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MEMBRANE ASSISTED MICROAERATION FOR SULFIDE REMOVAL IN ANAEROBIC DIGESTION PROCESS

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Summary and thesis outline

ABSTRACT

The continuously increasing energy consumption rate, vital for overall economic development, coupled with the projected decline of fossil fuel stocks, predicts an unfavorable world situation. Renewable energies offer an opportunity to mitigate such potential crisis. Biogas, produced through anaerobic digestion is a feasible energetic alternative that has the potential to become a relevant actor in forthcoming renewable energy market. The quality of biogas obtained is a critical property that defines the applications where this biofuel can be applied. The presence of impurities may require a conditioning/treatment stage, providing the necessary quality standards for specific uses. Several up-grading processes are nowadays available, which are effective in removing impurities and increasing energetic value of this gaseous biofuel. However, implementation of these treatment stages may turn biogas production more complex and expensive. The possibility to intervene biogas production stage in order to promote a better biogas quality may same costs and facilitates later biogas use. This thesis explores novel alternatives to implement the micro aeration process. That process to consist in the application of small amount of oxygen and was implemented at different anaerobic reactors configurations, with the aim of hydrogen sulfide removal. This strategy was significantly reduced the content of the pollutant without to affect the yield of anaerobic process

TABLE OF CONTENTS

CHAPTER I

Introduction

1. INTRODUCTION

1.1. BIOGAS AS RENEWABLE ENERGY SOURCE

Expedite access to energy represents a key factor for the development of nations. Energy demand is under constant and continuous increase (Scarlat *et al*., 2017), greatly influenced by the development of highly populated and therefore energetic demanding countries (Miguez *et al*., 2006).

The International Energy Agency (IEA, 2020) estimates that oil demand for year 2021 will be 2.1 mb/d. On the other hand, the estimations on fossil fuel availability predicts that reserves for oil, coal and natural gas will start decreasing in 35, 105 and 37 years, respectively (Shafiee and Topal, 2009). It is clear then that availability of energetic resources will turn into increasing strategic elements; that will play a significant role in the political and socioeconomically decisions that future governments will have to take (Lefèvre, 2010).

An alternative to cope with the forthcoming energetic crisis is the development of renewable energy sources such as solar, wind, hydro, geothermal and fuels derived from biomass (Kåberger 2018, Handayani *et al* 2019). However, technologies enabling the production of renewable energies must be economically feasible, in order to have a share into an already extremely competitive energetic market (Destouni and Frank, 2019).

Several types of biofuels can be derived from biomass. Liquid biofuels such as biodiesel, bioethanol, biobutanol, biomethanol, pyrolysis oils, or gaseous biofuels such as synthesis gas (product of thermochemical processes), biogas or biohydrogen. Among the benefits of the use of biofuels are the reduction of fossil fuel utilization, reduction of greenhouse

emissions, employment generation (Destouni and Frank 2019, Moreno and Lopez, 2008), development of local capacities, decentralization of energy production and increase of energy availability in rural or isolated areas (Bruun, *et al.*, 2014, Demirbas, 2009).

Anaerobic digestion process has received important levels of attention, since biogas production represents an interesting source of renewable energy, which can contribute to the goal of decreasing our dependence from fossil fuels (Bruun *et al* 2014, Arthur *et al*, 2010, Stewart *et al*., 1995). Indeed, nowadays anaerobic digestion in more commonly regarded as an energy production process than just a waste treatment technology. Biogas can be used for various applications, including: generation of heat/steam for industrial use (Capodaglio *et al* 2016, Holm-Nielsen *et al*., 2009), co-generation of heat and electric power (Wu *et al*, 2016, Kang *et al* 2014, Gebrezgabher *et al*., 2010; Walla and Schneeberger, 2008), fuel for home use (Raboni and Urbina 2014, Holm-Nielsen *et al*., 2009; Lantz *et al*., 2007), vehicule use (Scarlat *et al*. 2017, Osorio and Torres, 2009; Lantz *et al*., 2007), fuel cells substrate (Wu *et al*, 2016, Papurello *et al* 2016, Holm-Nielsen *et al*., 2009).

Anaerobic digestion is nowadays an established technology in several parts of the world. For example, the European Community has identified anaerobic technology as a powerful technology that can contribute to achieve the commitments described in the Kyoto agreement, as a reducing agent of the emissions of greenhouse gases. In 2015, the EU produced 18 billion m^3 methane, which represents half of world biogas production (Scarlat *et al*, 2019). Moreover, biogas market is expected to positively develop in the near future. According to Global Information Inc, (2019) the global market for biogas will have an increment of almost 6% by the year 2023

Anaerobic digestion is a process that converts organic matter into biogas, a mixture of methane (CH_4), carbon dioxide (CO_2), and a marginal proportion of other gases (Meier *et al* 2017, Rasi *et al*., 2007). Biogas represents a renewable energy carrier that can be used for different purposes, and can be produced from a wide variety of sources of organic matter, including biomass or energy crops. This is not the case of ethanol or biodiesel, that require appropriate substrates such as carbohydrates and lipids, respectively. Anaerobic technology can be then integrated into chains of production, linked to the transformation of waste biomasses, positively impacting the cost structure of business, and consequently generating a greater competitiveness in several sectors (Neumann and Jeison 2015, Jeison 2015, Yiridoe *et al*., 2009, Albertson *et al*., 2006, Chynoweth *et al*., 2001). Among the many environmental benefits that this technology offers, one could include: mitigation of greenhouse gases by biogas utilization (Moraes *et al* 2017, Bruun *et al* 2014; IPCC 2014), reduction of odors from waste (Appels, *et al*, 2008; Demirer and Chen, 2005), pathogen reduction (Côte *et al*, 2006), increase of the sustainability of other biofuels (Neumann and Jeison 2015; Power and Murphy, 2009; Stoeglehner and Narodoslawsky, 2009).

Biogas produced by anaerobic digestion usually contains the following compounds:

CH_4: It is a hydrocarbon, the main component of biogas. It can be fornd in biogas at concentrations ranging between 50 and 70% v/v. This compound is a potent greenhouse gas, that may contribute to global warming if discharged into the atmosphere (IPCC, 2001). It has a calorific value of 38 MJ/kg, and can be used as fuel.

CO_2: It is the most oxidized for of carbon, its content in biogas varies between 30 and 50% v/v.

H_2S: Hydrogen sulfide can reactive with most metals, causing corrosion in compressors, gas storage tanks and engines. Its reactivity is enhanced by concentration and pressure, the presence of water and elevated temperatures. It can be found in biogas usually at concentrations below 1 % v/v. (Camiloti *et al.,* 2018, Persson *et al.,* 2006, Rasi *et al*., 2007)

NH_3: The combustion of ammonia leads to the formation of nitrous oxide (NOx), precursors of acid rain and also is a greenhouse gas (Kobayashi *et al.;* 2019). Then, when preset in biogas at high concentrations, this compound compound must be removed.

Halogenated and Organic silicon compounds: During the combustion process the halogens (e.g. carbon tetrachloride, chlorobenzene, chloroformand trifluoromethane) compounds are oxidized. Additionally, in presence of water, can cause corrosion in downstream pipes and equipments. They can eventually generate dioxins and furans (Persson *et al.,* 2006).. In some cases, biogas has to be treated to remove these compounds, since they can be converted into inorganic siliceous deposits, causing serious damage in engines..

Depending on the substrates used, biogas can presents big variations on its composition. Depending on its use, some components may need to be removed. Table 1.1 presents guidelines regarding the content of CO_2, H_2S an NH_3 required for same biogas used, for different applications.

Table 1.1. Requirements for biogas treatment depending on its energetic use

Para-meter	Content in biogas	Fuel Cell	Vehicle Use	Power-Heating co-generation	Electricity Generator (engine or turbine)	Introduction into natural gas grid	Heating
CO_2	30-50 % v/v	O.R. (MCFC)	<3% v/v (ISO)	O.R. (Cirne *et al*., 2008)	O.R (Cirne *et al*., 2008)	< 6% v/v (Ch1) <6% v/v (Ch2) <6% v/v (G) <2% v/v (F)	O.R. (Cirne *et al*., 2008)
H_2S	0-1 % v/v	$6.5*10^{-7}$ %v/v (MCFC)	0.003 %v/v (Sw)	<0.05% v/v (D&S, 2008)	<0.05% v/v (D&S, 2008)	<0.003% v/v (S) <0.003% v/v (s)	<0.05% v/v (D&S, 2008)
NH_3	< 100 ppmv					<29 ppmv (Sw)	

G: German standard G260/G262; Sw: Swedish estandar SS 15 54 38; ISO: "Green gas" for vehicle ISO/DIS 15403, Ch1 : Swiss national standard for unlimited gas injection; Ch2: Swiss national standard for limited gas injection; F: French national regulation for gas injection; MCFC: Molten-carbonate fuel cell; O.R.: Optional removal,; D&S, 2008: Deublein and Steinhauser, 2008.

Therefore, for many biogas applications, upgrading technologies need then to be applied, involving further processes and therefore costs. However, biogas composition may be manipulated, within some ranges, by means of applying different operational strategies. Such approach, if successful, would reduce the needs of upgrading processes, increasing the economic feasibility of biogas production as renewable energy carrier. Such approach may be applied when a reduction in the H_2S content is required. Sulfide is very reactive compound, and conditions may be provided within the reactor in order to induce its transformation is compounds that do not jeopardize the energetic use of biogas.

When it comes to the reduction of biogas content of H_2S, one option could be the implementation of a pre-treatment stage, involving precipitating the oxidized sulfur compounds, and thus preventing sulfur to enter the digester. Another option is the expose the H_2S to oxidizing agents after or during the anaerobic process. These can be either biological or chemical, and may be used to oxidize the sulfide and then to separate the insolubilized sulfur forms the liquid media (Jeníček1 *et a.,l* 2017, Krayzelova *et al.,* 2015).

1.2. ALTERNATIVES TO REDUCE BIOGAS HYDROGEN SULFIDE

Sulfate is present in may waste(waters) generated by wide variety of production activities, such as fermentation, seafood processing, tannery, edible oil refinery, among others (Cirne *et al*., 2008; Sabumon, 2008; Liamleam and Annachhatre, 2007, Lens *et al*., 1998). Sulfate, is usually considered as an environmentally benign compound. It is nonvolatile, nontoxic, chemically inert and very abundant in nature (Silva *et al*., 2002).

The presence of oxidized sulfur species in organic waste causes negative effects on anaerobic digestion. This is the result of the activity of a group of microorganisms presents in anaerobic consortia known as sulfate-reducing bacteria (SRB). The SRB uses oxidized sulfur compounds as electron acceptors, reducing them to hydrogen sulfide. Hydrogen sulfide is toxic, cause corrosion of steel and concrete, increases the chemical oxygen demand (COD), and is responsible for odor problems sometimes associated with anaerobic digestion (Krayzelova *et al* 2015, van der Zee *et al*., 2007, Janssen *et al*., 1998).

Presence of sulfate in wastes induces competition between methanogenic (MB) and sulfate reducing bacteria, since the SRB can metabolize H_2, and acetate and other intermediate products of anaerobic digestion, such as volatile fatty acids, methanol and ethanol (Jeong *et*

al., 2008; Liamleam and Annachhatre, 2007, Wang and Banks, 2007). Considering that SRB do not have the thermodynamic limitations of acetogenic bacteria (Table 2.2), and the fact that they have a respiratory metabolisms, they usually succeeds such competition. This result, in most cases, in complete sulfate reduction. .

Table 1.2. Stoichiometry and change of free energy ($\Delta G'_0$) of hydrogen and acetate conversion under different conditions (Adapted from Zeeman, 1998)

Reaction			$\Delta G'_0$ (Kj/mol substrate)
Sulfate reducing bacteria			
$H_2 + ¼\ SO_4^{2-}$	→	$¼\ HS^- + H_2O$	-9.5
$CH_3COO^- + ¼\ SO_4^{2-}$	→	$HS^- + 2HCO_3^-$	-48
Methanogens			
$H_2 + ¼\ HCO_3^-$	→	$¼\ CH_4 + ¾\ H_2O$	-8.5
$CH_3COO^- + H_2O$	→	$CH_4 + 2HCO_3^-$	-31

H_2S is toxic for both MB and SRB. Such toxicity has been reported to be related with variation of intracellular pH due to sulfur assimilation (Cirne *et al*., 2008). Reported inhibition concentrations are between 83 and 550 mg/L (Rinzema and Lettinga, 1988; Reis *et al*., 1992; MacCartney and Oleszkiewicz, 1993; Montalvo and Guerrero, 2003). A value of COD/sulfate ratio below 10 is normally considered as a condition promoting a substantial generation of H_2S (Velasco *et al*., 2008; O `Reilly and Colleran, 2006; Valdes *et al*., 2006, Speece, 1996) and a competition between the BSR and BM (Sabumon, 2008).

Since biogas is in contact with the liquid phase within the reactor, part of the produced H_2S will be transferred to the biogas, producing operational problems such as odors and corrosion (Dawoud *et al.*, 1992). As already commented, H_2S presence in the biogas

restricts its direct use as fuel engine, and can actually damage the digesters, distribution lines and equipment. The usual H_2S content of untreated biogas is between 0 and 0.5% v/v, being heavily dependent on the composition of substrate (sulfate content).

Removal of H_2S from the biogas can be achieved by contact with chemical agents (Al Mamun and Torii 2015, Osorio and Torres, 2009). Such process is usually performed in packed columns or spray mist systems. Zinc oxide, carbon activated, zeolites or other traditional commercial adsorbents can be used for such purpose (Morgan-Sagastume. and Noyola, 2006; Yuan and Bandosz, 2007; Truong and Abatzoglou, 2005; Cosoli *et al*., 2008). Biological processes have been also commercially developed, based on the bio-H_2S oxidation activity of organisms such as *Thiobacillus* (Chung *et al*., 1996; Nishimura and Yoda, 1997; Chung *et al*., 1997; Oyarzún *et al*., 2003; Lee *et al*., 2006). Table 1.3 compares the various sulfur removal technologies.

Thiobacillus are autotrophic and can use the CO_2 present in the biogas as a carbon source to oxidize the H_2S to elemental sulfur and sulfate, using oxygen as electron acceptor, as described in Figure 1.1. Even though there are several technologies on the market having the capacity to efficiently remove H_2S from biogas, their implementation increases complexity of biogas production facilities, increasing biogas associated costs (Diaz *et al.*, 2015). Then, alternatives promoting the management or control of the H_2S net production within the digester have the potential to be a more suitable alternative.

