*) was observed at a DMDS concentration of 0.1 mM (i.e. 0.2 mM-S) (Fig. 3A; Table 5). This was 15 times lower compared to a *Thioalkalivibrio mix* that was studied in chapter 3 (Table 5).

The critical effluent DMDS concentrations that were found in Run 1 and 2 were 3-6 times higher than the *Ki* DMDS for *Thioalkalivibrio* sp. K90-mix (Figs. 1A, B, D and E and 3; Table 5). Apparently, the microbial populations in Run 1 and 2 were less susceptible to DMDS toxicity compared to the pure culture of *Thioalkalivibrio* sp. K90-mix. This may also be the case for the *Thiopaq* sludge and the pure culture of *Thioalkalivibrio sulfidophilus*. Higher *Ki* DMDS values were found for the *Thiopaq* sludge (dominated by *Thioalkalivibrio sulfidophilus* [130]) compared to the pure culture of *Thioalkalivibrio sulfidophilus* compared to (Figs. 3B and C, Table 5).

For *Thioalkalivibrio* sp. K90-mix, *Thioalkalivibrio sulfidophilus* and *Thiopaq* sludge it was found that MT inhibits biological sulfide oxidation more severely than DMDS (Table 5). MT was for example 2.5 times more toxic to *Thioalkalivibrio* sp. K90-mix than DMDS with *Ki* values of 0.04 and 0.1 mM, respectively (Fig. 3A; Table 5). This is most likely due to the stronger nucleophilic properties of MT [71]. The *Ki* MT for *Thioalkalivibrio* sp. K90-mix was similar to a *Thioalkalivibrio mix* (Table 5), while lower *Ki* values of 8 μM were reported for the neutrophilic SOB *Thiobacillus thioparus* [74, 207].

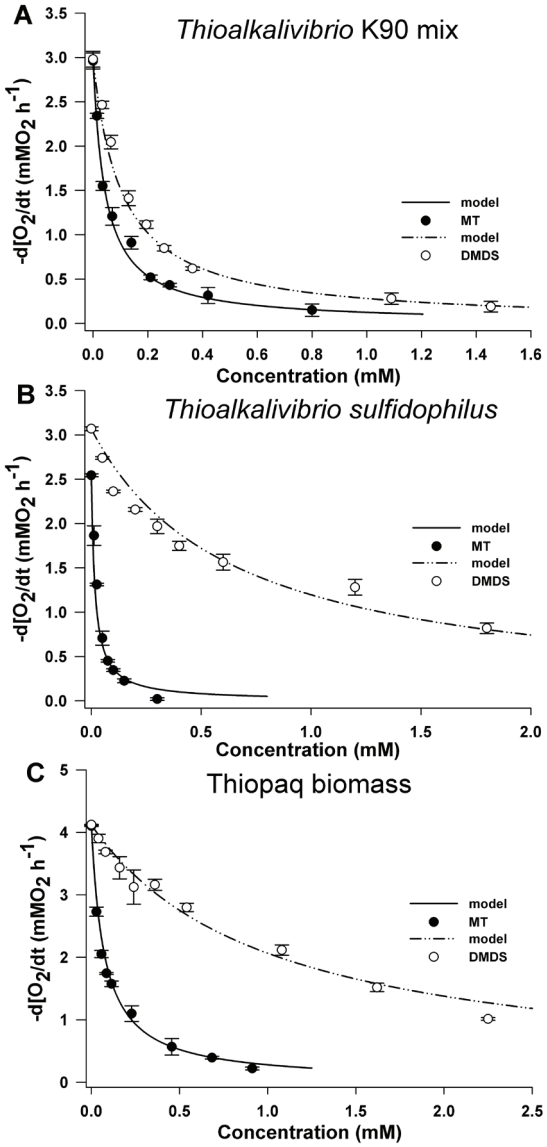


Figure 3: The relative biological oxidation rates during sulfide oxidation (0.25 mM sulfide) in the presence of varying MT and DMDS concentrations. The respiration experiments were performed for the pure cultures *Thioalkalivibrio* sp. K90-mix (A) and *Thioalkalivibrio sulfidophilus* (B), as well as for sludge derived from a full-scale installation for H_2S removal (*Thiopaq*). The estimated *Ki* values and the outcome of the respiration experiments were used to fit the model as shown in eq. 2.

From table 5 it can be seen that the *Ki* values for DMDS and MT for *Thioalkalivibrio* sp. K90-mix and *Thioalkalivibrio sulfidophilus* are lower compared to *Thiopaq* sludge. It might be that these differences in inhibition are related to the different stages of sulfide oxidation i.e. DMDS is less toxic for S^0 formation than for SO_4^{2-} formation and thus to the different enzymatic systems that are involved [130, 216]. *Thioalkalivibrio* sp. K90-mix and *Thioalkalivibrio sulfidophilus* for instance oxidized sulfide to SO_4^{2-} while the *Thiopaq* sludge oxidized sulfide partially to S^0.

From previous studies it is known that MT concentrations in SSC's can be as high as 0.4 M [36, 47]. These concentrations are considerably higher than the *Ki* values found for the tested HA-SOB and for the *Thiopaq* sludge. Therefore, treatment of SSC's containing elevated levels of MT and DMDS seems to have a detrimental effect on the sulfide removal efficiency. From Table 5, it follows that the DMDS concentration needs to be below 0.1 mM to ensure stable process conditions. In the event of higher influent concentrations, dilution can be achieved by recirculating a part of the effluent to the influent of the system.

Table 5: Inhibition coefficients (*Ki*) for biological sulfide oxidation for pure cultures of *Thioalkalivibrio* sp. K90-mix and *Thioalkalivibrio sulfidophilus* as well as for *Thiopaq* sludge. **Ki* values for *Thioalkalivibrio* mix as discussed in chapter 3.

	K_i DMDS (mM)	K_i MT (mM)
Thioalkalivibrio sp. K90-mix	0.1±0.08	0.04±0.06
Thioalkalivibrio sulfidophilus	0.6±0.16	0.02±0.18
Thiopaq sludge	1.0±0.15	0.07±0.16
Thioalkalivibrio mix*	1.5	0.05

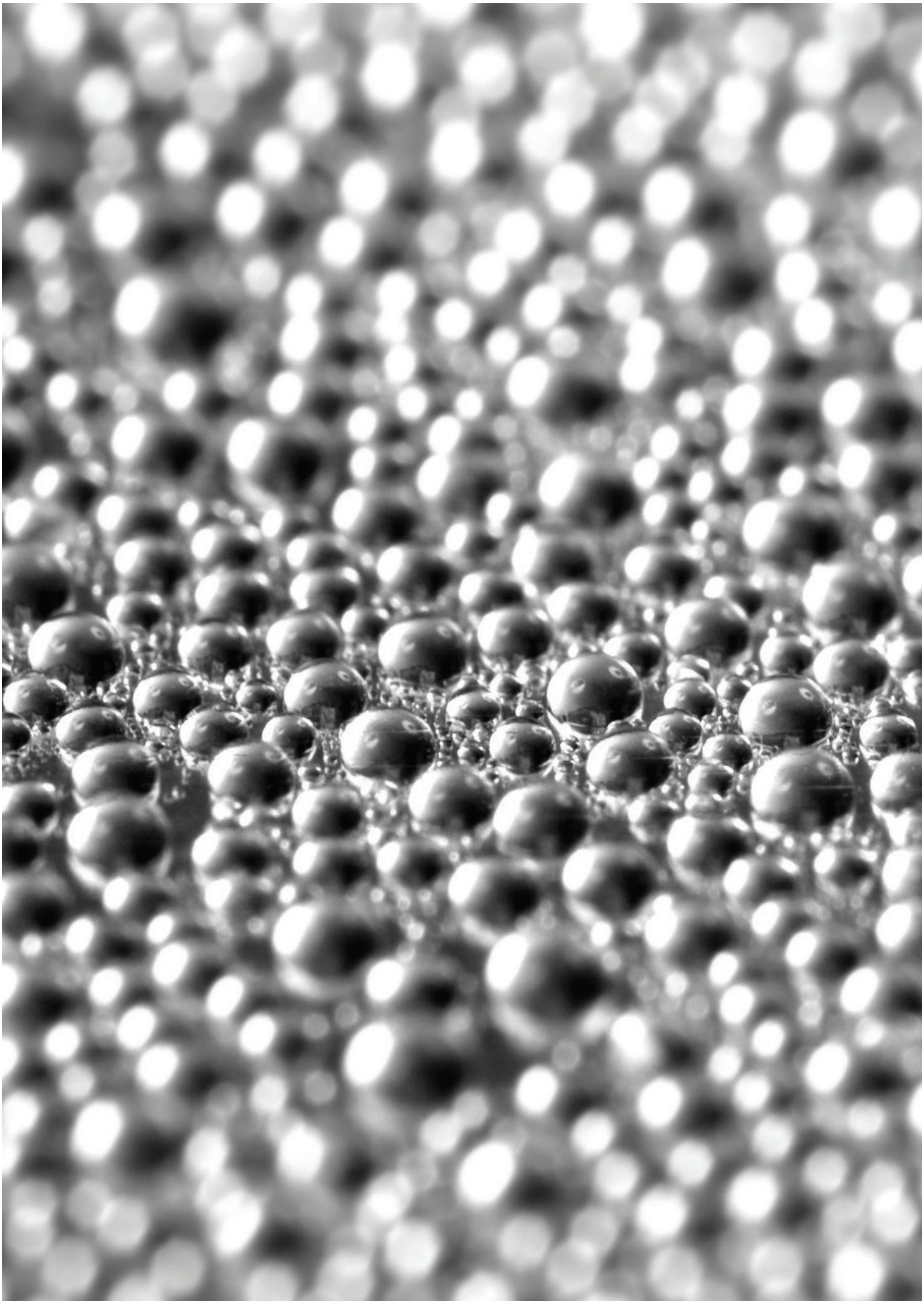
4.4 Conclusions

This study shows that complete biological conversion of sulfide to SO_4^{2-} in the presence of low concentrations of DMDS under haloalkaline conditions is possible. The sulfide conversion was accomplished by HA-SOB belonging to the genus *Thioalkalivibrio*. Severe inhibition of the biological sulfide oxidation capacity resulting in a subsequent process deterioration occurred at DMDS effluent concentrations between 0.1 and 0.9 mM. Apart from air stripping (max. 25%), it is yet unclear what other removal processes contributed to the total DMDS removal (40-70%). It can therefore only be speculated that part of the observed DMDS removal is due to biological conversion and/or adsorption.

For *Thioalkalivibrio* sp. K90-mix, *Thioalkalivibrio sulfidophilus* and *Thiopaq* sludge it was found that MT inhibits biological sulfide oxidation more severely than DMDS. The results obtained in this study indicate that MT and DMDS concentrations need to be below 0.02 and 0.1 mM, respectively to ensure stable process conditions.

Acknowledgements

This work was performed in the TTIW-cooperation framework of Wetsus, Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs, the European Union Regional Development Fund, the Province of Fryslân, the City of Leeuwarden and the EZ/Kompas program of the "Samenwerkingsverband Noord-Nederland". The authors like to thank the participants of the research theme "Concentrates" for the discussion and their financial support. The authors also want to acknowledge J.B.M. Klok (Wageningen University, The Netherlands) and Prof. dr. G.J.W. Euverink (University of Groningen) for discussing the results and M. Haan (Wetsus, The Netherlands) and B. Abbas (Delft University of Technology, The Netherlands) for their technical support.