Table 1.3. Comparation of sulfide removal technologies

Technique for H_2S removal	Efficiency	Capital Cost	Operational Cost	Complexity
Chemical Agents (Iron oxide; Zinc Oxide)	High	Medium	High	Medium
Activated Carbon	High	Medium	High	High
Biological oxidation	High	High	Low	High
Microaeration (Anerobic digestion of slurry)	High	Low	Low	Medium

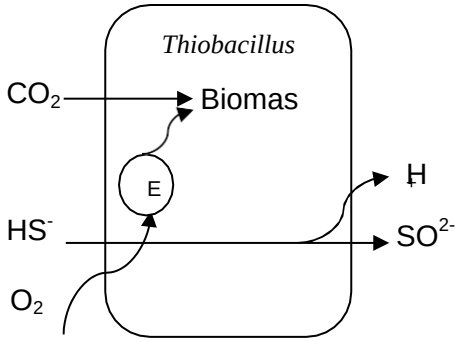

Figure 1.1. Scheme of oxidation of sulfide by *thiobacillus.*

When considering in-situ control of H_2S production in an anaerobic digester, 2 options may be considered: preventing the formation of H_2S, or the transformation of the produced H_2S into a harmless compound.

First alternative could be addressed by controlling the rate of sulfate reduction. This may be accomplished by inhibiting biomass responsible for the sulfate reduction itself. This could be implemented by the use of specific inhibitory agents such as molybdate (Ranade *et al*., 1999; Isa and Anderson, 2005). However, there are obvious financial and practical constraints to implement such alternative. The transformation of the produced H_2S seems to be then a more suitable alternative. This may be done by the use of iron salts, to promote the oxidation of the H_2S within the digester (Gutierrez *et al.*, 2010; Speece, 1996). However, this option would not be practical, since it would require continuous dosage of big amounts of chemicals. Moreover, long-term feasibility would be also doubtful as a result of a reduction of the effective volume of the reactor, due to accumulation of inert solids. A more convenient way to induce H_2S transformation is the addition of compounds that may promote specific changes in the redox potential, to induce sulfide oxidation, without affecting the anaerobic digestion process.

A way to increase redox potential is the addition of nitrate or nitrite, which can be obtained from, a nitrifying reactor. These compounds are highly soluble in aqueous medium (Diaz *et al* 2010, Cirne *et al*., 2008). The use of nitrate and nitrite to promote sulfide oxidation has been previously reported (Franke *et al* 2016, Diaz *et al* 2010, Cirne *et al*., 2008, Jing *et al*., 2009, Lu *et al*., 2009, Wang *et al*., 2009, Mahmood *et al*., 2007; Vaiopoulou *et al*., 2005; Reyes-Avila *et al*. 2004). Reports from Lens *et al*. (Lens *et al*., 2000) and Reyes-Avila *et al*. (Reyes-Avila *et al*., 2004) indicate that under conditions of high concentrations of sulfur and electron donating compounds -nitrate or nitrite-, nitrate/nitrite are reduced via denitrification through a mixotrophic mechanism (heterotrophic and autotrophic). Technologies have been developed based on these principles, such as denitrifying ammonium oxidation (DEAMOX), which uses autotrophic microorganisms, and involves the Ammonium Anaerobic Oxidation (ANAMOX) process and the reduction of nitrate to nitrite by sulfur oxidation. DEAMOX process, reported by Kalyuzhnyi *et al*., (2006a, 20006b) consists of an initial stage where organic nitrogen is oxidized under anaerobic conditions, followed by ammonia oxidation to nitrate, in a nitrifying reactor. By injecting a stream rich in sulfur, nitrate generated in the first phase is reduced to nitrite using electrons donated by the sulfur. Finally, both nitrite and ammonia generated in the earlier phases are removed by the activity of the ANAMOX biomass.

However, probably the most obvious way to promote sulfide oxidation is the simple addition of oxygen. By injecting air or oxygen in the recirculation line or in the headspace of a digester, it is possible to vary the redox potential and consequently create conditions

promoting H_2S oxidation, enabling a simple technological solution for sulfide removal (Krayzelova *et al* 2015, Cirne *et al*., 2008; van der Zee *et al*., 2007, Jansen *et al*., 1998).

The controlled doses of oxygen into an anaerobic digester is normally knows as micro-aeration (Diaz *et al.*, 2015; Diaz *et al.*, 2014; Zhu *et al*., 2009; Johansen and Bakke, 2006; Tang *et al*., 2004) or micro-oxygenation (Khanal and Huang, 2003; Janssen *et al*., 1998). Oxygen can enter to the reactor in different parts and in different ways. Some of the alternatives are presented in Figure 1.2 and Table 1.4

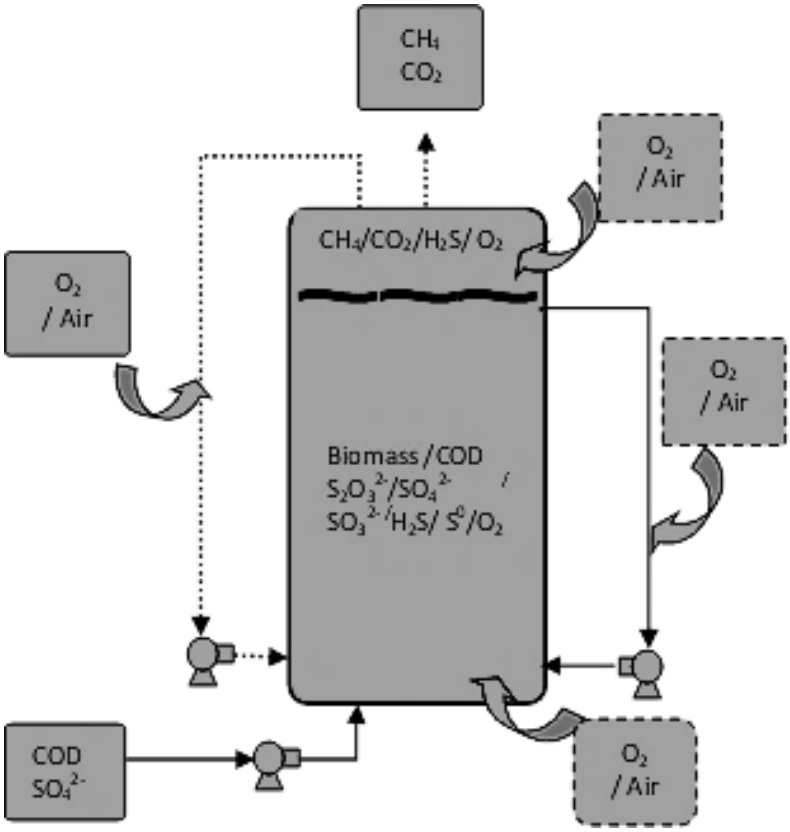

Figure 1.2. Different ways for oxygen up-take

Table 1.4. Effect of oxygen supply strategies in anaerobic process on biogas quality

O2-Source	Reactor system	Substrate	OLR ($mg\ COD\ L^{-1}\ d^{-1}$)	SLR ($mg\ S\ L^{-1}\ d^{-1}$)	HRT (day)	Injection Point	Redox Potential (mV)	% Removal H2S (Liquid phase)	Biogas Quality $CH_4/CO_2/H_2S/N_2/O_2$ (% v/v)	References
Air	FBR	Synthetic	6	1.3 mmol	5	IR	NR	66%	NR/NR/< 0.02 /NR/NR	van der Zee *et al.*, 2007
Air	ASD	Sludge	~ 52		20	HS	-515 to -492	97	62.8/35.3/0.0/0.4/1.5	Diaz *et al*,. 2008
O2	ASD	Sludge	~ 66		20	HS	-510 to -480	97	58.8/34.5/0.0/5.7/1.0	Diaz *et al*,. 2008
Air	CSTR	Sludge	NR		20	IR/LR	NR	NR	NR/NR/< 0.02 /NR/NR	Fdez.-Polanco *et al.*, 2009
O2	CH	Synthetic	0.6		15	BR	-280 to -180	100%	NR/NR/0.0 /NR/NR	Khanal and huang, 2003
Air	GCD	Synthetic MSW	NR	NR	15	IR	NR	99.28 %	50/NR/< 0.02 /NR/NR	Tang, 2004
Air	UHSR	Synthetic	2.5 - 3.75	1.9 2.85	1	IR	-225	> 80 %	NR	Sabumon, 2008
Air	CSTR	Sludge		6500	20	HS	NR	> 99%	58.6±1.5/34.5±1.7/0.00/5.7±1.4/1±0.5	Diaz *et al*, 2010
O2	CSTR	Sludge		7200	20	HS	NR	> 99%	62.8±0.8/35.3±0.9/0.00/0.4±0.2/1.5±0.2	Diaz *et al*, 2010
O2	UASB-MABR	Sybthetic	6			MABR	NR	90	54.2±14.7/34.2±11.7/<2/NR/NR	Camiloti *et al*, 2018
Air	AFSBR	Synthetic	3.5	0.17	0.9	MIR	NR	> 99 %	NR	Valdés *et al*, 2016

OLR: Organic Loading Rate; **SLR:** Sulfur Loading Rate; **FBR**: Fluidized Bed Reactor; A**FSBR**: Anaerobic Fixed Structured Bed Reactor; **ASD**: Anaerobic Sludge Digester; **CSTR:** Continuous Stirred-Tank Reactor; **CH:** Chemostat; **GCD:** Gas Circulation Digester; **UASB**: Up-Flow Anaerobic Sludge Bed; **UHSR**: Up-flow Hybrid Sulphidogenic Reactor; **MABR**: membrane aerated biofilm reactor, **MSW:** Municipal Solid Waste; **IR**: Into Reactor; **HS:** Headspace; **LR**: Liquid or Sludge Recirculation; **BR**: Biogas recirculation; **MIR**: Membrane Into Reactor; **NR**: No Reported.

Oxidation of hydrogen sulfide promoted by micro-aeration have been reported to be the result of both chemical and biological phenomena. A micro-oxic environment should triggers microbial population redistribution associated with a possible appearance of sulfur oxidizing microorganisms, affecting the phylogenetic diversity of the digester (Valdes *et al.*, 2017, Tang *et al*., 2004). The possible biological mechanism is mediated by micro-biological chemotrophs that has the ability to biologically oxidize H_2S. For both, chemical and biological mechanisms, process can be controlled in order to produce elemental sulfur (Krayzelova *et al.*, 2015, Diaz *et al.*, 2015, Diaz *et al.*, 2014, Cirne *et al*., 2008), instead of more oxidized compounds such as thiosulfate, sulfite and sulfate (Steudel *et al*., 1996). Studies conducted by Tang *et al* (2004) and Gonzalez-Sanchez and Raveh (2007) suggests that chemical mechanisms for H_2S oxidation would prevail, since the same results are achieved aerating a system with and without the presence of active microorganisms. However, this matter is not fully elucidated.

It is widely accepted that anaerobic digestion of complex substrates is a process that takes place in stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Parker, 2005; Batstone *et al*., 2000a; Batstone *et al*., 2000b). The controlled incorporation of oxygen would triggers aerobic metabolisms in facultative bacteria, increasing the amount of hydrolytic exo-enzymes, which ultimately leads to an enhancement of the hydrolysis and acidogenesis steps (Zhu *et al*., 2009, Johansen and Bakke, 2007). Such condition may lead to a reactor acidification, as was observed by different authors (Jagadabhi *et al*., 2010, Zhu *et al*., 2009).

Considering that methanogenic bacteria are strictly anaerobic, an uncontrolled increased of redox potential may severely and irreversibly reduced the activity of such organisms. Therefore, a controlled dosage of oxygen is required. Therefore, such effects should be considered when designing a system-oriented biogas production, because it may create a compromise between H_2S reduction and a reduction of biogas yield.

Previous antecedents indicate that microaeration has the potential to promote oxidation of sulfide, reducing the requirements of biogas treatment when its use as biofuel is on interest. However, oxygen dosage needs to be done in such a way that it does no disturb the anaerobic digestion process. This thesis studies the use of semipermeable membranes as a way to dose the oxygen required for sulfide oxidation.

1.3. HYPOTHESES

Considering that:

- The biogas cleaning is needed for her energetic use; in particular the sulfide removal is key for her utilization.
- The elimination of pollutants of biogas is a complex and expensive stage in the overall anaerobic process.
- The microaeration is a probed strategy for sulfide removal in sludge anaerobic digestion.
- In anaerobic reactors whit small head space, ie, UASB reactor must be to development a strategy of oxygen dosage in liquid phase.

The Following hypothesis is proposed:

Implementation of membrane-assisted oxygen transfer in anaerobic reactors for wastewater treatment can promote condition compatible with the removal of the sulfide generated by sulfate reduction

1.4. GENERAL OBJECTIVE

To determine the feasibility of reducing the content of H_2S of the biogas, by inducing the development of sulfide oxidizing bacteria, as a result of a strategy based on micro-aeration of anaerobic reactors assisted by membranes.

1.5. SPECIFICS OBJETIVES

1.5.1. To evaluate the effect of micro-aeration on the H_2S content in the biogas, elucidating the biological and/or chemical nature of the oxidative process.

1.5.2. To develop and validate a strategy for micro aerate anaerobic digester in order to promote sulfide oxidation in Up-flow Anaerobic Sludge Blanket (UASB) reactors, by using membrane to transfer oxygen

1.5.3. To evaluate the micro-aeration strategy assisted by membrane for H_2S removal in Anaerobic fixed-structured bed reactor (AFSBR).

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CHAPTER II

SULFIDE ABIOTIC OXIDATION ASSAYS

2. SULFIDE ABIOTIC OXIDATION ASSAYS

2.1. INTRODUCTION

The most reduced form of sulfur in the environment is the sulfide. When set in contact with an oxidant, like oxygen, it can be oxidized to forms like polysulfide, sulfite, thiosulfate and sulfate (Camiloti *et al.,* 2018). This oxidative process is very important in anoxic systems, like sediments and sea bottom (Luther *et al.,* 2011). It is also reported in micro aerobic digesters when micro-aeration is applied, and when sulfide oxidizing bacteria (SOB) are present (Krayzelova *et al.,* 2015; Valdés *et al.,* 2016; Jenicek *et al.,* 2017 and Camiloti *et al*., 2018). In abiotic conditions this process occurs spontaneously, and its main reaction products are elemental sulfur and sulfate (Dodds and Whiles, 2010).

Jorgensen (1994) reported the existence of sulfur abiotic oxidation at the presence of dissolved oxygen. However, the sulfate reduction process resulting from microbial action would occur more rapidly. This is consistent with results reported by Bernhard *et al*. (2000) regarding the accumulation of reduced sulfur species in the sediments.

Previous data reveals that both chemical and biochemical phenomena may be involved in the oxidation of sulfide in anaerobic digesters operating under micro-aeration conditions. Therefore, experiments were performed in order to elucidate the potential contribution of abiotic sulfide oxidation. Table 2.1 presents the forms of inorganic sulfur, along with their oxidation state.

Table 2.1. Oxidation state of inorganic sulfur compounds

Oxidation state	-2	0	+4	+6
Compounds	H_2S	S_0	SO_2	SO_3
			SO_3^{2-}	SO_4^{2-}

Equations 2.1, 2.2 and 2.3 show the typical oxidative reactions involving oxygen and sulfide.

$$H_2S + \frac{1}{2}O_2 \leftrightarrow S^0 + H_2O \qquad \text{Equation 2.1}$$

$$H_2S + \frac{3}{2}O_2 \leftrightarrow SO_2 + H_2O \qquad \text{Equation 2.2}$$

$$H_2S + 2O_2 \leftrightarrow SO_3 + H_2O \qquad \text{Equation 2.3}$$

2.2. METHODOLOGY

Oxidation tests of H_2S were performed under batch and continuous conditions. Assays were included involving only a gas phase, and a system gas-water, in order to observe the products of the oxidation reaction of H_2S. In the case of batch tests, they were performed at different molar ratios of H_2S and O_2, to elucidate the reaction mechanism of the oxidative process.

2.2.1. Batch abiotic oxidation assay of biogas sulfide in gas phase:

An Up-flow Anaerobic Sludge Blanket (UASB) reactor was implemented to provide sulfide-enriched biogas. The reactor was fed with diluted wine and sulfate. Initially, bottles of 125 mL capacity were only filled with biogas rich in sulfide and oxygen, and then biogas rich in sulfide, water, and oxygen. After vigorously shaking the vials, the composition of the resulting atmosphere was measured in a 10 min lapse.

Table 2.2 shows the conditions for each assay, in terms of amount of water and the molar ratio nH_2S/nO_2.

Table 2.2. Experimental set for abiotic sulfide oxidation assays in batch mode

Test Number	Water volume (mL)	molar ratio nH_2S/nO_2
1	0	0.5
2	0	1.0
3	0	2.0
4	0	8.0
5	0	16.0
6	6.25	0.5
7	6.25	1.0
8	6.25	2.0
9	12.5	0.5
10	12.5	1.0
11	12. 5	2.0

2.2.2. Continuous mode assay for abiotic oxidation of sulfide.

An assay was carried out, involving continuous fed to a 0.5 L glass reaction chamber of air and H_2S aqueous solutions. Figure 2.1 schematically represents the assay, during the necessary time for a steady state to be reached. System was operated at 3 hydraulic residence times: 6.25, 4.17, and 3.13 min, using a concentration of 100 mg $H_2S.L^{-1}$ and a molar ratio (nO_2 / nH_2S) of 1.1 in each of them.

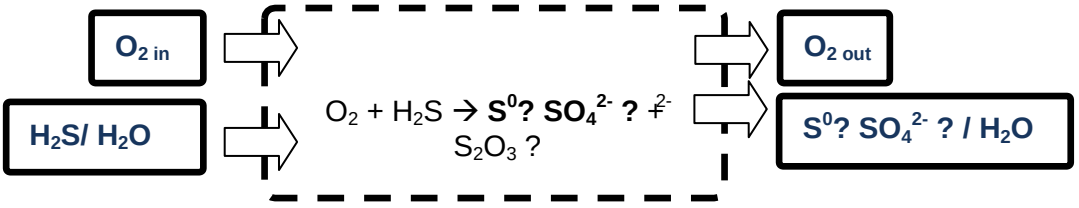

Figure 2.1. Scheme of oxidation test of H_2S with O_2

Once steady state is achieved, the following equations represent H_2S mass balance in the liquid phase:

In this case the involving mechanism is:

$H_2S_{(ac)} \leftrightarrow H_2S + HS^-$ (pKa ~ 7) Equation 2.4

$H_2S + OH^- \leftrightarrow HS^- + H_2O$ (Alkaline environment) Equation 2.5

$HS^- + \frac{1}{2}O_2 \leftrightarrow S^0 + OH^-$ Equation 2.6

$H_2S + \frac{1}{2}O_2 \leftrightarrow S^0 + H_2O$ (Neutral and/or acid environment) Equation 2.7

From a mass balance in steady state for H_2S, have:

$\frac{dH_2S_{in}}{dt} = -rH_2S + \frac{dH_2S_{out}}{dt}$ Equation 2.8

Assuming a first order kinetics:

$\frac{dH_2S_{in}}{dt} = K[H_2S] + \frac{dH_2S_{out}}{dt}$ Equation 2.9

Or

$K[H_2S] = \frac{dH_2S_{in}}{dt} - \frac{dH_2S_{out}}{dt}$ Equation 2.10

Considering two possibilities

Sub-stoichiometric oxygen

$K[H_2S] = \frac{dH_2S_{in}}{dt} - \frac{dH_2S_{out}}{dt}$ Equation 2.11

Super-stoichiometric oxygen

$K[H_2S] = \frac{dH_2S_{in}}{dt}$ Equation 2.12

Based on the equations above, for the liquid phase, considering volume of reactor, dilution factors, mass transfer process and a super stoichiometric amount of oxygen, the following equation is reached

$$\frac{dH_2S}{dt} = FC_{H_2S(in)} - VrH_2S + FC_{H_2S} - K_L\left(-C_{H_2S+}C^*_{H_2S}\right) \quad \text{Equation 2.13}$$

Where:

$\frac{d(H_2S)}{dt}$: Hydrogen sulfide consumption rate

F: Dilution factor

$C_{H_2S_{(in)}}$: Concentration of inlet aqueous sulfide

V: Reactor volume

r_{H_2S} −: Reaction rate

C_{H_2S}: Concentration of aqueous sulfide

$K_L\left(-C_{H_2S+}C^*_{H_2S}\right)$: Mass transfer component

Figure 2.2 shows a diagram explaining the microreactor setup and a photograph of the implementation of assay

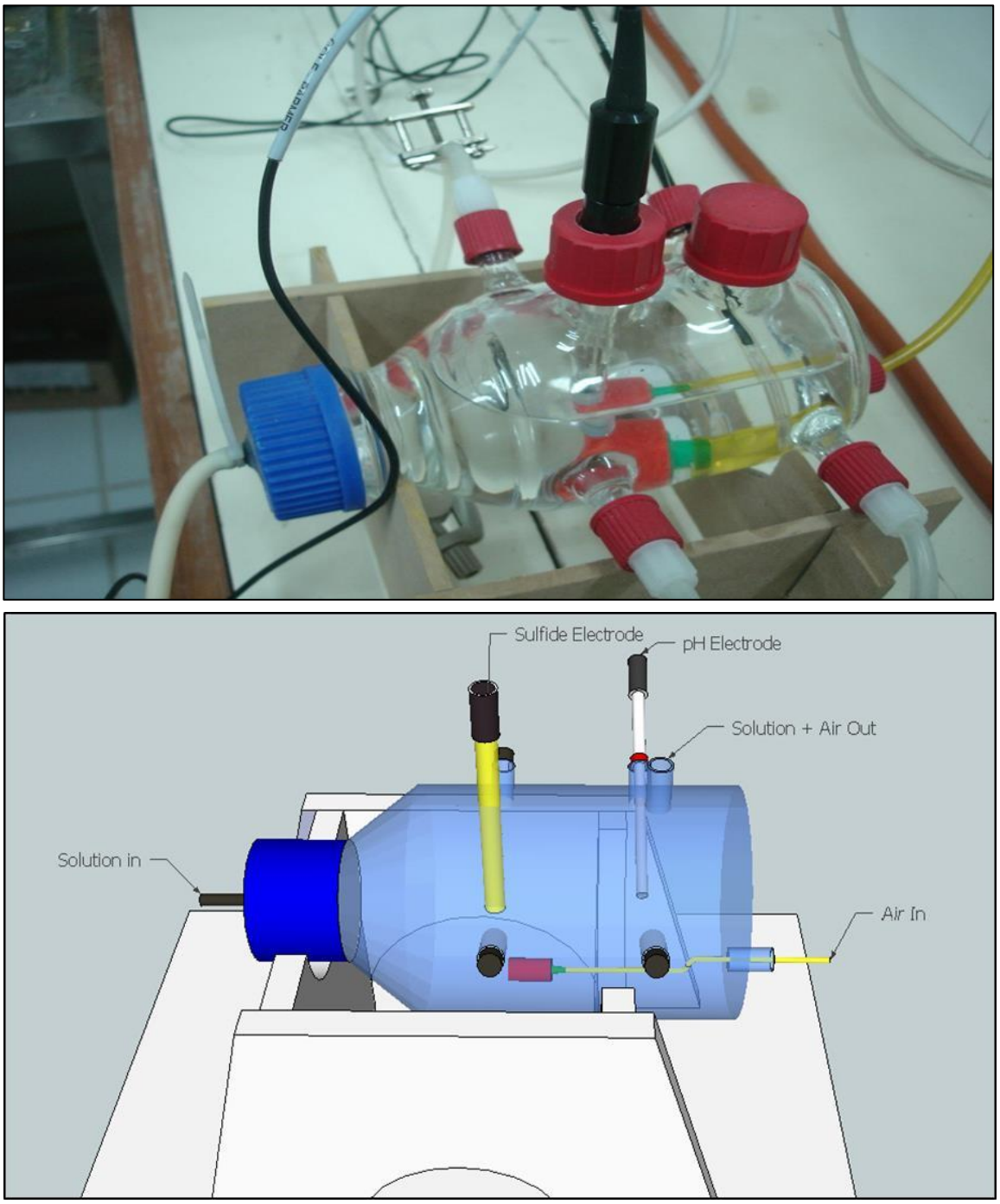

Figure 2.2.- Mini-reactor for continuous abiotic assays

2.2.3 Analytical methodology

Biogas H_2S content was determined using Rae System colorimetric columns. H_2S concentration in water was measured using an ion selective electrode (Orion, Thermo Scientific 9616 BNWP). Gas volume was measured by liquid displacement.

2.3. RESULTS AND DISCUSSIONS

Figure 3.3 shows batch tests results, involving contacting sulfide and oxygen in a gas phase (biogas) (assays 1-5 from Table 3.2). Data analysis indicate that the addition of oxygen to a biogas rich in H_2S produces only a dilution effect on the sulfide content. In other words, no reaction takes place between sulfide and oxygen.

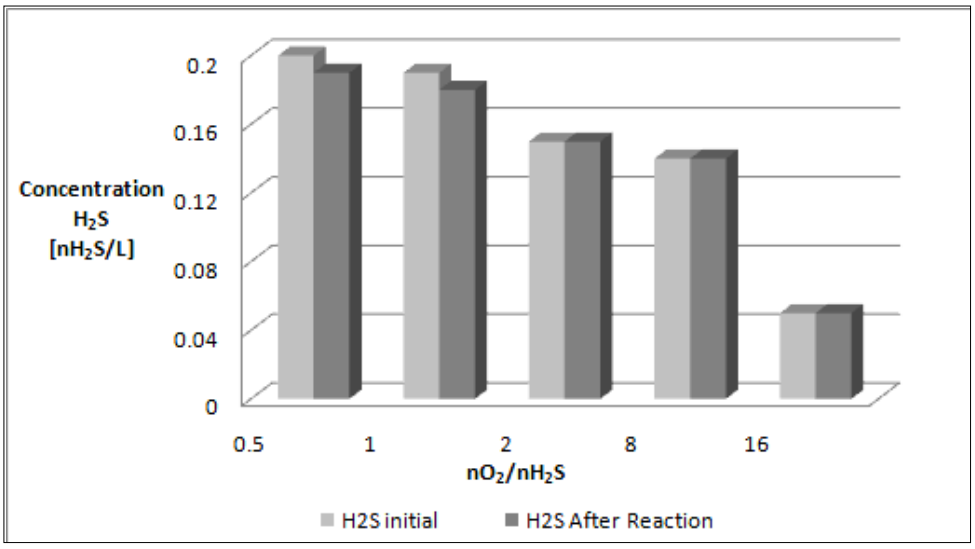

Figure 2.3. Effect of contacting sulfide and oxygen in gas phase

Figure 3.3 shows batch tests results, involving contacting sulfide and oxygen in a gas phase (biogas) (assays 1-5 from Table 3.2). Data analysis indicate that the addition of oxygen to a biogas rich in H_2S produces only a dilution effect on the sulfide content. In other words, no reaction takes place between sulfide and oxygen.

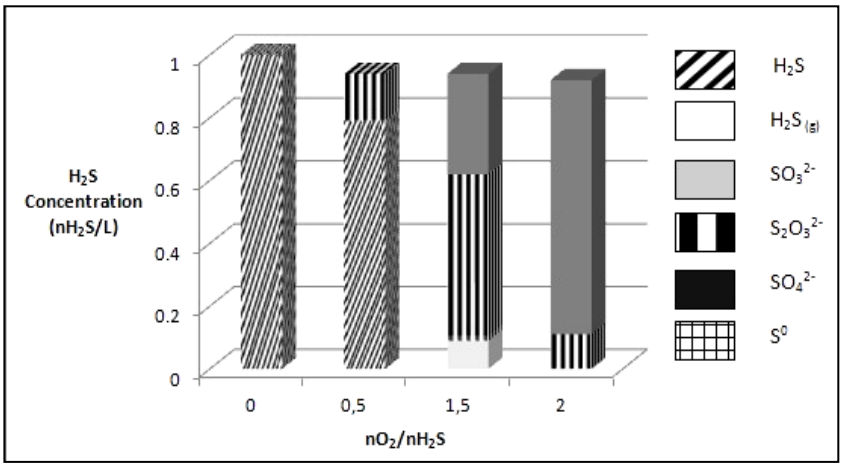

Figure 2.4. Oxidized forms of sulfur in abiotic essays, 5 % deaerated water in bottle

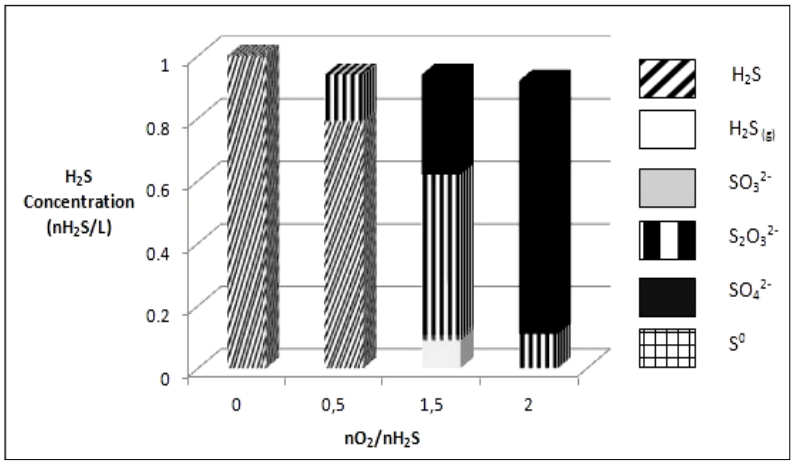

Figure 2.5. Oxidized forms of sulfur in abiotic essays, 10 % deaerated water in bottle

Results show that H_2S oxidation present in the biogas under abiotic conditions does not occur in the gas phase, necessarily requiring the presence of water to take place. This indicates that the reaction occurs in the aqueous phase.