Chapter 5

Application of a two-step process for the biological treatment of sulfidic spent caustics

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This chapter is published as:
Application of a two-step process for the biological treatment of sulfidic spent caustics
Water Research, Vol 46, 723-730, 2012

Abstract

This research demonstrates the feasibility and advantages of a 2-step process for the biological treatment of sulfidic spent caustics under haloalkaline conditions (i.e. pH 9.5; Na^+ 0.8M). Experiments with synthetically prepared solutions were performed in a continuously fed system consisting of two gas-lift reactors in series operated at aerobic conditions at 35 °C. The detoxification of sulfide to thiosulfate in the first step allowed the successful biological treatment of total-S loading rates up to 33 mmol L^{-1} day^{-1}. In the second, biological step, the remaining sulfide and thiosulfate was completely converted to sulfate by haloalkaliphilic sulfide oxidizing bacteria. Mathematical modeling of the 2-step process shows that under the prevailing conditions an optimal reactor configuration consists of 40% 'abiotic' and 60% 'biological' volume, whilst the total reactor volume is 22% smaller than for the 1-step process.

5.1 Introduction

Diluted caustic (NaOH, 5–10 wt%) solutions are commonly used in the oil refining industry for the desulfurization of hydrocarbon streams. During caustic scrubbing, gaseous sulfur compounds such as H_2S and thiols are absorbed resulting in waste streams known as sulfidic spent caustic. Without treatment, these streams may impose environmental problems because of their alkalinity (pH>12), salinity ([sodium] of 5-12% wt) and high sulfide (HS^- and S^{2-}) levels exceeding 2-3 wt% [36, 40, 91]. In addition, spent caustics may contain toxic organic sulfur compounds such as methanethiol and aromatic hydrocarbons like benzene [47, 58].

Recently, a specialized group of haloalkaliphilic sulfide oxidizing bacteria (HA-SOB) obtained from soda lake sediments was successfully applied in a continuously fed reactor system for the biological treatment of refinery spent caustics (chapter 2, [58]). In this 1-step process (Fig. 1A), HA-SOB oxidize dissolved sulfide to elemental sulfur (S^0; eq. 1) and/or sulfate (SO_4^{2-}; eq. 2). Sulfate formation occurs at elevated oxygen (O_2) levels in combination with low specific sulfide loading rates, whilst elemental sulfur is formed at oxygen-limiting conditions or at sulfide loading rates exceeding the maximum specific biological conversion rate to sulfate [127]. Sulfate is the preferred end product since it does not represent COD (chemical oxygen demand), which may allow the bioreactor effluent to be discharged into the environment. Moreover, protons formed during sulfate formation will result in pH neutralization of the alkaline solution.

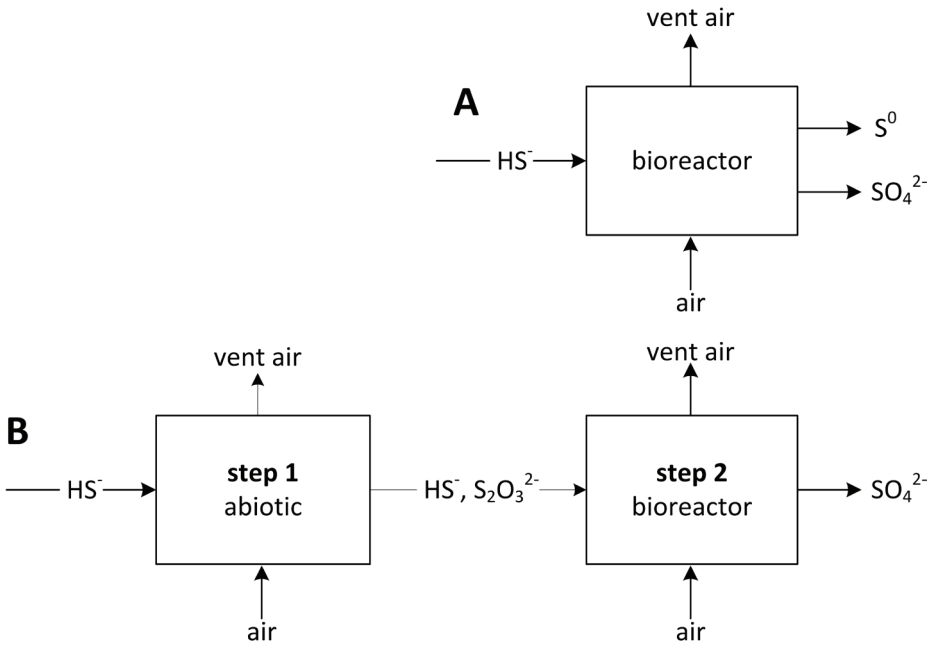


Figure 1: Schematic representation of the 1-step (A) and 2- step (B) process

Since in practice, e.g. at refineries, the sulfide influent concentration may vary in time, the formation of elemental sulfur may be difficult to prevent (chapter 2, [58]). Moreover, sulfide is toxic to microorganisms and known to inhibit several enzymatic systems, including cytochrome c oxidase, at already low concentrations [16, 217]. To ensure complete sulfate formation at all times, it is proposed to first auto-oxidize sulfide to the non-toxic thiosulfate ($S_2O_3^{2-}$; eq. 3) [68]. The remaining sulfide and thiosulfate are then fed to a second reactor where it is completely oxidized to sulfate (eq. 2 and 4) by HA-SOB:

$HS^- + ½O_2 \rightarrow S^0 + OH^-$ (eq. 1)

$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$ (eq. 2)

$2HS^- + 2O_2 \rightarrow H_2O + S_2O_3^{2-}$ (eq. 3)

$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2H^+ + 2SO_4^{2-}$ (eq. 4)

It was shown by González-Sánchez and Revah (2007) [218] that sulfide inhibits the activity of alkaliphilic sulfur oxidizing bacteria at least six times more than thiosulfate.

The objective of this study was to demonstrate the feasibility of a 2-step process (Fig. 1B) for the treatment of sulfidic spent caustics and to demonstrate its envisaged advantages, i.e. lower total installed reactor volume and increased robustness as a result of the application of a detoxification step. Consequently higher sulfide loading rates can be applied. A long-term

experiment of 190 days was performed using synthetically prepared sodium sulfide solutions in a continuously fed system consisting of two reactors in series that were operated under haloalkaline conditions (pH 9.5; Na^+ 0.8M) using HA-SOB originating from soda lakes. The experimental data were used to validate a mathematical model that was developed for the 2-step process which can be used to design an optimized process.

5.2 Methods

5.2.1 Reactor set-up

A continuously fed system consisting of a bubble column and a gas-lift reactor in series was used (Fig. 2). The system was operated under haloalkaline conditions (pH 9.5; Na^+ 0.8M) using HA-SOB originating from soda lakes. The reactors have a maximum wet volume of 2 L (Ø=10 cm) (Fig. 2). Reactor 1 (R1) serves as an abiotic reactor and reactor 2 (R2) as biological reactor. The temperature was maintained at 35 °C by using a water-jacket and a thermostat bath (Haake, Germany). The influent was fed to R1 and the effluent from R1 was fed into R2 using peristaltic pumps (Masterflex® L/S®, Cole-Parmer instruments, USA). The influent was added to the downcomer section of the reactors to assure proper mixing. The pH was monitored using a pH sensor (Endress+Hauser orbisint CPS12D, Naarden, The Netherlands). The percentage of oxygen saturation (% sat) was monitored with an oxygen sensor (Mettler Toledo Inpro 6050) and controlled (R1 100% sat.; R2 80% sat.) by supplying pure oxygen via mass flow controllers (Bronkhorst, The Netherlands). To prevent the release of volatile compounds from the system, the gas phases were continuously recycled by using a small compressor (N820 (20 L min^{-1}), KNF pumps, Germany; Fig. 2A). The recycled gas first passed a condenser (10 °C) to recover volatile compounds (e.g. sulfide) that may be stripped from the bioreactor suspension (Fig. 2B). R1 was equiped with a level controller in order to control the liquid volume at respectively 2, 1 and 0.5 L (Fig. 2C).

5.2.2 Inoculum and influent

The inoculum consisted of a concentrated biomass of haloalkaliphilic sulfide oxidizing bacteria dominated by *Thioalkalivibrio* sp. strain K90-mix that was grown in previous experiments and described in chapter 2 [58]. The original inoculum originated from a mixture of soda lake sediments (Kulunda Steppe, Altai, Russia) [156].

The mineral medium consisted of a sodium carbonate (Na_2CO_3) solution (pH 9.5; Na^+ 0.8M). 1 g L^{-1} NaCl was added to the Na_2CO_3 solution to meet the chloride requirements for growth of haloalkaliphilic sulfide oxidizing bacteria [128]. The influent was prepared by supplementing mineral medium with $Na_2S.9H_2O$ (Sigma-Aldrich, The Netherlands) to obtain a sulfide concentration of 65±1 mM. Traces of thiosulfate and sulfate (<1%) have been measured in the influent, most likely due to in-situ oxidation of sulfide and impurities in the sodium sulfide flakes. Macro-nutrients were continuously added to the bioreactor (R2) in the following amounts: 1 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgCl_2.6\ H_2O$ and 0.2 g L^{-1} urea. 0.5 ml L^{-1} of trace element solution was added as described elsewhere [157].

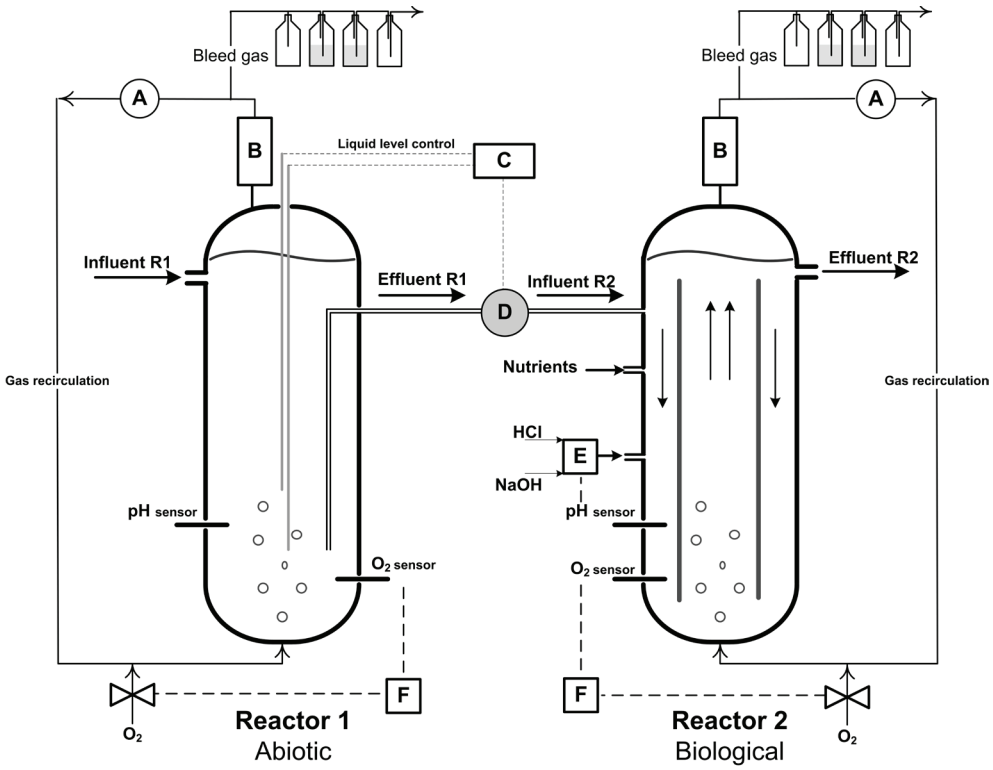


Figure 2: Schematic overview of the abiotic bubble column reactor and the gas-lift bioreactor as employed in the 2-step process. Controlling units for oxygen, pH and redox are shown as well as the gas recirculation pumps (A), condensers (B), liquid level control unit (C), influent pump for bioreactor R2 (D), pH (E) and oxygen (F) controlling units.