Accepting that the oxidative process occurs only in the presence of water (under abiotic conditions), formation products of oxidative reaction can be identified comparing the H_2S consumption with Equations 2.1, 2.2 and 2.3. Such analysis indicates that the oxidative reaction would produce mainly $S_2O_3^{2-}$, and eventually SO_3 when an excess of oxygen is provided. Apparently, the product of most interest, elemental sulfur, was not produced.

Table 2.3 shows the result of the continuous assays. Data analysis shows significant removal of H_2S. Sulfide was not detected in the liquid phase, neither in the gas phase leaving the reaction vessel. This indicates a high reaction rate that may validate the assumption of an instantaneous reaction.

Table 2.3. Removal of H_2S at different Hydraulic Residence Time

HRT (min)		6.25	4.17	3.13
nO_2/nH_2S	-------	1.1	1.1	1.1
H_2S_{in}	mg/L	100	100	100
H_2S_{out}	mg/L	< 10	< 10	< 10
$H_2S_{(g)}$	ppmv	0	0	0
$H_2S_{Removal}$	(%)	> 90	> 90	> 90

2.4 CONCLUSSIONS

The results of this study suggest the following:

- Sulfide oxidation was shown not to occur without the presence of water. Under abiotic conditions, the reaction products were sulfate and thiosulfate.
- If water is present and the required amount of oxygen is satisfied, the reaction is instantaneous.

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2.6. REFERENCES

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CHAPTER III

"Evaluation of sulfide oxidation in ASFBR assisted by membrane"

3. EVALUATION OF SULFIDE OXIDATION IN ASFBR ASSITED BY MEMBRANE

3.1. INTRODUCTION

With the development of more efficient and stable bioreactors, the use of anaerobic processes for wastewater treatment is increasing worldwide. However, one of the biggest problems related to the application of anaerobic biotechnology is the generation of sulfide, mainly when sulfate-rich wastewater is processed. High sulfide concentrations can compromise the quality of the liquid effluent and biogas, thus preventing the direct release of the anaerobically treated wastewater into the environment and the immediate use of biogas for energy generation. Unpleasant odors, corrosion and direct toxicity are associated with liquids and gases containing sulfide, even at low concentrations.

Several biotechnological alternatives have been developed for the removal of sulfur compounds in liquid effluents. These processes are generally based on the formation of insoluble products, such as sulfide metals or elemental sulfur, that can be separated from the liquid phase. Moreover, such technologies allow for the recovery of sulfur and some metals of interest, thus combining environmental and economic benefits.

One of the most promising processes is the conversion of the sulfide to elemental sulfur, an insoluble product that can be separated from the liquid phase and reused (Janssen *et al*. 1999). Elemental sulfur can be obtained by the partial biological or chemical oxidation of sulfide. Partial biological sulfide oxidation can proceed under aerobic, anoxic, and even anaerobic conditions. Under anoxic and aerobic conditions, nitrate and oxygen are used as electron acceptors, and the reaction can be carried out by colorless sulfur bacteria, such as those of the genera *Thiobacillus* (Lens *et al*. 1998), which have been studied previously

(Alcantara *et al*. 2004; Buisman *et al*. 1990, 1991; Janssen *et al*. 1999; Nishimura and Yoda 1997).

The partial aerobic conversion of sulfide to sulfur proceeds under oxygen-limiting conditions (Janssen *et al.* 1995), as sulfide can be oxidized to sulfate in the presence of excess oxygen. However, in practical situations it is difficult to maintain microaerobic conditions, and microaeration is the main challenge to process feasibility. Different sulfide oxidizing systems have been employed such as stirred tank reactors, up-flow anaerobic sludge blanket (UASB) reactors, expanded granular sludge bed (EGSB) reactors, fluidized bed reactors (FBR). Krayzelova *et al.* (2015) report that the reactors configuration used to the sulfide removal could be divided within two categories: (1) where oxygen/air is directly supplied into the reactor where the whole anaerobic digestion takes place, and (2) those configurations which comprise a chamber or separate unit where microaeration is performed. The direct aeration can be into the headspace (Díaz *et al.* 2011b; Ramos *et al.* 2012) or liquid phase (Díaz *et al.* 2011b; Krayzelova *et al.* 2014; Zee *et al.* 2007). Krayzelova *et al*. (2015) also report that the contact between oxygen and liquid phase is also intensified in digesters mixed by biogas recirculation (Díaz *et al.* 2011a, b; Fdz-Polanco *et al.* 2009). The separated unit is used in most cases to avoid the turbulence in the liquid phase (Annachhatre and Suktrakoolvait 2001; Xu *et al.* 2012). In all strategies, the main goal is to improve the selectivity for elemental sulfur, maximizing its recovery while generating minimal sulfate.

The application of membranes has been studied in bioreactors for wastewater treatment and is used to provide bubbleless oxygen mass transfer (Côté *et al.* 1989). Silicone membranes have been reported to be ideal for membrane based bubbleless aeration and to control the mass transfer. In a dense polymer membrane, the gas is absorbed into the polymer on the gas

side and is carried into the liquid by diffusion across the membrane (Côté *et al*. 1989). The oxygen mass flow through the silicone membrane has been described using a resistance in series model (Brookes and Livingston 1995; Camiloti *et al.* 2015; Côté *et al.* 1989). Camiloti *et al*. (2015) reported that, for silicone membranes with wall thickness of 2.4 mm, the alteration of liquid film thickness by hydraulic condition variation had little or no influence on the mass transfer process. Consequently, membrane wall resistance was responsible for oxygen transfer. In this way, the silicone membrane can limit the overall oxygen transfer and the aeration can be controlled in the reactor.

Oxygen transfer by means of silicone membrane tubes has been evaluated for different purposes: for the extraction of organic pollutants (Brookes and Livingston 1995), in the partial nitrification process (Cotter 2010) and for the removal of H_2S and volatile organic sulfur compounds (Manconi and Lens 2009). However, this technique has not yet been applied in the partial sulfide conversion process to elemental sulfur.

This study presents an innovative Internal Silicone Membrane Reactor for sulfide conversion. A silicone membrane was used for microaeration of the liquid medium, providing an environment suitable for colonization by sulfide-oxidizing bacteria.

3. 2. MATERIALS AND METHODS

3. 2.1. Reactor setup

A novel bioreactor, employing a silicone membrane for microaeration, was studied for partial sulfide oxidation to elemental sulfur. The reactor integrated a continuously fed Anaerobic Structure Fixed Bed Reactor (ASFBR, (Camiloti *et al*. 2013) and an Internal Membrane Reactor (IMR), as shown in Figure 3.1.

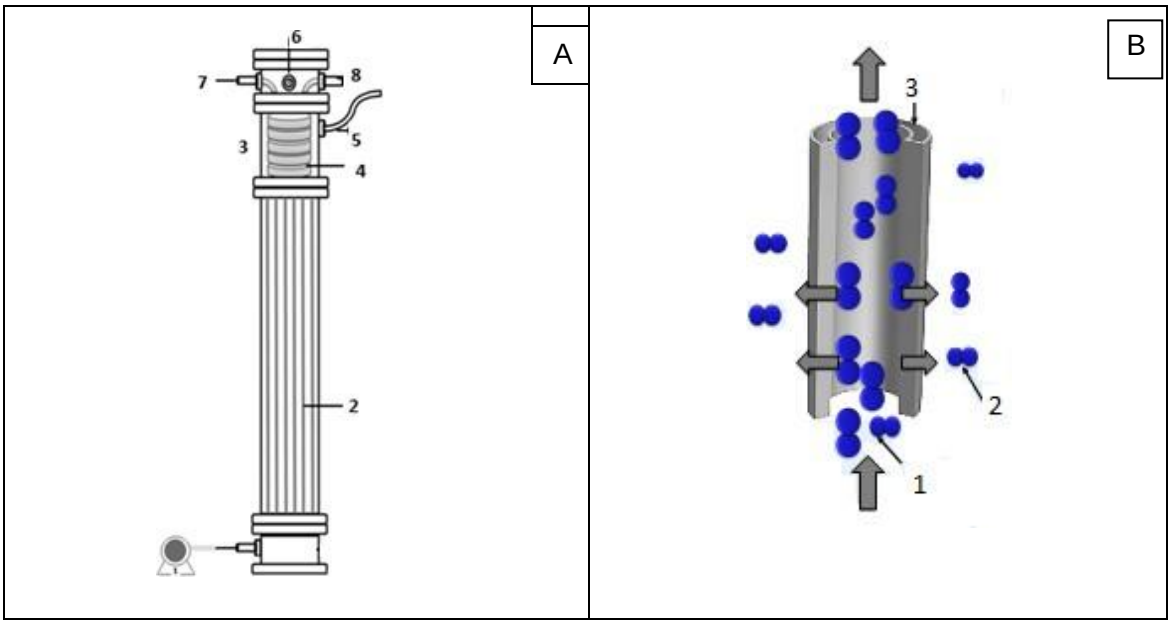

Figure 3.1: Schematic diagram of the anaerobic/microaerobic bioreactor system (A): 1- Influent pump; 2- Anaerobic bioreactor with a structured-fixed bed (ASFBR); 3- Microaerobic reactor (Internal Membrane Reactor, IMR); 4- Silicone membrane tube; 5- Effluent; 6- Gas outlet; 7- Air inlet; 8- Air outlet), schematic diagram of (C): 1- pressurized oxygen supply, 2- dissolved oxygen, 3- silicone membrane.

The ASFBR had a 9 cm internal diameter and a working volume of 6.2 L. The IMR had 2.0 L and a headspace of 0.3 L. The biomass support medium in the ASFBR was composed of 17 fixed strips of polyurethane foam, 70 cm in length. The ASFBR and IMP were connected so liquid could freely flow from one to the other.

The IMP was fitted with a 0.25 cm thick silicone tube membrane of 1.25 cm external diameter and 1.0 cm internal diameter. The silicone tube membrane was 2.1 m long with 5.2 cm^2/cm^3 of specific area and was maintained immersed in the liquid. Air, driven by a peristaltic pump, was circulated through the lumen of the membrane at a flow of 75 $mL.min^{-1}$, under a pressure of 150 mbar. The pressure was maintained by a 1.5 m column of water. To increase the gas permeability, the silicone membrane was subjected to a chemical

treatment prior to use, consisting of submerging the tube in a 70% ethanol, 30% water solution for 36 hours.

The system was operated continuously using the operational parameters listed in Table 3.1. The operation was divided into two stages: start-up (development of sulfate-reducing biomass in the ASFBR) and the application of microaeration.

Table 3.1 Operating conditions and parameters of the anaerobic/microaerobic system.

Parameter	Unit	Stage 1	Stage 2
COD_{inlet}	$mg\ L^{-1}$	3500	3500
OLR	$gCOD\ L^{-1}\ day^{-1}$	3.5	3.5
$Sulfate_{inlet}$	$mg\ L^{-1}$	150	150
Sulfate Load Rate	$gSO_4^{2-}\ L^{-1}\ day^{-1}$	0.17	0.17
HRT	day	0.9	0.9
$K_L a$	h^{-1}	0	0.15
Feeding flow	$ml.min^{-1}$	6.6	6.6
Air Flow[a]	$ml.min^{-1}$	0	75
Pressure[b]	mbar	0	150
Duration	days	21	29

(a) Air circulated into the membrane; (b) pressure inside on the membrane; COD: chemical oxygen demand, OLR: organic load rate, HRT: hydraulic retention time, $K_L a$: volumetric oxygen transfer coefficient

3.2.2. Inoculum and influent

The reactor was inoculated with granular sludge from a full-scale upflow anaerobic sludge blanket (UASB) reactor used for treating poultry slaughterhouse wastewater. The inoculation procedure was carried out by grinding the granules and immersing the support

medium in the crushed biomass for 2 hours at room temperature, following the method described by Zaiat *et al*.(1994).

The bioreactor was fed with synthetic wastewater as described by Camiloti *et al*.(2013). The feed stream was prepared to obtain a chemical oxygen demand (COD) of 3500 $mg.L^{-1}$. Sulfate was added to the wastewater as Na_2SO_4.

3.2.3. Analyses

Sulfate and thiosulfate were measured using a CS 5000 ion chromatograph (Thermo Fisher Scientific) equipped with an ionpac AS25 analytical column. The flow rate of the eluent (carbonate/bicarbonate - 4.5 mM Na_2CO_3/ x 0,8 mM $NaHCO_3$) was 1 ml min^{-1}. The sulfide concentration was determined colorimetrically in accordance with method 4500-S2-D (APHA 1995). Elemental sulfur was measured as described by Bartlett & Skoog (1954) and the dissolved oxygen concentration was analyzed with a luminescence sensor (Hach, LDO HQ10).

3.2.4. Determination of the volumetric oxygen transfer coefficient (K_La)

To describe oxygen transfer in the IMP, KLa provided by the silicone membrane was determined at three levels of pressure (50, 100 and 150 mbar) and three levels of air flow rate flowing through the membrane (24, 60 and 96 ml/min).

The influence of the chemical pretreatment in the silicone membrane with ethanol on the mass transfer was evaluated. A membrane with 200 mm of length was exposure to ethanol 95º for 24 hours and the K_La was determines at a pressure of 150 mbar and air flow of 96 mL/min.

The K_La was obtained using the dynamic gassing-in method (Atkison and Mavituna 1983). The oxygen concentration was measured with a Hach HQ40D® equipped with a

luminescent dissolved oxygen sensor (LDO101). The experimental data were examined using the one-way ANOVA statistical technique to verify the influence of the pressure and the flow rate on the mass transfer.

3.2.5. Biological analysis by 16S rRNA gene 454-pyrosequencing

16S rRNA pyrosequencing analyses were carried out to identify the microorganisms that were present in the reactor participating in the transformation of sulfur. The samples were collected at the end of reactor operation from the Internal Membrane Reactor (IMR) and the polyurethane foam from the Anaerobic Structure Fixed Bed Reactor (ASFBR).

The collected samples were retrieved by successive washing with phosphate-buffered solution and subsequent centrifugation. The extraction of total DNA was made using the phenol: chloroform-based protocol described by Griffiths (2000). DNA quality was assessed by the 260/280-nm >1.8 method, measured by an ND-2000 spectrophotometer (Nanodrop Inc., Wilmington, DE). The rRNA genes were amplified for pyrosequencing using a primer set that flanked the V4 hypervariable region of the 16S rRNA gene at corresponding Escherichia coli positions 563 and 802: primers 563F (5' – AYTGGGYDTAAAGNG - 3') and 802R (5'- CAGGAAACAGCTATGACC -3').

The pyrosequencing was performed at Instituto de Agrobiotecnologia Rosario (INDEAR) (Rosario, Argentina) using a 454 Genome Sequencer FLX (Roche). Barcodes that allow sample multiplexing during pyrosequencing were incorporated between the 454 adapter and the forward primers.

Sequences were processed with the Ribosomal Database Project (RDP) Pyrosequencing Pipeline (http://pyro.cme.msu.edu/index.jsp) (Cole *et al*. 2009). Sequences were first trimmed to remove the adaptor, barcodes, primers and sequences containing ambiguous 'N'

or shorter than 200 bps (Pipeline Initial Process). The minimum read Q score adopted was 25 (Phred Quality). Chimera sequences were removed using the DECIPHER program (Wright 2012). For alignment of the sequences, the "secondary structure aware Infernal aligner" tool (Nawrocki & Eddy, 2007) 19 was used. To determine the operational taxonomic units (OTU), "hierarchical clustering" with 97% similarity was used. Singleton sequences (OTU with one sequence) that may represent sequencing errors (Dickie 2010) were removed.

For the taxonomic classification of sequences representative of each OTU, RDP-Classifier was used. The confidence threshold adopted was 80% for genus and 50% for other taxonomic levels (Phylum-Family).

The phylogenetic tree was constructed using representative sequences of some OTUs (related to oxidation of sulfide and reduction of sulfate and/or sulfur) based on the Weighbor Joining method (RDP Tools – Tree Builder; (Bruno 2000) - .http://rdp.cme.msu.edu/treebuilderpub/treeHelp.jsp). Alfa (Chao1, Shannon, Simpson and Dominance) and Beta (Bray-Curtis dissimilarity index) diversity were quantified using Past software (Hammer 2001).

The sequences generated in this study were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk) under accession numbers ERS527222 (IMRblack), ERS527224 (ASFBRPF) and ERS527223 (IMRwhite). The project accession number is PRJEB6985.

3.3. RESULTS AND DISCUSSION

3.3.1. Membrane oxygen mass transfer capacity

Figure 3.2 presents the effect of air pressure and flow over K_La. It is clear that both factors affect the oxygen transfer capacity of the membrane. Increased pressure in the lumen of the

tube will increase the partial pressure of oxygen on the membrane surface, increasing the driving force for oxygen mass transfer. The results are consistent with those reported by Wilderer *et al*. (1985), who used reinforced silicone rubber tubes for the oxygenation of sequencing batch reactors. Cotter (2010) also reported that the influence of air pressure on mass transfer in synthetic membranes has a more significant effect on KL_a than the mixing. Consequently, it is inferred that pressure can be used to improve the capacity of oxygenation of the membrane.

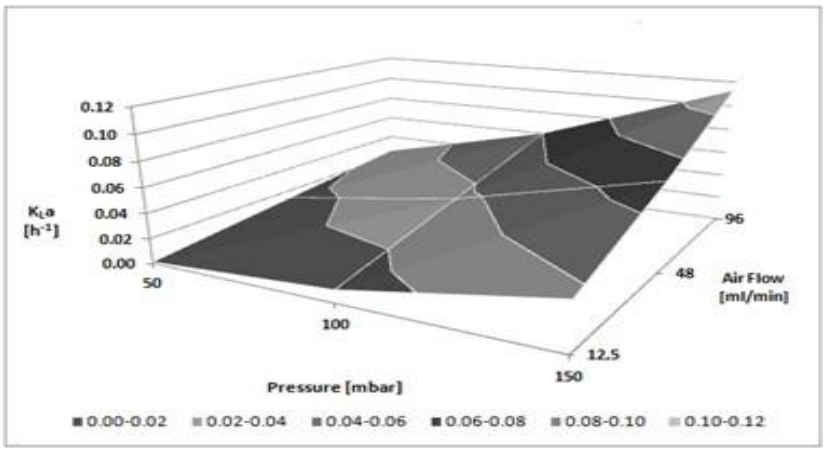

Figure 3.2: Experimental results for oxygen transfer coefficient (kLa) plotted at different pressure (mbar) and air flow (ml/min) for a silicon membrane. The kLa (h^{-1}) values are represented by grey scale.

Air flow can also improve the oxygen transfer, as a result of the increased turbulence in the membrane that reduces the depth of the stagnant gas layer on the membrane wall. Raghunath & Hwang (1992) reported that the boundary layer mass transfer resistance depends on the hydrodynamic conditions existing at the membrane.

The chemical pretreatment of the silicone membrane with ethanol was effective in increasing mass transfer capacity. Indeed, at a pressure of 150 mbar and an air flow of 96 mL/min, the pretreatment increased K_La from 0.10 to 0.22 h^{-1}. The increase in the mass

transfer capacity can be explained by the ethanol exposure destroying a protective film present in the commercial silicone tubing.

3.3.2. Performance of anaerobic/microaerobic system

The combined ASFBR-IMR system was continuously operated for 50 days. During Stage I (from day 0 to 21), there was no oxygen supply, and the dissolved sulfide stayed at 51.3 ± 1.6 mg/L (Figure 3.3). During Stage 2 (from day 22 to 50), microaeration was applied and the relationship between the oxygen and the sulfide concentrations was observed. The microaeration produced a sharp decrease in sulfide concentration, achieving an almost complete removal (99%) on day 30 (Figure 3.3). It is important to emphasize that operational period of reactor operation was function as the sulfide effluent concentration, in the other word, the sulfide removal capacity of system.

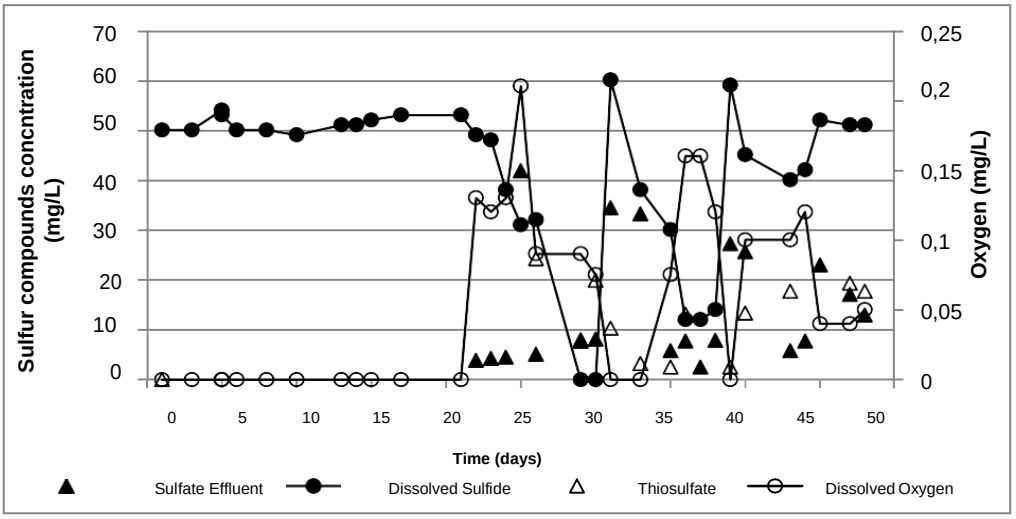

Figure 3.3. Sulfide removal under microaerated conditions in a fixed bed anaerobic reactor.

In stage 2 (days 22 – 50), the air was circulated through the silicone membrane at a pressure and air flow of 150 mbar and 75 mL.min-1, respectively. These conditions would provide a K_La of 0.15 h^{-1}, based on a clean membrane. On the 22nd and 24th days the oxygen concentration achieved 0.13mg.L^{-1}, and a decrease in the sulfide concentration was observed

to values below the detection limit. Janssen *et al*., (1995) and de Graaff *et al*., (2012) suggest the use of dissolved oxygen as a control parameter for the reaction products. When the dissolved oxygen value is below 0.1 $mg.L^{-1}$, the main product of the reaction is elemental sulfur; however, when that oxygen value is exceeded, sulfate and thiosulfate are the main products.

In stage 2, the sulfur outputs also show changes during the system operation. It is possible to observe in Figure 3.3 an increase in the sulfide concentration between the 31th and 35th days, followed by a decrease in the sulfide concentration and then, once again, an increase on the 39th day. After the 45th day, the oxygen concentration was maintained at 0.05 $mg.L^{-1}$ and the sulfide concentration was, on average, 48.6 $mg.L^{-1}$. It is important to highlight that the increase in the sulfide concentration was followed by an increase in the sulfate concentration, and a gradual increase in the thiosulfate concentration was also observed. The sulfate and thiosulfate concentrations show, on average, concentrations of 14.3 ± 12.2 $mg.L^{-1}$ and 13.1 ± 7.3 $mg.L^{-1}$, respectively, and sulfite was not detected. Three different factors might explain these observations: the decrease in the membrane transfer capacity, the sulfur reduction to sulfide, the sulfur reaction with sulfide and oxygen and lost of continuity of air flow.

The decrease in the membrane transfer capacity may occur gradually, however, an increase in the oxygen concentrations occurs again on the 35th and 40th days and the concentration was maintained at 0.05 $mg.L^{-1}$. The decrease in the mass transfer capacity may be due to the biomass and elemental sulfur settling, contributing extra resistance to oxygen transfer; however, this decrease is not the main factor that led to the increased sulfide concentrations.

The sulfur outputs also reveal the increase in the sulfide concentration and the other reaction intermediates. The increase can be associated with the sulfur consumption, which can occur by reduction or oxidation. The sulfur reduction to sulfide can be mediated by members of

the genus *Dethiosulfovibrio*. This genus was found in the samples drawn from the membrane wall and will be discussed in the next section.

The other intermediates can result from the reaction of hydrogen sulfide and sulfur, as shown in equations 3.1 and 3.2. Kleinjan *et al*. (2005) demonstrated that polysulfide formation may occur and that a heterogeneous reaction occurs between dissolved hydrogen sulfide and biologically produced sulfur. In contrast, the reaction between polysulfide and oxygen can produce thiosulfate (equations 3.2). The polysulfide formation was not measured, and the color alteration caused by the formation of polysulfide (Chen and Morris 1972) is difficult to detect because the effluent had a yellowish coloration.

$$HS^- + (X-1)S^0 \rightarrow S_X^{2-} + H^+ \qquad \text{Equation 3.1}$$

$$S_X^{2-} + \frac{3}{2}O_2 \rightarrow S_2O_3^{2-}(X-2)S^0 \qquad \text{Equation 3.2}$$

The decrease in microorganism ability to oxidize the sulfide to sulfur and consequent sulfur conversion to other intermediates can be related to the surface contact between sulfide and bacteria and oxygen. Pokasoowan *et al*. (2009) reported that the recovered sulfur on the bacteria surface reduced contact between sulfide and oxygen, preventing the sulfide oxidation to sulfur. Therefore, the recovered sulfur could react with sulfide and oxygen, and transform into polysulfide and other intermediaries.

The results, both for sulfur production and consumption, suggest that the formation of distinct microbial communities in the IMR occurred and suggest that the oxygen-limited conditions promoted colonization by sulfide-oxidizing bacteria (SOB) in the biomass deposited in the membrane, which enhanced the sulfide conversion. These results showed that the reactor configuration can develop SOB under microaerobic conditions and can improve and reestablish the sulfide conversion to elemental sulfur.

3.3.3. Bacterial community

The composition of the bacterial community demonstrates the capability of the reactor configuration to promote colonization by sulfide-oxidizing bacteria from an anaerobic sludge. The samples were drawn from biomass attached at the polyurethane foam in the anaerobic reactor ($ASFBR_{PF}$) and from the IMR. In the IMR, the biomass deposited in the membrane wall shows different features: a sample with white color – IMR_{white} (probably due to elemental sulfur formation) and a black sample – IMR_{black}.

The analysis of samples drawn from the IMR (IMR_{white} and IMR_{black}) and from the biomass attached to the polyurethane foam in the anaerobic reactor ($ASFBR_{PF}$) showed different genera related to the sulfur cycle. Regarding the sulfide oxidation genera, some Operational Taxonomic Units (OTUs) were affiliated with *Acidithiobacillus, Pseudomonas* and *Sulfuricurvum* genera. For the genera associated with the sulfur reduction, some OTUs were affiliated with the *Dethiosulfovibrio* genus. These results suggest the formation of distinct microbial communities in the IMR and that the anaerobic biomass is capable, under microaerobic conditions, of supporting colonization by sulfide-oxidizing bacteria and reinforce the operational results that suggest the sulfide conversion to sulfur is followed by the sulfur consumption.