Table 1: Overview of the different operating periods, the type of process during every period, hydraulic residence times (HRT) of abiotic reactor R1 and bioreactor R2, the total liquid volume of the system (V_{tot}=V_{R1}+V_{R2}) in which V_{R2} is 2 L and the total-S load (sum of sulfide, thiosulfate and sulfate) based on the total system volume.

Period	I	II	III	IV	V	VI	VII	VIII
time (days)	0-64	64-97	97-136	137-158	158-168	168-171	171-180	180-190
process	2 step	2 step	2 step	1 step	1 step	-	2 step	2 step
HRT_{R1} (days)	3.5	1.8	0.9	-	-	-	1	0.5
HRT_{R2} (days)	3.5	3.5	3.5	3.5	2	-	2	2
V_{tot} (L)	4	3	2.5	2	2	-	3	2.5
total-S load (mmol L^{-1} day^{-1})	9±1	12.5±1	15±1	19±1	33±1	-	22 ±1	26±1

5.2.3 Reactor conditions

A long-duration experiment of 190 days was performed. Table 1 shows the operating conditions at different periods for R1 and R2. The liquid volume of R2 (V_{R2}) was always 2L, while the liquid volume of R1 (V_{R1}) varied (2, 1 and 0.5 L). The influent flow was changed from 0.4 to 0.7 mL min^{-1} at period V (day 158-168). In period VI (day 168-171), the influent was interrupted to allow the system to recover from a process disruption.

5.2.4 Respiration tests

Respiration tests were performed as described in chapter 3 and 4. A 4 mL reaction chamber was used. Active cell suspensions (20 µL; 10 mg N L^{-1}) were added to a carbonate buffer solution (pH 9.5; Na^+ 0.8 M). The temperature was controlled at 35 °C. A pure culture of the haloalkaliphilic sulfide oxidizing bacterium *Thioalkalivibrio* K90-mix was provided and cultured (pH 10; Na^+ 1.2 M). Oxygen consumption rates (mMO_2 h^{-1}) were obtained for different sulfide concentrations (0-0.4 mM). Biological sulfide oxidation rates were calculated by subtracting the measured rates in the absence of cells (abiotic oxidation) from the values measured in the presence of cells (combined abiotic and biological oxidation rate).

5.2.5 Analytical procedures

Biomass concentrations were measured as total-nitrogen using the Hach Lange cuvette test LCK238 (Hach Lange, Germany). Biomass samples were washed 3 times with a Na_2CO_3 solution (pH 9.5; Na^+ 0.8 M) to remove any dissolved nitrogen compounds. This method was calibrated by standard addition of ureum and nitrate to reactor samples as well as fresh medium. Total sulfide was analysed using Hach Lange cuvette test LCK653 (Hach Lange, Germany) and sodium concentrations were determined using ICP-OES (Perkin Elmer Optima 5300 DV). Sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$) were determined by ion chromatography on a 761 compact IC with a 762 IC interface (Metrohm, Switserland) and equipped with a conductivity detector. A metrosep A supp 5 column was used at ambient temperature and at a flow rate of 0.7 mL min^{-1}. A pre-column (metrosep A supp 4/5 guard) was used. The injection volume was 20 µL. The eluent comprised of 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$ and 1% (v/v) acetone. In addition, suppressors for eluent conductivity and CO_2 were used (Metrohm, Switzerland). At steady state conditions, the elemental sulfur concentration was calculated according to the following equation (eq. 5):

$$[S^0] = [\text{Influent S}]-[SO_4^{2-}]-2*[S_2O_3^{2-}]-[HS^-] \quad \text{(eq. 5)}$$

5.2.6 Development of a mathematical model for sulfide removal

Sulfide removal in both the abiotic and the bioreactor was described according to the following mass balance (eq. 6):

$$\frac{d[HS^-]}{dt} = \frac{Q}{V} \cdot \left([HS^-_{in}] - [HS^-]\right) - K_{ab} \cdot [HS^-]^{\alpha} - R \cdot X_b \qquad \text{(eq. 6)}$$

where

$$R = \frac{\mu_{max}}{Y} \cdot \frac{[HS^-]}{\frac{[HS^-]^2}{K_i} + K_m + [HS^-]} \qquad \text{(eq. 7)}$$

Consequently, the maximum specific sulfide consumption rate (k) is defined by

$$k = \frac{\mu_{max}}{Y} \qquad \text{(eq. 8)}$$

V	volume (L)
Q	flow (L h^{-1})
$[HS^-_{in}]$	influent sulfide concentration (mM)
$[HS^-]$	effluent sulfide concentration (mM)
K_{ab}	chemical reaction constant (h^{-1})
α	reaction coefficient (-)
R	specific biological reaction rate (mmol mgN^{-1} h^{-1})
X_b	biomass concentration (mgN L^{-1})
k	maximum specific sulfide consumption rate (mmol mgN^{-1} h^{-1})
K_m	substrate affinity constant (mM)
K_i	inhibition coefficient (mM)
μ_{max}	maximum specific growth rate (h^{-1})
Y	Yield (mgN $mmol^{-1}$)

The reaction kinetics for the abiotic sulfide oxidation were earlier described by O'Brien and Birkner (1977) [203], where K_{ab} is a lumped variable containing the oxygen concentration. Steady state values of the abiotic reactor R1 were used to estimate the parameters K_{ab} and α, using a non-linear, non-weighted, least-square-parameter estimation method. The algorithm

has been written in Matlab. The biological sulfide oxidation rate was described by a second order Monod-Haldane equation (eq. 7).

The outcome of the biological sulfide oxidation experiments (i.e. respiration tests; paragraph 2.4) were used to estimate the kinetic parameters in the Monod-Haldane curve (eq. 7 and 8). The substrate affinity constant (K_m), the substrate inhibition constant (K_i), and the maximum specific sulfide consumption rate (k) were estimated using a non-linear, non-weighted, least square parameter estimation. From our experiments it becomes clear that sulfate is the sole end product of biological sulfide oxidation provided that a complete conversion takes place, i.e. without any remaining sulfide in the effluent ($[HS^-]<0.001\ [HS^-_{in}]$). Hence, equation 2 applies to describe the biological oxidation of sulfide to sulfate.

The model was developed in Matlab and used to assess the overall sulfide removal rate by varying the ratio of the abiotic reactor volume over the total reactor volume.

5.3 Results and discussion

The results of abiotic reactor R1 show that sulfide is oxidized with thiosulfate as the sole, non-toxic, end product (Fig. 3A and 4A). This is in agreement with several other studies, which show that thiosulfate is the main product of chemical sulfide oxidation under alkaline conditions [68, 203, 219]. Since the oxygen concentration was kept constant, the amount of sulfide oxidized (i.e. oxidation efficiency) in R1, was fully dependent on the hydraulic residence time (HRT=V/Q) and the sulfide loading rate (Tables 1 and 2), thereby following first-order reaction kinetics [68, 203, 218, 220]. Hence, lowering the HRT resulted in lower removal efficiencies. This becomes apparent in R1 where decreasing oxidation efficiencies of 85% , 70%, 47% and 35% were found at HRT's of respectively 3.5, 1.9, 0.9 and 0.5 days (Fig. 3A and 4A; Tables 1 and 2). For similar HRT's, e.g. 1 day, the oxidation efficiency in R1 became 18% higher when the sulfide loading rate was increased 1.7 times (Figs. 3A and 4A, Table 2). From the experimental data (Tables 2 and 4) and the mass balance for the abiotic reactor (eq. 6), the chemical reaction rate constant (K_{ab}) and the reaction coefficient (α) were estimated as shown in Table 4. The experiments revealed that the value found for K_{ab} (0.21 h^{-1}) is in the same order of magnitude as compared to the value of K_{ab} (0.87 h^{-1}) found by Janssen et al. (1995) [68]. An explanation for the differences in both values can be found in differences in the reaction conditions including pH, temperature and the type of solution in which the reaction takes place [203, 218, 220].

The 2-step process was tested in period I-III. Steady state effluent sulfide and thiosulfate concentrations were reached for R1 indicating a stable process operation (Fig. 3A; Table 2). Moreover, a complete conversion to sulfate was achieved in bioreactor R2 (Table 3; Figs. 3B). Biomass concentrations in R2 were in general stable and not significantly affected by variations in the sulfide to thiosulfate influent ratios (Fig. 3C). This is most likely due to the constant S-load (i.e. sum of sulfide and thiosulfate) that was applied in these periods.

Table 2: Sulfide load and the average sulfide and thiosulfate concentrations in the effluent of abiotic reactor R1 during steady state as well as the sulfide to thiosulfate oxidation efficiency

Period	I	II	III	VII	VIII
time (days)	27-64	70-97	98-137	171-180	180-190
sulfide load (mmol L^{-1} day^{-1})	19±1	38±1	67±1	66±1	132±1
effluent-sulfide (mM)	9±1	18±2	34±2	23±2	42±2
effluent-thiosulfate (mM-S)	56±2	44±1	30±3	42±2	23±2
oxidation efficiency (%)	85±1	69±1	46±2	65±1	35±1

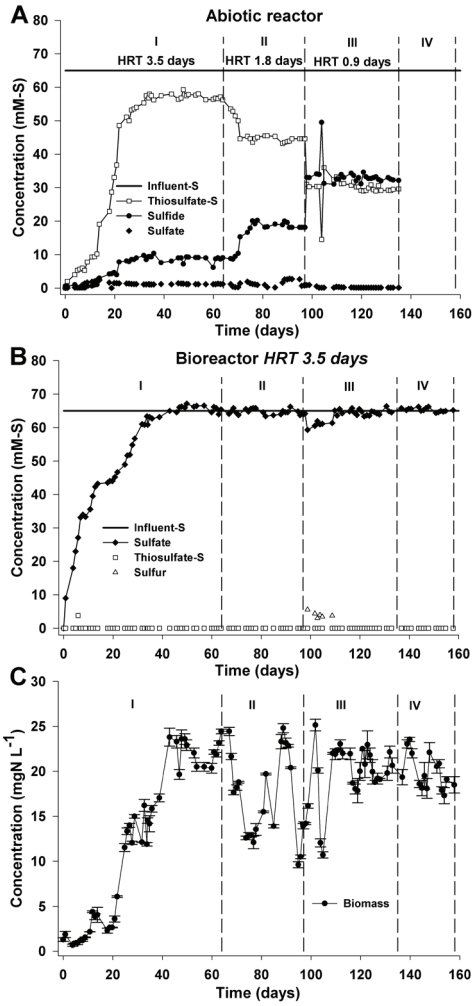


Figure 3: Performance of the 2-step (period I-III) and 1-step (period IV) process during the first 158 days of the experiment. Total influent-S concentration (i.e. sum of sulfide, thiosulfate and sulfate), effluent concentrations of sulfide, thiosulfate, sulfate, sulfur (A and B) and biomass concentrations (C)