In total, of the three samples, 3114-5892 raw sequences were generated with an average length of 224±1 bp (Table S1). After trimming (filtering parameters and chimera check) 2683-5027 sequences remained to determine the OTUs with 97 % of similarity (total of 176-253 OTUs). From all of the OTUs, 20-23% were representing singletons and were not used in the taxonomical classification. Good's estimator values ranged from 98 to 99%, indicating a high coverage of the diversity. Rarefaction curves indicate that at 80 % of similarity (phylum level) the number of sequences was enough to access all diversity of phylum (Figure S1). However, the rarefaction curves at 95% (genus level) and 97% (species

level) of similarity showed that more sequence is necessary to characterize the full diversity of genera and species. The sample taken from the biomass attached to foam polyurethanes ($ASFBR_{PF}$) showed higher diversity than the samples taken from the IMR. Therefore, the rarefaction and Chao1 values observed in the $ASFBR_{PF}$ sample (283 and 372, respectively) were higher than those observed in the IMR samples (IMR_{black} – 206 and 264; IMR_{white} – 223 and 277, respectively). The diversity index values (Shannon and Simpson) indicated a slight difference between IMR_{black} (3.9 and 0.9) and IMR_{white} (3.7 and 0.9) while the highest values were observed in the sample $ASFBR_{PF}$ (4.0 and 1.0). The dominance index value was highest in the IMR_{white} sample (0.07), while the other samples had values of 0.04-0.05. The dominance was related to the OUT affiliated with *Sedimentibacter* genus (Table S2).

By using a Bray-Curtis similarity dendrogram (Figure 3.4a) and Venn diagrams of OTUs (Figure 3.4b) the differences and similarities among the communities were determined. The Bray-Curtis similarity dendrogram shows a high similarity (64%) between the black biomass deposited in the membrane wall (IMR_{black}) and the biomass drawn from the biomass attached foam polyurethanes ($ASFBR_{PF}$). Moreover, 15% of OTUs were common between these samples (IMR_{black} and $ASFBR_{PF}$). These results may be related to the features of biomass attached in the polyurethane foam and black biomass on the IMR, which could maintain several members of the microbial community despite the different sampling site.

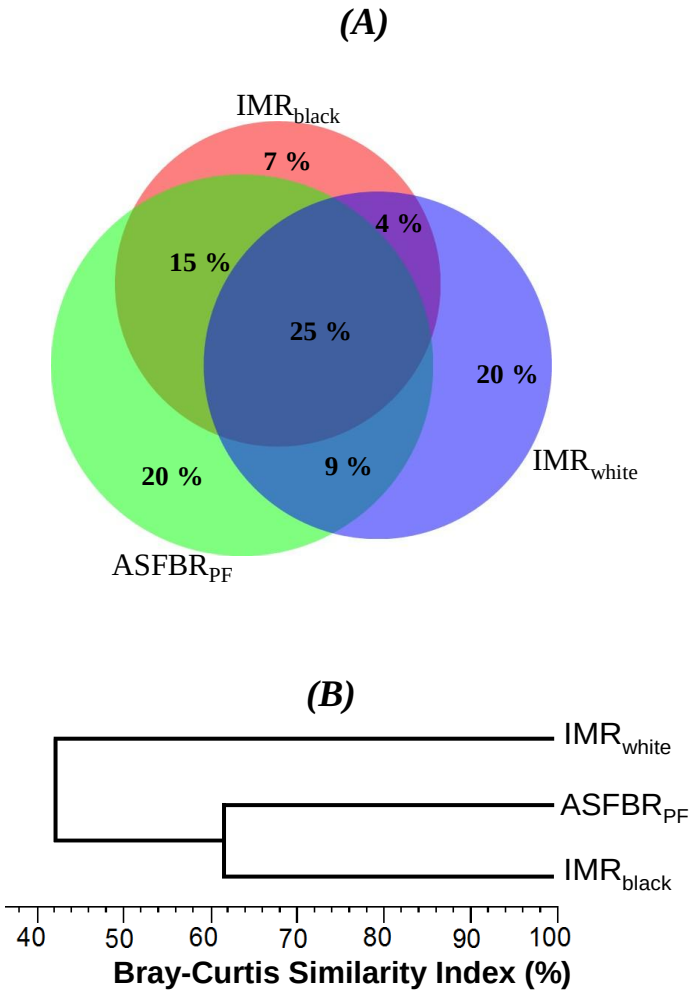

Figure 3.4 Comparison of microbial communities from samples taken from Internal Membrane Reactor (IMR) with white color (IMRwhite), IMR with black color (IMRblack) and anaerobic structure fixed bed reactor (ASFBR) attached at polyurethane foam (ASFBRPF), at 97% similarity level: (A) The area-proportional Venn diagram showing overall overlaps of OTUs between samples. A total of 279 OTUs were defined at 97% similarity. (B) Dendrogram based on Bray–Curtis similarity index.

In contrast, the two samples taken from the IMR membrane (IMR_{black} and IMR_{white}) showed a Bray-Curtis similarity value of only 42% and only 4% of exclusive shared OTUs (Figure 4a). These results suggest the formation of distinct microbial communities in the IMR and that the anaerobic biomass is capable, under microaerobic conditions, of supporting

colonization by sulfide-oxidizing bacteria. In addition, the samples taken from the IMR showed that 31% of OTUs were exclusive (7% for IMR_{black} and 20% for IMR_{white} and 4% were common between IMR_{black} and IMR_{white}) while the sample taken from ASFBR had 20 % exclusive OTUs. Furthermore, only 9% of exclusive shared OTUs were common between IMR_{white} and $ASFBR_{PF}$ reflecting the selection effect due to microaeration.

Sulfide oxidizer genera were found attached to the polyurethane foam in the reactor ASFBR and the samples from IMR (black and white), highlighting the genera *Acidithiobacillus*, *Sulfuricurvum* and *Pseudomonas* (Table 3.2). The relative proportions of these genera were higher in IMR (0.11 – 1.16 %) than in ASFBR (0.04 – 0.14 %). By using the phylogenetic tree (Figure 3.5) these genera were affiliated to the species *Acidithiobacillus thiooxidans*, *Sulfuricurvum kujiense* and *Pseudomonas stutzeri*.

The members of *Acidithiobacillus* have been employed for the aerobic treatment of H_2S in biotrickling filters (Sercu *et al*., 2005). The genus of *Acidithiobacillus* had originally been classified as *Thiobacillus* but was recently reclassified by Kelly and Wood (2000). The *Acidithiobacillus* are colorless sulfur bacteria and have long been considered the typical bacteria responsible for the oxidization of sulfide to elemental sulfur or sulfate using oxygen or nitrate as final electron acceptors (Lens *et al*., 1998). It should be noted that the members of *Acidithiobacillus* were found mainly in the sample drawn from the deposited biomass (samples IMR black and white), confirming the presence of SOB in the anaerobic biomass under the microaerobic conditions.

The genus *Sulfuricurvum* was found mainly in the biomass deposited in the membrane. The genus has been described by Kodama & Watanabe (2004) as a facultative anaerobic sulfur oxidizing bacterium that grows anaerobically and microaerobically by oxidizing reduced

sulfur species, such as sulfide, elemental sulfur and thiosulfate. Another genus involved in the sulfide oxidation was *Pseudomonas stutizeri,* which has been reported to oxidize sulfur

Table 3.2 Relative abundance of genera and number of OTUs found in samples taken from the Internal Membrane Reactor (IMR) with white color (IMRwhite), IMR with black color (IMRblack) and anaerobic structure fixed bed reactor (ASFBR) attached to polyurethane foam (ASFBRPF), at 97% similarity level.

Phylum	Genus	Relative Abundance			Number of OTUs		
		IMR black	ASFBR PF	IMR white	IMR black	ASFBR PF	IMR white
Bacteroidetes	*Bacteroides*	0.15	0.28	-	1	2	-
Firmicutes	*Sedimentibacter*	17.18	14.96	37.95	8	10	8
	Gracilibacter	0.07	-	-	1	-	-
Proteobacteria	*Geobacter*▲	3.32	1.01	0.15	3	3	1
	*Acidithiobacillus**	1.16	0.14	0.10	1	1	1
	*Sulfuricurvum**	0.30	0.04	0.12	1	1	1
	*Pseudomonas**	0.11	0.04	0.42	1	1	2
	Desulfovibrio▲	0.07	0.62	0.24	1	5	2
	Aeromonas▲	-	-	0.17	-	-	1
	Desulfobulbus▲	-	-	0.10	-	-	1
	Smithella▲	-	0.04	-	-	1	-
	Syntrophobacter▲	-	-	0.10	-	-	1
Synergistetes	*Aminiphilus*	3.91	4.40	1.07	6	6	5
	Dethiosulfovibrio□▲	0.11	0.20	0.71	1	1	2
Unclassified	Unclassified	68.32	73.56	50.57	101	130	109
Others	Others	5.29	4.71	8.30	16	33	27

* Related to sulfide oxidation; ▲ related to sulfate oxidation; □ related to sulfur reduction

compounds with nitrogen. The genus was isolated from an anoxic reactor in nitrite reduction conditions (Mahmood *et al.*, 2009).

In addition, it is important to highlight that in the sample drawn from IMR_{white} were found mainly members of *Dethiosulfovibrio (Dethiosulfovibrio peptidovorans;* Figure 4.5*)*. The *Dethiosulfovibrio* genus is capable of reducing sulfur to sulfide. This result indicates the presence of elemental sulfur attached to the membrane and that this genus is not desirable for the process.

In relation to sulfate-reducing bacteria, seven genera were found. *Geobacter*, *Dethiosulfovibrio* and *Desulfovibrio* genera were present in all three samples indicating that the sulfate reduction occurred in the ASFBR reactor and IMR. In contrast, the genera *Aeromonas*, *Desulfobulbus* and *Syntrophobacter* were present only the IMR_{white} sample, and the *Smithella* genus was found exclusively in the $ASFBR_{PF}$ sample.

Finally, our findings regarding the bacterial composition in an Internal Silicone Membrane Reactor (ISMR) combined with an Anaerobic Structure Fixed Bed Reactor (ASFBR) indicate that, under microaerobic conditions, sulfide-oxidizing bacteria can grow in anaerobic biomass. The biomass drawn from the membrane wall could have been deposited there, and the microaerobic conditions provided the opportunity for the sulfide oxidizing bacteria to establish in the membrane.

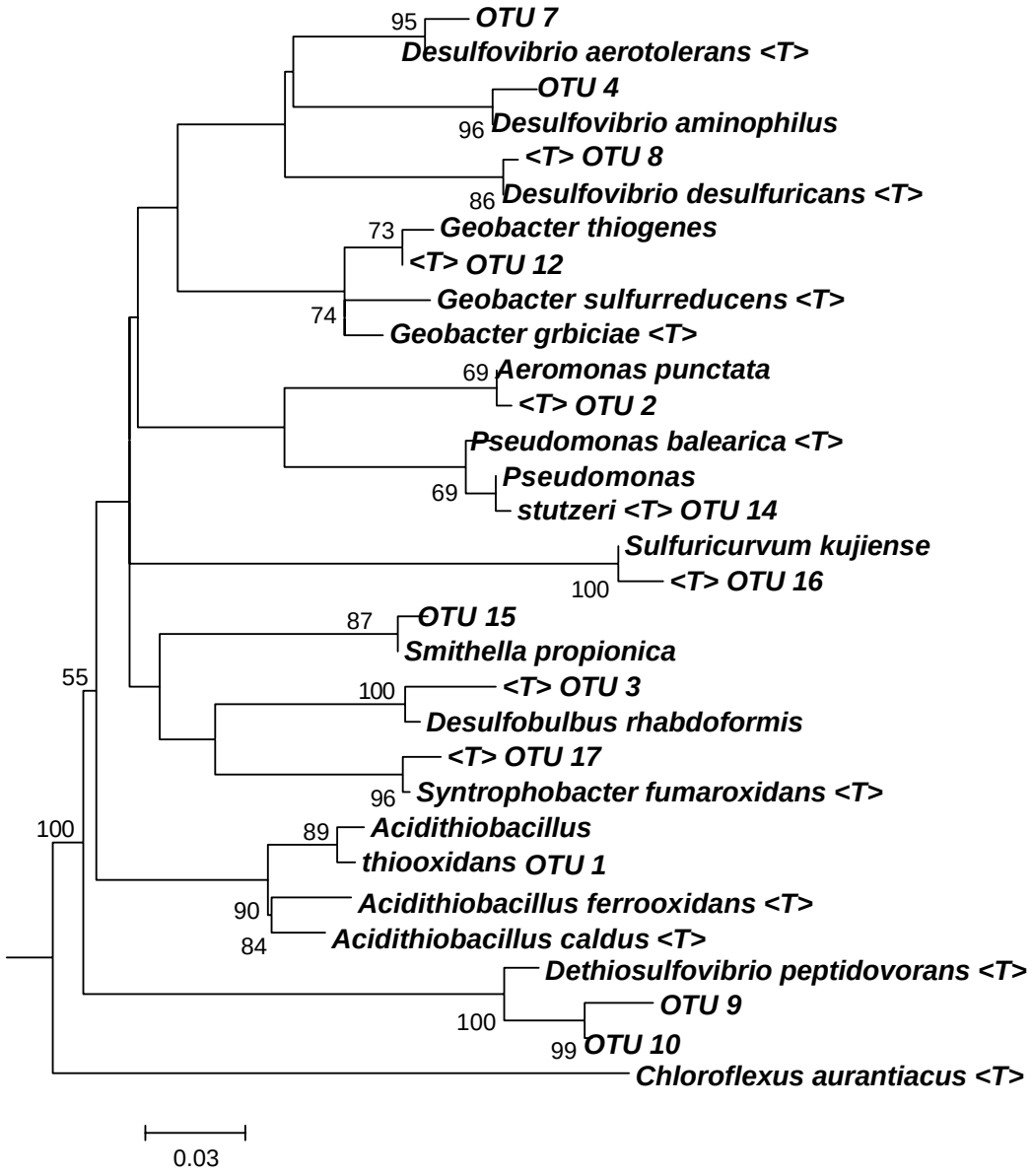

Figure 3.5. Phylogenetic tree using representative sequences of some OTUs based on the Weighbor Joining method (RDP Tools – Tree Builder). Chloroflexus aurantiacus <T> was used as an outgroup. <T>, type strain. Bootstrap values (100 replicate runs, shown as %).

3.4 CONCLUSSIONS

The ISMR combined with an ASFBR was demonstrated to be able to remove dissolved sulfide and to support membrane colonization by sulfide-oxidizing bacteria from the anaerobic biomass. The pyrosequencing analysis identified various species related to sulfide oxidation, highlighting the genera *Acidithiobacillus*, *Sulfuricurvum* and *Pseudomonas*. These results suggested that the anaerobic biomass is capable, under microaerobic conditions, of supporting colonization by sulfide-oxidizing bacteria. However, the development of microorganisms that is capable to use the formed sulfur can occurs, therefore, the frequently remove of sulfur from the system is necessary.

In this way, the strategy of microaerating an anaerobic reactor through the use of permeable membranes was effective, and the sulfide could be converted to elemental sulfur in addition to having the advantage of performing a bubble-free aeration.

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3.6 SUPPLEMENTARY MATERIAL

Table 3.3: Pyrosequencing result analysis for samples taken from the Internal Membrane Reactor (IMR) with white color (IMR_{white}), IMR with black color (IMR_{black}) and anaerobic structure fixed bed reactor (ASFBR) attached to polyurethane foam ($ASFBR_{PF}$).

	IMR_{black}	**$ASFBR_{PF}$**	**IMR_{white}**
Description of results			
Good's estimated coverage (%)	98.7%	98.8%	99.0%
Total of sequences (raw data)	2.748	5.116	4.157
Total of sequences (after trimming data)	2.683	5.027	4.095
Sequence length (after trimming data)	224±1.1	224±1.1	224±0.9
Total of Chimera	31	84	54
Total of OTUs	176	253	202
Singletons	35	59	41
Total of OTUs (taxonomical classification)	141	194	161
Unique OTUs	19	56	56
Richness Estimation			
Chao1	264 ± 67	372 ± 44	277 ± 66
Rarefaction	206 ± 27	283 ± 16	223 ± 36
Diversity index			
Shannon (H)	3.9 ± 0.1	4.0 ± 0.1	3.7 ± 0.2
Simpson (1–D)	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Dominance	0.05 ± 0.01	0.04 ± 0.01	0.07 ± 0.01

Table 3.4: Relative abundance and number of OTUs of different phyla and genera found in samples taken from Internal Membrane Reactor (IMR) with white color (IMR_{white}), IMR with black color (IMR_{black}) and anaerobic structure fixed bed reactor (ASFBR) attached at polyurethane foam ($ASFBR_{PF}$), at 97% similarity level.

	IMR_{black}		**$ASFBR_{PF}$**		**IMR_{white}**	
	% of Sequence	Nº of OTUs	% of Sequence	Nº of OTUs	% of Sequence	Nº of OTUs
0_unclassified	26.798	43	44.201	47	25.079	36
0_unclassified	26.798	43	44.201	47	25.079	36
Acidobacteria	0.149	1	0.577	6	2.369	5
Gp3	-	-	0.040	1	-	-
Gp4	-	-	0.318	1	-	-
Gp6	0.149	1	0.040	1	-	-
Gp7	-	-	0.099	2	0.464	1
Holophaga	-	-	0.080	1	1.905	4
Actinobacteria	-	-	-	-	0.147	1
0_unclassified	-	-	-	-	0.147	1
Armatimonadetes	0.075	1	0.477	2	-	-
Armatimonadetes	0.075	1	0.477	2	-	-
Bacteroidetes	0.820	5	1.134	9	0.659	5
0_unclassified	0.671	4	0.756	5	0.440	3
Bacteroides	0.149	1	0.278	2	-	-
Parabacteroides	-	-	0.040	1	0.171	1
Sediminibacterium	-	-	0.060	1	0.049	1
Chlorobi	-	-	0.080	1	0.049	1
Ignavibacterium	-	-	0.080	1	0.049	1
Chloroflexi	0.298	2	0.099	2	-	-
Bellilinea	0.224	1	0.099	2	-	-
Leptolinea	0.075	1	-	-	-	-
Euryarchaeota	-	-	0.458	5	0.122	2
Methanospirillum	-	-	0.458	5	0.122	2
Firmicutes	26.426	42	20.788	59	47.766	43

0_unclassified	6.523	24	4.416	34	7.839	28
Anaerovorax	-	-	0.060	1	-	-
Carnobacterium	-	-	0.040	1	0.073	1
Clostridium IV	0.075	1	0.080	1	-	-
Gracilibacter	0.075	1	-	-	-	-
Lactococcus	0.634	2	0.080	2	1.074	1
Lutispora	0.745	1	0.298	1	0.391	1
Megasphaera	0.261	2	0.060	1	-	-
Papillibacter	-	-	0.040	1	-	-
Peptostreptococcus	-	-	0.139	2	0.244	2
Proteocatella	-	-	0.060	1	0.098	1
Sedimentibacter	17.182	8	14.959	10	37.949	8
Succinispira	0.224	1	0.080	1	-	-
Syntrophomonas	0.708	2	0.477	3	0.098	1
Proteobacteria	26.761	15	12.194	23	7.228	34
0_unclassified	21.804	8	10.185	8	2.613	7
Acidithiobacillus	1.155	1	0.139	1	0.098	1
Aeromonas	-	-	-	-	0.171	1
Azonexus	-	-	-	-	0.073	1
Bradyrhizobium	-	-	-	-	0.293	1
Coxiella	-	-	-	-	0.098	1
Desulfobulbus	-	-	-	-	0.098	1
Desulfococcus	-	-	-	-	0.195	1
Desulfonema	-	-	-	-	0.049	1
Desulfovibrio	0.075	1	0.656	6	0.244	2
Dongia	-	-	0.040	1	-	-
Geobacter	3.317	3	1.015	3	0.147	1
Methylophilus	-	-	-	-	0.098	1
Novosphingobium	-	-	-	-	0.171	1
Pleomorphomonas	-	-	-	-	0.049	1
Pseudomonas	0.112	1	0.040	1	0.415	2
Rhodoplanes	-	-	-	-	0.317	1

Salmonella	-	-	-	-	0.147	1
Smithella	-	-	0.080	2	-	-
Sulfuricurvum	0.298	1	0.040	1	0.122	1
Syntrophobacter	-	-	-	-	0.244	2
Syntrophorhabdus	-	-	-	-	1.148	5
Yersinia	-	-	-	-	0.440	1
Spirochaetes	2.609	4	2.506	8	0.440	4
0_unclassified	1.491	2	1.094	3	0.098	1
Leptonema	1.118	2	1.074	2	-	-
Treponema	-	-	0.338	3	0.342	3
Synergistetes	11.666	23	11.001	25	13.553	24
0_unclassified	1.081	5	1.333	5	1.148	5
Aminiphilus	3.914	6	4.396	6	1.074	5
Aminobacterium	0.224	1	0.219	1	0.366	1
Aminomonas	1.640	3	0.895	4	2.295	3
Cloacibacillus	0.075	1	0.080	1	0.879	2
Dethiosulfovibrio	0.112	1	0.199	1	0.708	2
Synergistes	4.622	6	3.879	7	7.082	6
Thermotogae	0.075	1	-	-	0.049	1
0_unclassified	0.075	1	-	-	0.049	1
Verrucomicrobia	4.324	4	6.485	7	2.540	5
0_unclassified	4.249	3	6.326	5	1.538	2
Subdivision3	0.075	1	0.159	2	1.001	3
Nº of OTUs		141		194		161
Nº of Sequence	2683		5027		4095	

Figure S1

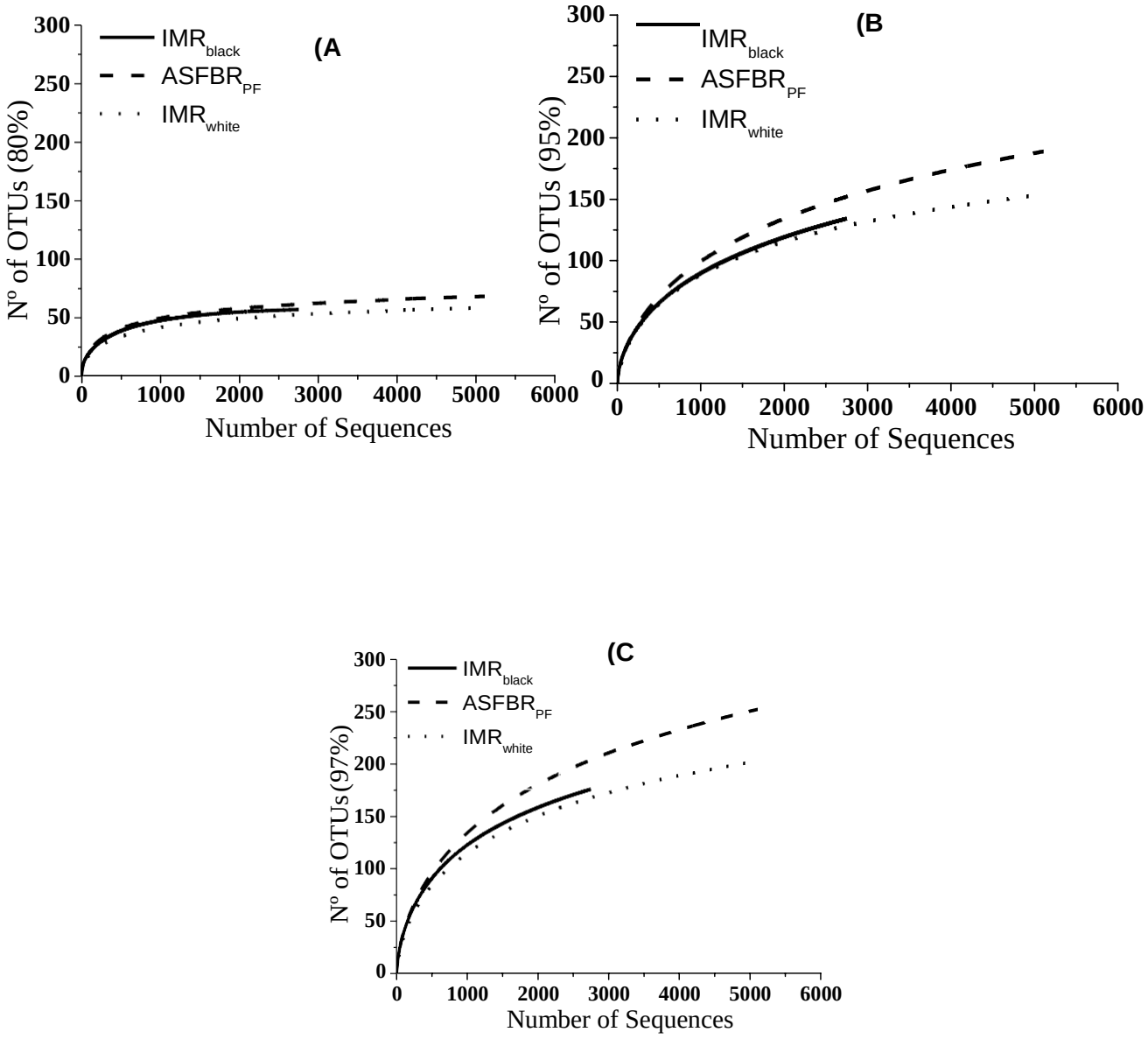

Figure 3.6: Rarefaction curves of samples taken from Internal Membrane Reactor (IMR) with white color (IMR_{white}), IMR with black color (IMR_{black}) and anaerobic structure fixed bed reactor (ASFBR) attached to polyurethane foam ($ASFBR_{PF}$). **(A)** 97% similarity (species level), **(B)** 95% similarity (genus level) and **(C)** 80% similarity (phylum level).

CHAPTER IV

"Microaeration using membrane for sulfide oxidation in UASB Reactor"

4. MICROAERATION USING MEMBRANE FOR SULFIDE OXIDATION IN UASB REACTOR

4.1. INTRODUCTION

High sulfate concentrations can be found in wastewater from different industries including pharmaceutical, food, tannery, edible oil refinery, among others (Krayzelova *et al.*, 2014; Cirne *et al.*, 2008; Sabumon *et al.*, 2008 and Liamleam *et al*., 2007). Sulfate is generally found in nature, is chemically inert, non-toxic and non-volatile. However, it could affect the anaerobic digestion process during (waste)water treatment by promoting competition between methanogens archaea and sulphate-reducing bacteria (SRB). SRB could be thermodynamically enhanced by oxidizing organic compounds (*e.g.*, volatile fatty acids) using sulfate as the electron acceptor to produce hydrogen sulfide (H_2S) (Liamleam *et al*., 2007; Wang *et al*., 2006; Jeong *et al*., 2008). H_2S is toxic to the methane producing microbial community. Normally, maintaining a COD/sulfate ratio over 10 prevents reaching inhibition concentrations in anaerobic reactors, which, depending on the pH, can be between 50 and 550 mg/L (Valdés *et al.*, 2006; Velasco *et al*., 2007; van der Zee *et al*., 2007; Montalvo y Guerrero, 2003). High concentrations of H_2S in the produced biogas also reduces its quality, causing corrosion in the distribution lines and equipment, as well as generating undesirable odors (Dawoud, 1992).

Different strategies have been used to mitigate H_2S production in anaerobic reactors. They include the removal of sulfate from wastewater (before feeding anaerobic digester) by chemical precipitation (Benatti *et al*., 2009; Perry; 2000; Tchobanoglous *et al*., 1995), inhibition of the SRB using specific compounds such as molybdate (Ranade *et al*., 1996; Isa *et al*., 2005) and use iron salt to oxidize the H_2S (Speece, 1983). Although these techniques are efficient in preventing or mitigating H_2S production, they are costly and may

be unsustainable.

An interesting alternative involves the microbial oxidation of H_2S to elemental sulfur (S^0), through the injection of micro-dose oxygen into the system (Krayzelova *et al*., 2015). Pure oxygen or air can be supplied in the recirculation line, in the liquid phase or in the headspace of the anaerobic reactor. The latter is the most used option due to its operational simplicity, since the bacterial community capable of oxidizing H_2S can grow as a biofilm on the walls of the headspace (Cirne *et al.*, 2008; van der Zee *et al*., 2007; Muñoz *et al*., 2015 and Zhu *et al*., 2009). A minimal residence time of the biogas in the headspace ($\geq$ 5 hours) is decisive for achieving removal efficiencies above 97% (Krayzelova *et al*., 2015 and Muñoz *et al*., 2015). Even though several authors have reported successful results by injecting air/oxygen into the headspace of continuous stirred tank reactors (CSTRs), limited research has been reported involving upflow anaerobic sludge blanket reactors (UASB) (Krayzelova *et al.*, 2014; Bacab *et al.*, 2020 and Pokorna-Krayzelova *et al*., 2017). While high H_2S removal efficiencies are normally reported when applying micro-aeration to CSTR reactors, in UASB reactors 75% is rarely exceeded (Krayzelova *et al.*, 2014; Ramos *et al*., 2014 and Diaz *et al*., 2010). Probably the design and the smaller headspace of UASB reactors do not provide an adequate biogas residence time, required for an effective H_2S removal (Muñoz *et al*., 2015).