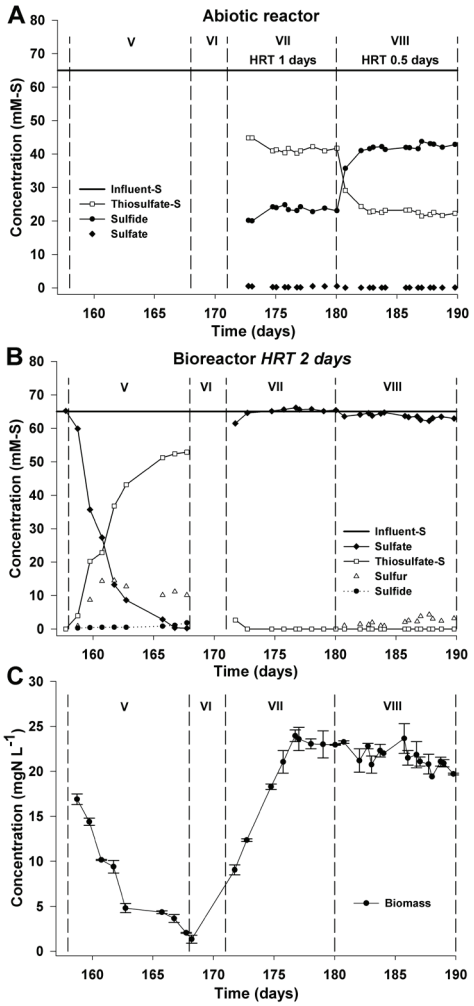


Figure 4: Performance of the 1-step (period V) and 2-step (period VII,VIII) process for days 158-190. Total influent-S concentration (sum of sulfide, thiosulfate and sulfate), effluent concentrations of sulfide, thiosulfate, sulfate, sulfur (A and B) and biomass concentrations (C)

Table 3 The total-S load (i.e. the sum of sulfide, thiosulfate and sulfate), sulfide load and the average effluent sulfide, thiosulfate and sulfate concentrations of bioreactor R2 during steady state of periods I-IV and VII-VIII

period	**I**	**II**	**III**	**IV**
time (days)	27-64	70-97	108-137	137-158
total-S load (mmol L^{-1} day^{-1})	19±1	19±1	19±1	19±1
sulfide load (mmol L^{-1} day^{-1})	2.5±0.3	5±0.4	10±0.5	19±1
effluent-sulfate (mM)	64±2	64±2	64±2	65±0.5
sulfate selectivity (mol %)	99±1	99±1	98±2	99±1
Period	**V**	**VI**	**VII**	**VIII**
time (days)	158-168	168-171	171-180	180-190
total-S load (mmol L^{-1} day^{-1})	33±1	-	33±1	33±1
sulfide load (mmol L^{-1} day^{-1})	33±1	-	12±1	21±1
effluent-sulfate (mM)	decrease	-	65±0.5	63±1
sulfate selectivity (mol %)	0	-	99±1	98±2

Even after switching from a 2-step to a 1-step process (period IV), all incoming sulfide was completely converted to sulfate in R2 at a total sulfide loading rate of 19 mmol L^{-1} day^{-1} (Fig. 3B; Table 3). It was, however, essential to determine the critical sulfide load (i.e. the load at which sulfur will be formed) for bioreactor R2 to allow a proper comparison between the 1-step and the 2-step process. The onset of sulfur formation was observed when the sulfide load for R2 was increased to 33 mmol L^{-1} day^{-1}, resulting in a rapid decline in sulfate selectivity that was accompanied by the accumulation of sulfide (0.25 to 1.8 mM between day 158-168) and the abiotic formation of thiosulfate (Period V; Fig. 4B, Table 3). Similar to van den Bosch et al. (2007) [127], the HA-SOB in R2 switched to sulfur formation (20±3% of the total-S) at effluent sulfide concentrations above 0.25 mM (Period V, Fig. 4B). Complete process deterioration occurred when the biomass was washed out at day 168 (Fig. 4C). The wash out of HA-SOB can be explained by a severe decrease in growth rates after switching from sulfate to sulfur forming conditions [127].

Finally, a total-S loading rate of 33 mmol L^{-1} day^{-1} was applied to R2 in a 2-step process (periods VII-VIII; Tables 1-3). It is shown that this resulted in a complete recovery of the sulfate selectivity, i.e. more than 98 mol% sulfate after reaching steady state (Table 3; Fig. 4B). Hence, it can be concluded that as a result of the partial detoxification of sulfide in the first step, a higher total-S loading rate could be applied to R2 compared to the 1-step process (Tables 1 and 3).

The biomass concentration increased after switching to the 2-step process on day 171 before reaching steady state concentrations around 22±1 mgN L^{-1} (Table 1; Fig. 4C). Based on the original inoculum, it is assumed that members of the genus *Thioalkalivibrio* dominated R2. From literature it is known that *Thioalkalivibrio* are able to oxidize sulfide and thiosulfate under a broad range of haloalkaline conditions [143]. Previous research shows the potential of *Thioalkalivibrio* strains for the removal of hydrogen sulfide at loading rates above 50 mmol

L^{-1} day^{-1} [221]. This load was applied at sulfur-forming conditions and was significantly higher than the values found in this study (Table 3). The reason for this is that under sulfur forming conditions 4 times less oxygen is consumed than for a sulfate producing system (eq. 1 and 2).

Table 4: Parameter values and standard deviations (SD) for the chemical reaction rate constant (K_{ab}), reaction coefficient (α), maximum specific reaction rate (μ_{max}), substrate affinity constant (K_m) and the inhibition coefficient (K_i). In addition, the coefficients of determination (R^2) for measured versus calculated results for the abiotic reactor (R1) and bioreactor (R2) are included.

Parameter	Average	SD
K_{ab} (h^{-1})	0.21	0.15
α	0.51	0.21
k (mmol mgN^{-1} h^{-1})	1.48	0.38
K_m (mM)	0.23	0.08
K_i (mM)	0.09	0.03
R^2 R1	0.91	-
R^2 R2	0.99	-

Biological sulfide oxidation showed a maximum rate of 3.5 mmol O_2 L^{-1} h^{-1} (0.35 mmol O_2 mgN^{-1} L^{-1} h^{-1}) at a sulfide concentration of about 0.2 mM (Fig. 5). This is a factor 2 higher compared to experiments performed with a mixed culture of *Thioalkalivibrio* at higher salt concentrations (chapter 3, [207]). However, the mixed culture also contained non-sulfur oxidizing, heterotrophic bacteria resulting in lower oxidation rates [129, 216]. In the absence of cells (abiotic controls), the oxidation rates increased with increasing sulfide concentrations (Fig. 5). The Monod-Haldane equation (eq. 7) was used to fit the respiration data (Fig. 5). Values for K_m, K_i, and k were calculated and are shown in Table 4. From simulations with the newly developed model for a generic case, it follows that a total reactor volume reduction of 22% can be achieved for the 2-step process (Table 5). Furthermore, a 2-step process could also be used for the detoxification of toxic organic sulfur compounds that can be found in refinery spent caustics [36, 41, 47]. Methanethiol (CH_3-S) for example, rapidly auto-oxidizes to dimethyldisulfide (CH_3-S-S-CH_3), which is known to be almost 40 times less toxic to *Thioalkalivibrio* strains than its precursor [47, 207]. In this way, overall toxicity of the waste solution is reduced before it enters the bioreactor.

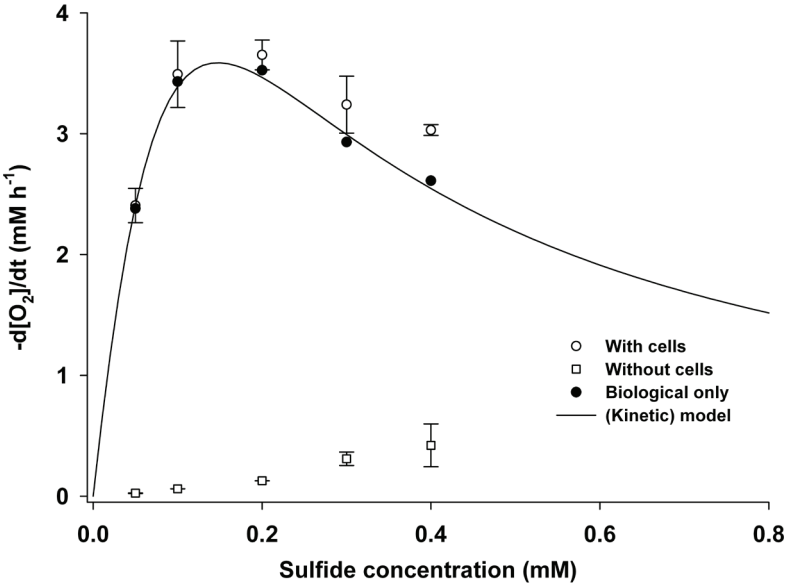


Figure 5: Oxidation rates in the absence and presence (10 mg N L^{-1}) of cells at different sulfide concentrations. The Monod-Haldane model (kinetic model) was fitted to the biological only oxidation rates for sulfide concentrations 0-0.4 mM.

Table 5: Simulation results for several reactor configurations of the 2-step process ([HS^-_{in}]=65 mM, X_b = 10 mg N L^{-1}). The percentages of the total reactor volume (V_{total}), compared to the bioreactor volume ($V_{biochem.}$), needed to remove 99.9% of the influent sulfide are shown.

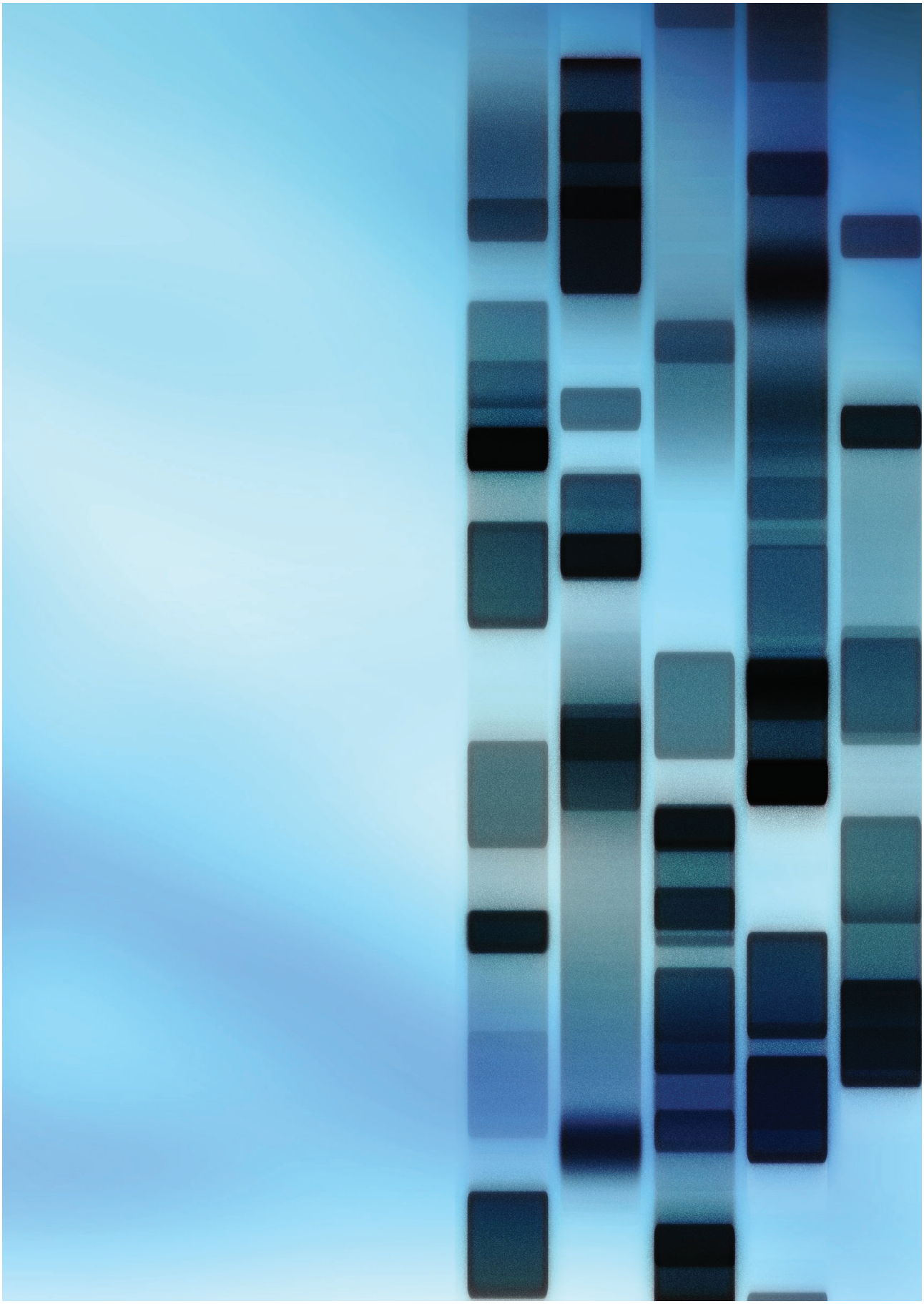
$V_{abiotic}$ (%)	$V_{biochem.}$ (%)	V_{total} (%)	Process
0	100	100	1-step
20	80	83	2-step
40	60	78	2-step
60	40	80	2-step
80	20	94	2-step

5.4 Conclusions

This study shows that a newly developed 2-step process for the biological treatment of sulfidic spent caustics allows higher sulfide removal efficiencies compared to a 1-step process. In the first step, thiosulfate was the sole end product of abiotic sulfide oxidation. The oxidation efficiency in the abiotic reactor is, amongst others, determined by the HRT and the sulfide-loading rate. In contrast to the 1-step process, total-S loading rates of 33 mmol L^{-1} day^{-1} were successfully applied in the 2-step process. Haloalkaliphilic sulfide oxidizing bacteria belonging to the genus *Thioalkalivibrio* were most likely responsible for the sulfide conversion. Mathematical modeling of the 2-step process shows that under the prevailing conditions an optimal reactor shall consist of 40% 'abiotic' and 60% 'biological' volume whilst the total reactor volume (V_{R1} + V_{R2}) is 22% smaller than for the 1-step process.

Acknowledgements

This work was performed in the TTIW-cooperation framework of Wetsus, Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs, the European Union Regional Development Fund, the Province of Fryslân, the City of Leeuwarden and the EZ/Kompas program of the "Samenwerkingsverband Noord-Nederland". The authors like to thank the participants of the research theme "Concentrates" for the discussion and their financial support. The authors also want to acknowledge Dr. D.Y. Sorokin (Delft University of Technology, The Netherlands and Winogradsky Institute of Microbiology, RAS, Moscow, Russia) for culturing *Thioalkalivibrio* K90-mix and for his support and discussion regarding this manuscript.



Chapter 6

General conclusions and discussion

Marco de Graaff

6.1 Introduction

The need for desulfurization of fossil fuels, such as coal and crude oil, became apparent when many industrialized nations were confronted with environmental problems related to smog formation and acid rain. Although renewable energy sources (solar, wind and geothermal) are increasingly implemented, most countries are still strongly fossil fuel dependent [1, 2]. Moreover, the need for fossil fuels will increase with the increasing energy demand due to a growing population. This means that as long as fossil fuels are used at large scale, there is the need and obligation from a political and an environmental point of view to desulfurize raw fuel sources. The sulfur emission control strategies and corresponding legislation may differ for various countries. The European Union, for example, set the maximal allowable sulfur content for fuels at 10 ppm while highway diesel fuel in the United States may not contain more than 15 ppm (chapter 1).

In the oil and gas industry, caustic solutions can be used to remove (in)organic sulfur compounds from fossil fuels. Once the sulfur compounds are absorbed, the saturated solution is called a sulfidic spent caustic (SSC). Depending on the origin of the treated hydrocarbon stream, spent caustics may contain various volatile organic sulfur compounds (VSC's) of which H_2S is the most pronounced. Typical examples of VSC's are methanethiol (MT) and dimethyl disulfide (DMDS). In addition, aromatic compounds such as benzene may be present (Table 3, Chapter 1). SSC's are commonly treated by physico-chemical processes that are often characterized by high investment and operating cost [39].

The aim of the work described in this thesis is to develop a biotechnological process for the treatment of SSC's. Haloalkaliphilic bacteria originating from soda lakes were employed because at high pH and salinity levels little, if any, fresh water is required to dilute the SSC before biotreatment. Especially in areas where fresh water is scarce, this offers major advantages. In addition to bioreactor experiments this thesis also describes respiration experiments to assess the effects of commonly found pollutants in SSC's (i.e. MT, DMDS and benzene) to biological sulfide oxidation. Finally, the development of a 2-step process for biological sulfide treatment will be presented as well as recommendations for further research.

6.2 Sulfur (S^0) and sulfate (SO_4^{2-}) formation

Sulfide conversion is the dominant process in a biological process for the treatment of SSC's. Depending on the oxygen concentration, sulfide can be partially converted to elemental sulfur (S^0, eq. 1) or completely to sulfate (SO_4^{2-}, eq. 2).

$$HS^- + \tfrac{1}{2}O_2 \rightarrow S^0 + OH^- \qquad \text{eq. 1}$$

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad \text{eq. 2}$$

$$HS^- + O_2 \rightarrow \tfrac{1}{2}S_2O_3^{2-} + \tfrac{1}{2}H_2O \qquad \text{eq. 3}$$

$$HS^- + (x\text{-}1)S^0 \rightleftarrows S_x^{2-} + H^+ \qquad \text{eq. 4}$$

$$S_x^{2-} + 1.5\ O_2 \rightarrow S_2O_3^{2-} + (x\text{-}2)S^0 \qquad \text{eq. 5}$$

The advantages of S^0 formation in biological H_2S removal processes such as the *Thiopaq* process are:

- Regeneration of caustic (eq. 1) allows its reuse in the sulfide extraction step [127].
- S^0 formation requires four times less O_2 than SO_4^{2-} formation. Therefore, the O_2 transfer rate limits the maximum oxidation rate of S^0 formation to a lesser extent and higher loading rates can be applied.
- Biologically produced S^0 is a potentially valuable end-product since it can be directly used for the synthesis of sulfuric acid (H_2SO_4) or re-used as a fertilizer. Half of the world's H_2SO_4 production is used in the fertilizer industry, mainly to obtain phosphoric acid from phosphate rock [222]. The worldwide market price for sulfur has been rising enormously during the last decade. In 2008, for example, sulfur prices temporarily increased from less than \$100 ton^{-1} to over \$800 ton^{-1} [222]. This was mainly due to a relative shortage of sulfur on the world market and the increased use of fertilizer in the United States and China.

The work described in this thesis aims to maximize SO_4^{2-} formation as it offers the following advantages:

- More metabolic energy is gained from the complete bio-oxidation of sulfide, which has a positive effect on biomass growth rates and hence the overall biomass concentration.
- Protons (H^+) are formed which result in a (partial) neutralization of the effluent.
- An almost complete removal of the chemical oxygen demand (COD) may be achieved. Under S^0 forming conditions, it is known that a (small) fraction of the sulfide is chemically (i.e. non-biologically) oxidized to thiosulfate ($S_2O_3^{2-}$, eq. 3) [68, 221]. In addition, polysulfides (S_x^{2-}) are formed in a S^0 forming bioreactors due to the reaction between (bi)sulfide (HS^-) and S^0 (x) (eq. 4). S_x^{2-} are unbranched chains consisting of S^0 atoms with average chain lengths of around 5 [127, 223]. Respiration tests show that the chemical oxidation rate of S_x^{2-} to $S_2O_3^{2-}$ (eq. 5) is about twice as high as that of sulfide (eq. 3) (chapter 3).

6.3 Biological treatment of SSC's

Characterization of two SSC's taken from the same refinery revealed sulfide concentrations of 0.16 and 0.24 M and sodium (Na^+) concentrations of 0.8 M (chapter 2). These specific SSC's also contained benzene concentrations (0.6 and 10 mM) but no organic sulfur compounds (chapter 2).

In the presence of benzene (15 µM; 71 µmol L^{-1} day^{-1}), complete sulfide to SO_4^{2-} was achieved at sulfide loading rates of 27 mmol sulfide L^{-1} day^{-1} at a hydraulic residence time (HRT) of 3.5 days (chapter 2). After the start-up phase, the raw refinery spent caustic solutions were mixed with a sodium (bi)carbonate solution to obtain sulfide concentrations that allowed us to reduce the HRT whilst working at the same sulfide load. Based on an average biomass concentration of 7 mgN L^{-1}, the specific conversion rate was 3.8 mmol sulfide mgN^{-1}

day^{-1}. This is somewhat lower compared to the value (4.56 mmol mgN^{-1} day^{-1}) that was found for a HA-SOB consortium (*Thioalkalivibrio* mix), in a S^0 forming fed-batch bioreactor [127]. From the influent benzene, about 93% was removed in the bioreactors. Part of the benzene removal was due to air-stripping. This indicates the need for an additional post-treatment step to remove benzene from the flue gas in order to avoid excessive emission rates. Physical treatment methods (e.g. incineration and carbon adsorption) and biotechnological methods are available for the removal of monoaromatic compounds [162]. Obviously, these post-treatment technologies will make the overall process more expensive.

Part of the benzene removal was most likely due to biodegradation (chapter 2). This was supported by microbial community analysis, which revealed the presence of haloalkaliphilic heterotrophic bacteria belonging to the genera *Marinobacter*, *Halomonas* and *Idiomarina* (chaper 2). Members of the genus *Marinobacter* have been shown to degrade benzene under moderately (halo)alkaline conditions [168, 169]. Members of the genera *Halomonas* and *Idiomarina* have been related to degradation of crude oil and diesel fuel under halophilic conditions [169, 170]. More specific, *Halomonas campisalis* was found to degrade catechol which is a common intermediate in benzene biodegradation [153].

Our results also show that DMDS concentrations exceeding 0.1-0.9 mM may cause severe inhibition to the biological sulfide oxidation capacity ultimately leading to complete process deterioration (chapter 4). DMDS was used in the bioreactor experiments since it is rapidly formed from the auto-oxidation of MT. DMDS is believed to be a key intermediate in MT biodegradation by *Thiobacilli* [47]. Although DMDS removal rates ranged from 40 to 70% it is yet unclear what the underlying mechanism is. Apart from air stripping (max. 25%), part of the removal of DMDS may be explained by the formation of undetected (organic-sulfur) compounds due to reaction with intermediate formed S^0 particles [45]. It can also be that the hydrophobic DMDS is absorbed, for example, in the lipid membrane layer of the bacteria. Biodegradation of DMDS may be another possibility. However, this hypothesis is not supported by the performed microbial community analysis and respiration experiments.

6.4 Biological sulfide oxidation

It was shown by denaturing gradient gel electrophoresis (DGGE) and cloning techniques that members of the genus *Thioalkalivibrio* were the dominant sulfide oxidizing bacteria (SOB) in the bioreactor experiments (chapter 2 and 4) and in the HA-SOB consortium that was employed for respiration experiments in chapter 3 [127, 129]. In the bioreactor experiments presented in chapter 2 and 4, sequences closely related to *Thioalkalivibrio* sp. K90mix were found. Members of the genus *Thioalkalivibrio* are a highly physiologically diverse group of bacteria that grow under extreme haloalkaline conditions (chapter 1). *Thioalkalivibrio* are relatively slow growing bacteria (maximum specific growth rates of about 0.2 h^{-1}) with efficient substrate conversion [128]. Respiration experiments showed that the maximal specific sulfide oxidation rate for a pure culture of *Thioalkalivibrio* sp. K90mix was 0.35 mmol O_2 mgN^{-1} L^{-1} h^{-1} at a sulfide concentration of approximately 0.2 mM (chapter 5).

For a HA-SOB consortium (*Thioalkalivibrio* mix) it was shown, that sulfide concentrations of 0.20-0.25 mM and above, result in biological S^0 formation. At sulfide concentrations around 0.05 mM and below, sulfide is completely oxidized to SO_4^{2-}. At intermediary sulfide

concentrations, both S^0 and SO_4^{2-} are formed (chapter 3). S^0 formation following sulfide accumulation was also observed when overloading the 1-step bioreactor process in chapter 5.

The DGGE gel for the bioreactor experiments fed with sulfide and benzene (chapter 2) revealed sequences related to different core strains within the genus *Thioalkalivibrio* (i.e. *Thioalkalivibrio* sp. ALBR_X3, *Thioalkalivibrio* sp. ALR20 and *Thioalkalivibrio* sp. K90mix) during different periods of the run (chapter 2). This means that different strains became dominant at different stages of the experiment. In addition, the DGGE gels showed different profiles over time (chapter 2 and 4), indicating that the species diversity in the microbial population changes over time. This is most likely the result of small changes in the operating conditions and might indicate subtle differentiations within the same niche (genetic microdiversity). Because not all bands were excised from the DGGE gels presented in chapter 2 and 4, it is most likely that the species diversity is probably even higher. This also becomes apparent from the clone libraries (chapter 2).

6.5 Effect of organic sulfur compounds

The effects of MT, DMDS and DMTS on biological sulfide oxidation were tested in respiration experiments. The main results described in chapter 3 and 4 and the corresponding experimental conditions are summarized in Table 1. It is shown that the biological sulfide oxidation rate is reduced by 50% (*Ki* value) at MT concentration between 0.02 and 0.07 mM. Regardless of the type of biomass that was tested, MT inhibited biological sulfide oxidation more than DMDS and DMTS. This may be explained by the lower nucleophilicity of DMDS and DMTS. As DMDS and DMTS are more hydrophobic compared to MT, hydrophobic interactions do not seem to play a major role in the inhibition of biological sulfide oxidation. MT and DMDS inhibition was found to be higher for the pure cultures compared to the mixed cultures (i.e. *Thiopaq* sludge and *Thioalkalivibrio* mix). Possibly, the mixed cultures are less susceptible to inhibition due to a higher diversity in sulfide oxidizing bacteria. Bioreactor biomass dominated by *Thioalkalivibrio* sp. K90-mix was severely inhibited at DMDS concentrations above 0.1 mM (chapter 4). This is similar to the *Ki* DMDS for the pure *Thioalkalivibrio* sp. K90-mix (Table 1).

The differences in the observed DMDS and MT inhibition may be related to the different stages of sulfide oxidation. In that case DMDS is less toxic for S^0 formation than for SO_4^{2-} formation and thus to the different enzymatic systems that are involved. This would explain the higher *Ki* DMDS and MT values for the *Thiopaq* sludge, which solely formed S^0, in contrast to the pure cultures.

Table 1: Inhibition coefficients (*Ki*) for biological sulfide oxidation for (i) pure cultures of *Thioalkalivibrio* sp. K90-mix and *Thioalkalivibrio sulfidophilus* as well as for (ii) *Thiopaq* sludge taken from a full-scale installation and (iii) a mix of *Thioalkalivibrio* strains. Also product formation, the experimental conditions during the experiments and the origin of the biomass are given.

	K_i DMDS (mM)	K_i MT (mM)	Product	Experimental conditions	Biomass	Chapter
Thioalkalivibrio sp. K90-mix	0.1±0.08	0.04±0.06	SO_4^{2-}	0.8 M Na^+ ; pH 9.5	pure culture	4
Thioalkalivibrio sulfidophilus	0.6±0.16	0.02±0.18	SO_4^{2-}	0.8 M Na^+ ; pH 9.5	pure culture	4
Thiopaq sludge	1.0±0.15	0.07±0.16	S^0	0.8 M Na^+ ; pH 9.5	full scale *Thiopaq* process	4
Thioalkalivibrio mix	1.5 1 (DMTS)	0.05	S^0	2 M Na^+/K^+ ; pH 9	S^0 forming bioreactor	3

6.6 Application of a 2-step process

Sulfide influent concentrations at operating units may vary in time leading to peak sulfide loads, which result in the formation of (unwanted) elemental S^0. Moreover, sulfide is toxic to microorganisms and known to inhibit several enzymatic systems, such as cytochrome c oxidase which, in turn, will lead to complete process deterioration (chapter 1). It is known that sulfide inhibits the activity of alkaliphilic SOB at least six times more than $S_2O_3^{2-}$ [218]. It was shown that applying a 2-step process for the biological treatment of SSC's allows significantly higher sulfide removal efficiencies compared to a 1-step process (Fig. 1). The detoxification of sulfide by its oxidation to $S_2O_3^{2-}$ (eq. 3) in the first chemical oxidation step and its subsequent biological oxidation in the 2nd step allows the successful biological treatment of total-S loading rates up to 33 mmol L^{-1} day^{-1} (chapter 5, Figure 1).

From simulations with a newly developed mathematical model, it follows that a total reactor volume reduction of 22% can be achieved for the 2-step process compared to the 1-step process. Optimal reactor configuration consists of 40% 'abiotic' and 60% 'biological' volume (chapter 5). Compared to a 1-step process, the 2-step process offers the following advantages:

- Prevention of sulfide toxicity due to a chemical detoxification step increases the robustness of the process (i.e. better anticipation to shock loads of sulfide).
- Higher sulfide loads and/or a lower total reactor volume may result in lower investment costs.
- Smaller reactors allow more efficient mixing which may increase the oxygen transfer rate leading to lower aeration requirements.
- By reducing the height of the reactors i.e. at lower static pressures, less energy is needed for aeration.

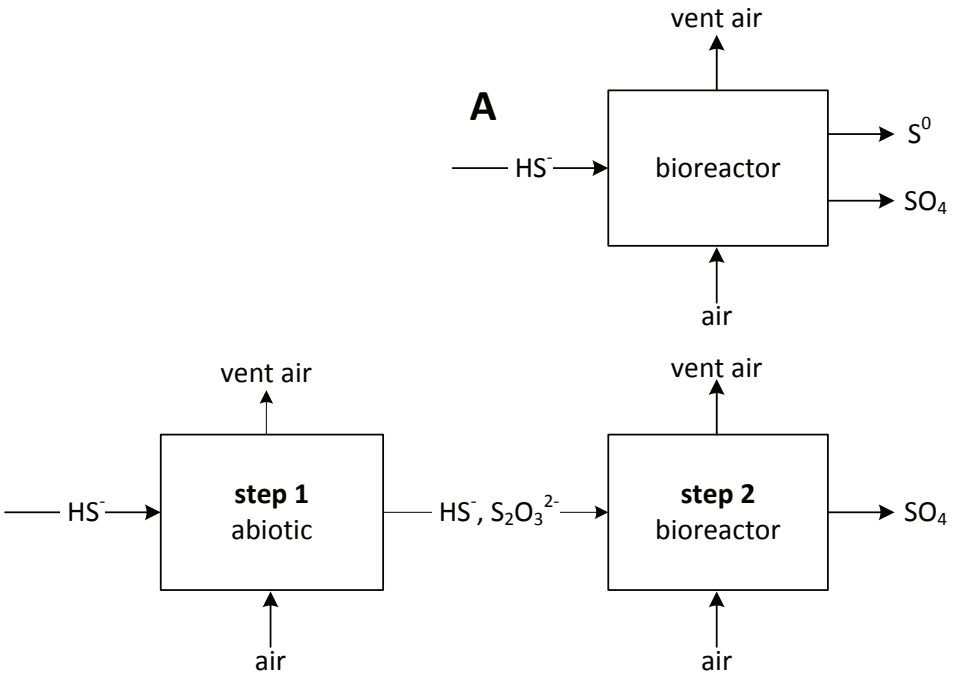


Figure 1: Schematic representation of the 1-step (A) and the developed 2-step (B) process for the treatment of sulfide (as bisulfide or HS^-) containing waste solutions such as SSC's.

The 2-step process would also be applicable for the complete oxidation of sulfide to SO_4^{2-} in gas- or wastewater streams that contain very low sulfide concentrations. These may include biogas, landfill gas and waste solutions produced in the pulp and paper industry, breweries and food processing industry. In addition, the first step could also be used for stripping out volatile compounds such as benzene or (partial) detoxification of MT (chapter 2 and 4). In this way, the overall toxicity of the waste solution is reduced before it enters the bioreactor.

6.7 Concluding remarks and recommendations

Our results show that biological sulfide oxidation in the presence of benzene and small amounts of organic VSC's is possible under haloalkaline conditions using soda lake bacteria. However, the results show that in order to ensure stable process conditions, MT and DMDS concentrations need to be below 0.02 and 0.1 mM, respectively. Hence treating SSC's with elevated MT and DMDS concentrations easily inhibits the sulfide oxidation capacity of the process. Auto-oxidation of MT or the chemical reaction of MT with S^0 particles will result in (partial) detoxification due to the formation of DMDS and DMTS. Although this may help to some extent, the effluent levels still need to be kept very low. Successful biological treatment of MT and DMDS containing SSC's may depend on the biological degradation of these compounds. When rapid biodegradation of organic VSC's can be achieved, the

concentrations in the reactor will remain below the critical levels.

The application of a 2-step process is expected to lead to a process that is more robust, allows higher sulfide loads and reduces the investment and operating costs compared to a conventional 1-step process. In order to assess the performance of a 2-step process for the handling of more complex SSC solutions (containing organic VSC's and aromatics), further research is needed. In addition, further research is also required to investigate the potential of heterotrophic soda lake bacteria for the degradation of organic VSC's. More knowledge on the actual mechanisms of toxicity by organic VSC's on soda lake bacteria would create more insight whether the observed toxic effects are (ir)reversible and whether there are differences in sensitivity between microbial populations.

Further research may investigate the effects of higher biomass concentrations. This would increase the overall treatment capacity of a full-scale installation enabling even smaller reactor volumes. To achieve higher biomass concentrations, a settler (with recirculation of biomass), a carrier material or a membrane for biomass retention can be considered.

Finally, the application of soda lake bacteria could be feasible for the removal of (in) organic contaminants from many industrial high saline and/or alkaline waste solutions. These may include the reject streams of reverse osmosis membranes [224] and saline waste water produced in the agro-food and leather industries which commonly contain high levels of organics [225].

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English summary

In this thesis, the development of a new biotechnological process for the treatment of undiluted sulfidic spent caustics (SSC's) using soda lake bacteria is described. SSC's are waste solutions that are formed in the oil and gas industry due to the caustic (NaOH) scrubbing of hydrocarbon streams for the removal of sulfur compounds. Without treatment, SSC's may impose serious environmental problems because of their alkalinity (pH>12), salinity (Na^+ 5-12 wt%) and high sulfide (HS^- and S^{2-}) levels. Depending on the hydrocarbon stream that is treated, SSC's may also contain organic sulfur compounds and monoaromatic hydrocarbons. Biological treatment of undiluted SSC's would be a cheaper and safer alternative to the currently applied physico-chemical treatment methods (e.g., wet air oxidation or deep well disposal) since no additional chemicals are needed and the process works at ambient pressure and temperature conditions.

In chapter 2 the biological treatment of refinery SSC's is described in continuously fed systems under haloalkaline conditions (i.e. pH 9.5; Na^+ 0.8 M). The experiments were performed in gas-lift bioreactors operated under aerobic conditions at 35 °C. Sulfide removal was complete up to 27 mmol L^{-1} day^{-1} by conversion to sulfate (SO_4^{2-}). The sulfide conversion was accomplished by haloalkaliphilic sulfide-oxidizing bacteria (HA-SOB) belonging to the genus *Thioalkalivibrio*. Members of the this genus are extremophiles that are able to oxidize sulfide under a broad range of haloalkaline conditions (0.3 - 4.3 M Na^+ and up to pH 10.6). In this chapter, it was also shown that benzene, at influent concentrations ranging from 100 to 600 µM, was removed by 93% due to air-stripping and biodegradation. Microbial community analysis revealed the presence of haloalkaliphilic heterotrophic bacteria belonging to the genera *Marinobacter, Halomonas* and *Idiomarina* which might have been involved in the observed benzene removal.

Sour gases and SSC's may also contain elevated amounts of methanethiol (MT; CH_3SH). Hence, knowledge on the potential toxic effects of these type of compounds on the performance of this biotechnological process is required. Under sulfur (S^0) forming conditions, MT reacts with biologically produced S^0 particles resulting in a mixture of inorganic polysulfides (S_x^{2-}), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). Respiration experiments with HA-SOB (*Thioalkalivibrio* mix) show in chapter 3 that biological oxidation of sulfide to S^0 is inhibited by 50% (*Ki* value) at 0.05 mM MT. The measured *Ki* values for DMDS and DMTS were 1.5 and 1.0 mM, respectively. As DMDS and DMTS are products from the reaction between MT and S^0, this reaction results in a partial detoxification of MT in a S^0-producing bioreactor. The results from the respiration experiments as shown in chapter 3 indicate that the application of the biotechnological process for the treatment of H_2S and MT containing gases and SSC's is feasible as long as MT, DMDS and DMTS do not accumulate in the bioreactor. Accumulation of MT can be prevented by auto-oxidation of MT to DMDS, by the reaction between MT and biosulfur particles or biodegradation.

Chapter 4 discusses the biological treatment of synthetically prepared SSC's containing both sulfide and DMDS. Continuously fed gas-lift bioreactor experiments showed that biological sulfide oxidation (4-10 mmol L^{-1} day^{-1}) is possible in the presence of low concentrations of DMDS under haloalkaline conditions (i.e., pH 9.5; Na^+ 0.8 M). Sulfide was completely oxidized to SO_4^{2-} by members of the genus *Thioalkalivibrio* (closely related

to *Thioalkalivibrio* sp. K90-mix). It was also shown that severe inhibition of the biological sulfide oxidation capacity and process deterioration occurs at DMDS effluent concentrations between 0.1 and 0.9 mM. The measured DMDS removal efficiency amounted up to 40-70% (0.05-0.37 DMDS-S L^{-1} day^{-1}), of which 25% could be attributed to air stripping. It is yet unclear what other processes contributed to the total DMDS removal and it can only be speculated that the remainder was removed by biological conversion and/or adsorption. Results from respiration experiments presented in chapter 4 reveal that pure cultures of HA-SOB (*Thioalkalivibrio* sp. K90-mix and *Thioalkalivibrio sulfidophilus*) as well as biosludge taken from a full-scale installation for H_2S removal (*Thiopaq*) are more severely inhibited by MT than DMDS. Furthermore, the *Ki* values for DMDS and MT were lower for *Thioalkalivibrio* sp. K90-mix and *Thioalkalivibrio sulfidophilus* compared to *Thiopaq* sludge. From bioreactor and respiration experiments it follows that, to ensure stable process conditions, MT and DMDS concentrations need to be below 0.02 and 0.1 mM, respectively. This clearly demonstrates that treating SSC's with elevated MT and DMDS concentrations will easily inhibit the sulfide oxidation capacity of the process. Although auto-oxidation of MT will result in (partial) detoxification due to the formation of DMDS, the effluent levels still need to be kept very low. Successful biological treatment of MT and DMDS containing SSC's will depend on the biological degradation of these compounds. When rapid biodegradation of organic VSC's can be achieved, the concentrations in the reactor will remain below the critical levels.

Chapter 5 shows that the application of a newly developed 2-step process for the biological treatments of SSC's using HA-SOB allows significantly higher sulfide removal efficiencies compared to a 1-step process. The detoxification of sulfide by the abiotic oxidation to thiosulfate ($S_2O_3^{2-}$) in the first chemical oxidation step and the subsequent complete biological oxidation in the second step allowed total-S loading rates up to 33 mmol L^{-1} day^{-1}. Experiments with synthetically prepared solutions were performed in a continuously fed system consisting of two gas-lift reactors in series. These reactors were operated at haloalkaline (pH 9.5; Na^+ 0.8M) and aerobic conditions at 35 °C. Mathematical modelling of the 2-step process shows that under the prevailing conditions an optimal reactor configuration consists of 40% 'abiotic' and 60% 'biological' volume, whilst the total reactor volume is 22% smaller than for the 1-step process. The major advantages of a 2-step process are the improved anticipation to shock loads of sulfide and lower investment and operational costs due to downsizing of the total reactor volume.

Further research regarding the biological treatment of SSC's may involve the potential of heterotrophic soda lake bacteria for the degradation of organic VSC's as well as the mechanisms of toxicity of these compounds.

Nederlandse samenvatting

In dit proefschrift wordt de ontwikkeling beschreven van een nieuw biotechnologisch proces voor de behandeling van sulfidic spent caustics (SSC's). Dit proces is gebaseerd op de toepassing van speciale bacteriën die afkomstig zijn uit soda-meren. SSC's zijn (afval-)waterstromen, die gevormd worden in de olie en gas industrie tijdens de ontzwaveling van koolwaterstoffen. Tijdens deze ontzwavelingsprocessen worden loogoplossingen (van NaOH) gebruikt. Vanwege de hoge concentraties aan sulfiden (HS^- en S^{2-}), de hoge zoutconcentraties (Na^+ 5-12 gewichts%) en de hoge alkaliniteit (pH > 12) is het vanuit milieuoverwegingen erg belangrijk dat SSC's worden behandeld. Naast sulfiden kunnen SSC's ook significante hoeveelheden organische zwavelverbindingen en mono-aromatische stoffen bevatten. Aangenomen mag worden dat een biologische behandeling van SSC's een goedkoper en veiliger alternatief zou zijn voor de huidige fysisch-chemische behandelingsprocessen (zoals wet air oxidation en deep well disposal). Bij een biologische behandeling hoeven immers geen additionele chemicaliën gebruikt te worden en kan het proces onder atmosferische druk en temperatuur plaatsvinden.

In hoofdstuk 2 wordt de biologische behandeling van SSC's in continu systemen onder haloalkaline condities (pH 9.5; Na^+ 0.8 M) gedemonstreerd. De experimenten werden uitgevoerd in gas-lift reactoren, onder aerobe condities bij een temperatuur van 35 °C. Sulfide werd geheel verwijderd bij een belasting van 27 mmol L^{-1} dag^{-1}. Hierbij werd sulfide door haloalkalifiele sulfide oxiderende bacteriën (HA-SOB), behorende tot het genus *Thioalkalivibrio*, geheel omgezet in sulfaat (SO_4^{2-}). *Thioalkalivibrio* zijn extreme bacteriën, die in staat zijn om sulfide te oxideren binnen een breed spectrum aan haloalkaline condities (0.3 - 4.3 M Na^+ en pH tot 10.6). De resultaten van hoofdstuk 2 laten ook zien dat 93% van de benzeen (influent concentraties variërend van 100-600 µM) werd verwijderd door middel van luchtstrippen en biologische afbraak. Microbiële gemeenschap analyse toonde de aanwezigheid van haloalkalifiele heterotrofe bacteriën aan, behorend tot de genera *Marinobacter, Halomonas* en *Idiomarina*. Het is zeer aannemelijk dat deze bacteriën een rol hebben gespeeld in de verwijdering van benzeen.

SSC's kunnen, naast sulfide, ook methaanthiol (MT; CH_3SH) bevatten. Daarom is het van belang om kennis te vergaren over de mogelijke toxische effecten van deze organische zwavelverbindingen op de prestaties van dit nieuwe biotechnologische proces. MT kan onder zwavelvormende condities reageren met zwavel (S^0), waarbij een mix ontstaat van anorganische polysulfiden (S_x^{2-}), dimethyldisulfide (DMDS) en dimethyltrisulfide (DMTS). Uit respiratie experimenten met HA-SOB (*Thioalkalivibrio* mix) in hoofdstuk 3 blijkt, dat de biologische oxidatie tot S^0 wordt geremd met 50% (*Ki*-waarde), bij 0.05 mM MT. De *Ki*-waarden voor DMDS en DMTS waren respectievelijk 1.5 en 1 mM. Omdat in vergelijking met MT voor DMDS en DMTS een hogere *Ki*-waarde is gevonden, kan worden gesteld dat de reactie tussen MT en S^0 in een S^0-vormende bioreactor (ten dele) een detoxificerend effect heeft. Uit hoofdstuk 3 blijkt dat de behandeling van sulfide en MT bevattende zure gassen en SSC's in een biotechnologisch proces mogelijk is zolang MT, DMDS en DMTS niet accumuleren in de bioreactor. De accumulatie van MT kan voorkomen worden door auto-oxidatie van MT tot DMDS, de reactie tussen MT en S^0 of biodegradatie.

Hoofdstuk 4 richt zich op de biologische behandeling van (synthetisch aangemaakte) SSC's, die zowel sulfide als DMDS bevatten. Continue bioreactor-experimenten tonen aan, dat biologische sulfide oxidatie (4-10 mmol L^{-1} day^{-1}) mogelijk is bij de aanwezigheid van kleine hoeveelheden DMDS. De experimenten werden uitgevoerd in gas-lift-reactoren onder aerobe condities bij 35 °C. Sulfide werd geheel omgezet in SO_4^{2-} door *Thioalkalivibrio* (nauw verwant aan *Thioalkalivibrio* sp. K90-mix). Uit de bioreactor-experimenten bleek, dat een zeer sterke remming van de biologische sulfide oxidatiecapaciteit, en daarmee een sterke verstoring van het proces, optreedt bij effluent DMDS-concentraties tussen de 0.1 en 0.9 mM. De gemeten DMDS verwijdering was 40-70% (0.05-0.37 DMDS-S L^{-1} day^{-1}), waarbij 25% werd veroorzaakt door luchtstrippen. Vooralsnog is het echter onduidelijk welke andere processen hebben bijgedragen aan de totale verwijdering van DMDS. Aansluitend laten respiratie-experimenten zien, dat pure cultures van HA-SOB (*Thioalkalivibrio* sp. K90-mix en *Thioalkalivibrio sulfidophilus*) en bioslib, afkomstig van een waterstofsulfide verwijderingsinstallatie (*Thiopaq*), meer geremd worden door MT dan door DMDS. Daarnaast blijkt dat de *Ki* waarden voor MT en DMDS lager zijn voor *Thioalkalivibrio* sp. K90-mix en *Thioalkalivibrio sulfidophilus* dan voor *Thiopaq* slib. Uit de resultaten van hoofdstuk 4 volgt dat om een stabiel proces te garanderen de concentraties van MT en DMDS lager moeten zijn dan respectievelijk 0.02 en 0.1 mM. Dit betekent, dat de behandeling van SSC's met hoge concentraties MT en DMDS de sulfide oxidatiecapaciteit van het proces gemakkelijk kan verstoren. Auto-oxidatie van MT tot DMDS kan de detoxificatie van MT ten dele bewerkstelligen. Het is echter wel zo, dat de effluent concentraties van deze stoffen nog steeds erg laag moeten worden gehouden. De succesvolle behandeling van SSC's met een hoge concentratie MT en DMDS is om die reden afhankelijk van een biologische afbraak van deze stoffen. Indien er een snelle biologische afbraak van deze stoffen plaatsvindt, zullen de concentraties in de bioreactor beneden de kritische grens blijven.

In hoofdstuk 5 wordt een nieuw tweestaps proces voor de biologische behandeling van SSC's onder haloalkaline condities gedemonstreerd. Er wordt aangetoond dat een tweestaps proces een significant hogere efficiëntie van sulfideverwijdering bereikt dan een éénstaps proces. Met de detoxificatie van sulfide door de oxidatie tot thiosulfaat ($S_2O_3^{2-}$) tijdens de eerste chemische oxidatiestap en de daarop volgende biologische oxidatie tot SO_4^{2-} tijdens de tweede stap, kon 33 mmol S L^{-1} day^{-1} worden behandeld. Experimenten met synthetisch aangemaakte SSC's (pH 9.5; Na^+ 0.8 M) werden uitgevoerd in een continu systeem, bestaande uit een in serie geschakelde bubbel-kolom reactor en een gas-lift-bioreactor, onder aerobe condities bij 35 °C. Mathematische modellering van het tweestaps proces laat zien, dat onder de onderzochte condities een optimale reactor configuratie bestaat, bestaande uit 40% chemisch en 60% biologisch volume, terwijl het totale reactorvolume 22% kleiner is dan bij het éénstaps proces. De belangrijkste voordelen van een dergelijk tweestaps proces zijn een betere anticipatie op piekbelastingen van sulfide en lagere investering en operationele kosten door een kleiner totaal reactor volume.

Nader onderzoek zou zich kunnen richten op biologische afbraak van organische zwavelverbindingen door haloalkalifiele heterotrofe bacteriën. In aansluiting hierop zou het interessant zijn om onderzoek te verrichten naar de mechanismen die een rol spelen bij de toxiciteit van organische zwavelverbindingen op micro-organismen.

Acknowledgements

My PhD thesis is finished. An almost surrealistic but great feeling. During my PhD period many people helped and supported me to achieve this goal.

First of all Albert, who initiated the spent caustics project in Leeuwarden. Thank you Albert, for giving me the opportunity to work on this project and for your full support. Your commitment to this project, expertise, strong and honest feedback and your (incredible) fast responses and revisions meant a lot to me.

Since we wanted to know everything about sulfur bacteria, that in fact had the most difficult job, we started to cooperate with the Delft University of Technology. Thank you Gerard, for sharing your passion for microbiology and your expertise with me. I always felt very welcome to come to Delft for a good discussion and to make use of the lab facilities. In addition, I want to thank Ben for his help and technical support with the molecular analysis. It was really fun and very instructive to work with you! And thank you Dimitry. Your help with culturing haloalkaliphilic sulfide oxidizers and the discussions we had, were a very valuable contribution to this project.

The spent caustics team expanded with the arrival of Martijn B to Wetsus. To me, this was a very important and positive turning point in my PhD period. Thank you Martijn, for helping me with this project and for getting me focused. Also for your contagious enthusiasm and your friendship.

The technical team (Wim, Harrie, Harm, Jan T, Foppe and Ernst) helped me tremendously during my whole PhD period with everything from building and designing reactor set-ups to repairing all the stuff that "somehow" broke. Also the analytical team (Jelmer, Janneke, Mieke and Marianne) played an absolutely vital role in this project. All those many different (toxic) samples and new methods! I can honestly say that I could not have done it without all of you. Thank you for being so patient with my "gepruts" and especially for the many fun and exhilarating moments. In addition I want to thank Vinnie, Bert, Katja and Ardy for their technical support in Wageningen whenever needed!

Many thanks to you, Pim, for introducing me in the world of sulfur. During my stay in Wageningen you shared all your scientific knowledge with me while we worked as a team on several experimental set-ups. Thank you for your patience and friendship! I also want to thank Robin, for helping me out with many mercaptan related issues.

I was also very fortunate that 3 MSc students helped me with the many reactor runs and other experiment: Diwan, Rungnapha (Pom) and Pedro. I really enjoyed working with you and also learned a lot from you. Your hard work and persistency was very important in the realization of this thesis. I am also very proud that you, Pom and Pedro, started a PhD at Wetsus yourself.

I want to thank Cees, Johannes and Gert-Jan for giving me the opportunity to work at Wetsus. It was (and is) inspiring to be part of Wetsus, which became a successful scientific institute due to the efforts of you and the rest of the great staff. I think that the good facilities, endless possibilities and the joy that Wetsus offers to PhD students, is something to cherish. Especially, I want to thank Gert-Jan for supervising me the first couple of years. It was always very motivating to talk with you about the project since we share the same microbio-

logy background. Besides that your eye for detail and human insight is amazing. In addition, I want to thank the participants of the research theme "Concentrates" for the discussion and their financial support.

In general, I want to thank all my colleagues at Wetsus and especially all the PhD's and alumni for all the fun moments we had during and outside work. I would have liked to thank you all individually but I will need at least 100 more pages to describe all the individual memories I have. However, I do want to thank my colleagues/ friends whom I shared an office with more specific: Elsemiek, Kamuran, Urania, David, Lucia and Luciaan. Especially Elsemiek for being a like-minded person, her incredible sense of humor and the many talks we had. And David and Urania for the many fun moments and dinners!

I also want to thank all my colleagues at the sub-department of Environmental Technology of the University of Wageningen. Especially to Liesbeth and Anita for always helping me out on distance!

Thanks to everyone in the Talent program team where I have been working part-time since last year. What a great job, creating chaos in the classroom with cool water experiments.

I am also grateful to SNP-travel (especially Joyce and Martijn) for giving me the opportunity and support to guide groups through the most beautiful places in the world. The trips I did during my PhD period were always a very welcome energy boost!

I want to thank my paranymphs Kamuran and Jan. Thank you Kamuran for your support and your friendship. Your enthusiasm for almost everything that deals with life and science and your contagious humor! (And your interpretation of an Indian accent). And thank you Jan, Matlab wizard, for being who you are. A great colleague and friend. I think we are an excellent team and I am very grateful that we worked together!

I especially want to thank my closest friends (windsurfing and crossfit buddies, matties, brothers). With you guys I experienced so many special, inspiring and crazy moments. Many more moments to come!

I also want to thank Peter and Coby for their support, discussions and hospitality whenever I needed a place to sleep near Wageningen.

In addition, thank you Erik-Jan for helping me with the preparation (cover and layout) of this thesis.

Finally, I am so grateful to my family for their unconditionally support and love! And Renske, for her love, support at all times and the many beautiful (travel) moments!

Take care, Marco

Havelte, November 2012

About the Author

Iceland, August 2012

Marco de Graaff was born on the 29th of April 1978 in Leeuwarden, the Netherlands. After finishing High School (mavo, havo and vwo), he started studying biology at the University of Groningen. After finishing his first year at university, he worked and travelled for one year in Australia and China. In 2007, he graduated with specializations in marine biology and microbial ecology. The aim of his first thesis was to study the application of microorganisms for the treatment of oil-polluted aquifers. For his second thesis he went, amongst others, to Patagonia (Argentina) to study the effects of UV radiation on the physiology and community dynamics of marine microalgae. During his study he participated in the minor-programs: Science, Business and Policy (University of Groningen), Arctic Studies (Arctic Centre, Groningen) and Japanese Studies (Center of Japanese studies, Groningen).

From 2007 till 2011, he was employed by Wageningen University (sub-department of Environmental Technology) to work at Wetsus, which resulted in the redaction of this thesis. Currently he is working for the Talent-program of Wetsus, aiming to inspire and educate children and teenagers about watertechnology. Besides this, he works as an Alpine tourleader for SNP-travel and as a freelance researcher and consultant.

List of publications

This thesis:

van den Bosch, P.L.F., de Graaff, M., Fortuny-Picornell, M., van Leerdam, R.C., Janssen, A.J.H. (2009), Inhibition of microbiological sulfide oxidation at natronophilic conditions by methanethiol and methylated polysulfides, Applied Microbiology and Biotechnology 83(3):579-587

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Villafane, V.E., Janknegt, P.J. de Graaff, M., Visser, R.J.W., van de Poll, W.H., Buma, A.G.J., Helbling, E.W. (2008), UVR-induced photoinhibition of summer marine phytoplankton communities from Patagonia, Marine Biology 154:1021–1029.

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This work was performed in the TTIW-cooperation framework of Wetsus, Centre of Excellence for Sustainable Water Technology (www.wetsus.nl). Wetsus is funded by the Dutch Ministry of Economic Affairs, the European Union Regional Development Fund, the Province of Fryslân, the City of Leeuwarden and the EZ/Kompas program of the "Samenwerkingsverband Noord-Nederland". Funding was also provided by the participants of the research theme "Concentrates".

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- PCDI Post-doc retreat

Didactic Skills Training
- Supervising 3 MSc students
- Supervising Projects for several MSc students groups

Research Skills and Training
- Reactor operation / Respiration tests
- Molecular tools / DGGE / Cloning

Oral Presentations
- Biological treatment of spent caustics, Internal Wetsus Congress, 2007 and 2010, Leeuwarden, The Netherlands
- Biological treatment of spent caustics, TU delft-biotechnology group, March 2011, Delft, The Netherlands
- Biological treatment of spent caustics, Concentrate theme meetings, Wetsus, The Netherlands
- EMBO Workshop on Microbial Sulfur Metabolism, TU delft-biotechnology group, April 2012, Noordwijkerhout, The Netherlands

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Cover design by Erik-Jan Frieser