Since UASB is a traditional treatment alternative, massively used worldwide for wastewater treatment, more research is needed to develop new strategies to improve H_2S removal efficiencies, such as the oxygen injection into the liquid phase of the reactor. In this context, the use of membranes has been proposed as an innovative strategy to provide micro-oxygenation. This research aimed to test silicon membranes as a micro-oxygenation mechanism to promote H_2S removal by microbial oxidation. Two configurations for micro-oxygenation were compared in a UASB reactor: immersed in the liquid phase and in an

external unit connected to biogas recirculation.

4.2 MATERIALS AND METHODS

4.2.1. Reactors setup

Two reactor configurations were implemented, to test the effectiveness of membranes for oxygen transfer to UASB reactors. Both configurations used chemically treated silicone tubes as membranes and involved identical UASB reactors of 2.2 L of useful volume. Chemical treatment of silicone tubes consisted of submerging them in a 70 % ethanol, 30 % water solution for 36 h, in order to increase the gas permeability.

In the first configuration (Reactor 1, Figure 1a) membrane was submerged in the sludge bed of the UASB reactor. The second configuration (Reactor 4.2, Figure 1b) included an external chamber that contained the membrane, through which UASB recirculation flowed. In both cases the silicone tubes had an external diameter and thickness of 9 and 2 mm, respectively. Membranes lengths were 1.1 and 2.2 m for Reactor 1 and 2, respectively. External membrane areas were then 0.031 and 0.062 m^2 for Reactor 1 and 2, respectively. Internal membrane areas were 0.017 and 0.035 m^2 for Reactor 1 and 2, respectively. A shorter membrane was used in Reactor 1, since a bigger one could not be fitted inside the reactor, due to space restrictions.

In both systems pure oxygen was used to promote microaerobic conditions in the UASB reactors. During Reactor 1 operation, oxygen was circulated within the membrane, by using a peristaltic pump, at a flow of 40 mL min^{-1}. Then, oxygen was transferred from the lumen of the membrane (inside/out operation for oxygen, see Figure 1a). Oxygen used for this purpose was stored in a 2 L container. In order to measure the consumed oxygen, a 1 L graduated cylinder with water was coupled to the 2 L oxygen container. Oxygen consumption in the system generated a vacuum in the 2 L container, which displaced water from 1 L graduated cylinder to the container. Then, a decrease of water volume indicated the volume of consumed oxygen.

Gas composition in the container was determined by gas chromatography to account for the potential transfer of carbon dioxide, methane or sulfide through the membrane. Container storing the gas was flushed with fresh oxygen every 1-3 days. Changes of gas volume present in the container, composition of the gas and the pressure were used to evaluate the rate of oxygen consumption, using ideal gas law.

In the case of Reactor 2, oxygen was circulated through the external chamber where membrane was placed. In this case, oxygen was then transferred towards the lumen of the membrane (outside/in operation for oxygen, see Figure 4.1b), where liquid from the UASB reactor was flowing (recirculation). A system to manage oxygen gas was set, similar as that described for Reactor 1, including a container for the oxygen, connected to a graduated cylinder containing water. As was the case of Reactor 1, content of the container was flushed every 1-3 days and the same approach was used to evaluate the rate of oxygen consumption. Oxygen was circulated between the mass transfer chamber and the oxygen container at a rate of 40 mL min^{-1}. Liquid recirculation of the UASB was set at 45 mL min^{-1}.

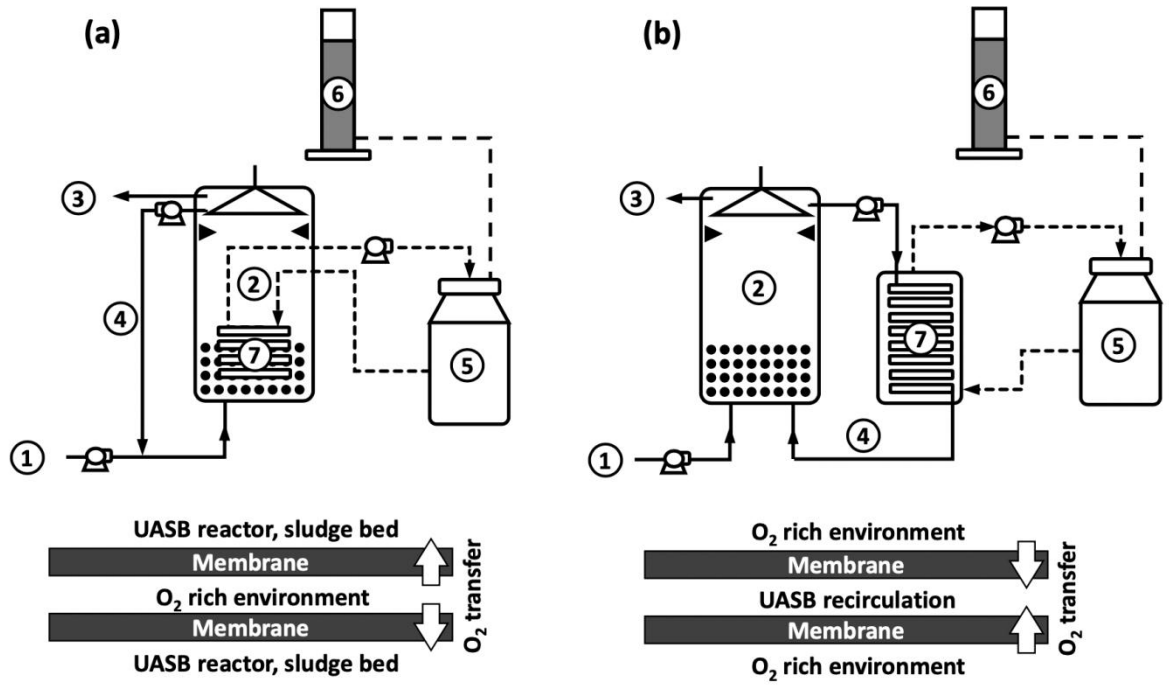

Figure 4.1. Schematic representation of reactors configuration tested in this study. (a)

Reactor 1, with submerged silicone membrane, (b) Reactor 2, with external mass transfer unit. 1: UASB feed, 2: UASB reactor, 3: UASB effluent, 4: UASB recirculation, 5: oxygen container, 6: graduated cylinder, 7: silicone membrane. Scheme below each configuration depicts the direction of oxygen transfer (inside/out or outside in).

4.2.2. Reactors operation

UASB reactors were operated at an organic loading rate (ORL) of 7.5 g-COD L^{-1} d^{-1}, and at a sulfur load of 0.075 g-S L^{-1} d^{-1} (COD/S ratio of 100). Diluted wine supplemented with anhydrous sodium sulfate was used as substrate. COD and sulfate concentrations were 7.2 and 0.19 g L^{-1}, respectively. UASB reactors were inoculated with anaerobic granular sludge from a full scale UASB reactor treating brewery wastewater. Both reactors were operated at 35 °C and pH was maintained at 7.2 by the addition of $NaHCO_3$ to the feed. During Reactor 1 operation, membrane oxygenation produced no o little effect on sulfide concentration in the biogas. As a result, operation of that reactor was stopped at day 36, as will be discussed below.

Operation included a startup period (not reported). During first 10 days of operation (after star-up) no micro-oxygenation was applied, to characterize the performance of the system without H_2S oxidation. On day 11, the described membrane based micro-oxygenation systems started their operation. Table 4.1 presents the parameters determined during systems operation, including the analytical methods used. By the end of Reactor 2 operation, the biofilm formed inside the silicone membrane was collected, for total solids and sulfur determination. Elemental sulfur content of the biofilm was determined by elemental analysis.

Table 4.1. Parameters determined during systems operation.

Parameter	Method	Periodicity
Total Chemical Oxygen Demand (COD)	APHA Standard Methods [27]	2-3 times/week
Biogas production	Liquid displacement	2-3 times/week
Biogas composition ($CH_4/CO_2/N_2/O_2$)	Gas Chromatography with TCD detector (Perkin Elmer, Clarus 500)	2-3 times/week
Sulfate	Ionic Chromatography (Methrom, Compact IC plus 882)	2-3 times/week
Thiosulfate	Ionic Chromatography (Methrom, Compact IC plus 882)	2-3 times/week
Dissolved sulfide	Colorimetric (Metilene-blue, Hach kit)	2-3 times/week
Biogas sulfide	Gas Chromatography with FPD detector (Perkin Elmer, Clarus 500)	2-3 times/week
Elemental sulfur	Elemental analyser (Eurovector , Isoprime-Euro EA 3000)	-

4.2.3. Mass balances

As already commented, oxygen consumption was determined by recording the changes in the volume of gas contained in the oxygen container (Figure 2). However, during operation, carbon dioxide, methane and sulfide were detected in the container of both reactors, because of back-transport of those species from the liquid phase of the reactor. Then, in order to properly determine the oxygen consumption, changes in total gas volume as well as changes in composition of the gas were considered, by means of a mass balance.

Sulfur mass balances were evaluated during Reactor 2 operation. The sulfur entering the system was determined considering the one present in the liquid influent (*i.e.* sulfate). The sulfur leaving the system was determined considering the sulfur contained in the biogas as H_2S, the one contained in the liquid effluent as sulfide, sulfate and sulfite, the one present in the biofilm developed inside the membrane and the one leaving the system through the membrane in the form of gaseous sulfide. Mass balances were evaluated for the period without oxygenation (days 1-10) and for the period with membrane assisted oxygenation. In the second case, data from day 16 until the end of the operation were considered. Mass

balances were calculated considering the whole mass of sulfur species entering and leaving the system, during the periods of time considered.

4.3. RESULTS

4.3.1. Reactors performance

Both reactors presented similar COD removals during the whole operation period (no oxygenation and oxygenation stages). During the first 10 days of operation (*i.e.* no oxygenation), average removal was 87.9% (s = 0.5%) and 91.3% (s = 0.5%), for Reactors 1 and 2, respectively. From day 11 onwards, values were 88.1% (s = 0.3%) and 93.9% (s = 1.2%), respectively. A t-student test confirmed no statistical difference between values with and without membrane micro-oxygenation for Reactor 1. However, in the case of Reactor 2 t-student test showed that the increase in COD removal was statistically significant ($\alpha = 0.05$). In addition, no changes were observed in the volumetric biogas production of both reactors, which remained around 6 L d^{-1} (volume in standard conditions).

Figure 4.2 presents contents of sulfide in liquid effluent and biogas, during the operation of both reactors. As shown in Figure 4.2A, Reactor 1 presented a small decrease in the sulfide content of the liquid effluent after membrane oxygenation was started, from 50 to 40 mg L^{-1} (*i.e.* after day 10). In the case of Reactor 2, sulfide in the liquid effluent decreased from 50 to 20 mg L^{-1}, in the same period. This decrease in sulfide concentration is most likely related to the increase in COD removal already commented. Moreover, as expected, the decrease in H_2S concentration levels in liquid phase of both reactors was related to the decrease in biogas levels. For example, H_2S in the biogas for Reactor 2 decreased from about 3100 to 1650 ppm (Figure 4.2B).

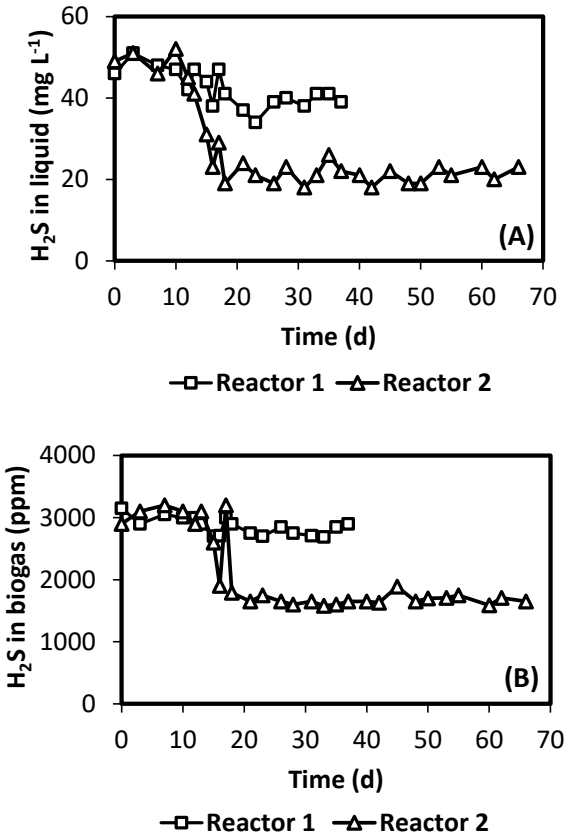

Figure 4.2. Concentration of H_2S in biogas produced in the UASB reactor.

Results presented in Figure 2 suggest that membrane oxygenation in Reactor 1 was somehow ineffective, considering the small reduction in sulfide concentration. Low membrane area (half of that in Reactor 2) may have contributed. Moreover, limited mass transfer on the external surface of the membrane may have influenced the response since membrane was submerged in the sludge bed, where mixing is limited. In the case of Reactor 2, phases were constantly on circulation, most likely providing better conditions for oxygen transfer and sulfide conversion. As a result of an ineffective sulfide oxidation, the operation of Reactor 1 was stopped at day 36, and only the operation of Reactor 2 continued. As operation of Reactor 2 advanced, formation of a biofilm was observed in the internal surface of the silicone membrane, that developed as the reactor operation progressed. Eventually, that biofilm clogged the lumen of

the membrane, blocking liquid circulation. For this reason, operation of Reactor 2 was stopped at that moment (day 66).

Table 4.2 presents the amounts of sulfide leaving the systems during Reactor 2 operation. Data indicate that micro-oxygenation promoted decreases on sulfide loads leaving the system of 44 and 57% for UASB liquid effluent and biogas, respectively. Overall sulfide reduction was close to 55%, since most of the sulfide left the system in the liquid phase.

Figure 4.3 shows O_2 transfer for Reactor 2, which varied between 1 and 1.8 g-O_2 d^{-1} (0.45 - 0.81 g-O_2 L^{-1} d^{-1}). Considering the sulfide produced by Reactor 2 during the first 10 days of operation (Table 4.2) it can be estimated that oxygen supply was in the range of 6-11 mol O_2 per mol sulfide, depending on the operation day. Latter ratio is much higher than that stoichiometrically required for complete sulfide oxidation to sulfate (2 mol O_2/mol sulfide). Table 4.2 shows that micro-oxygenation promoted a conversion of 0.0387 g-S L^{-1} d^{-1}. Then it can be determined that conversion per unit of membrane area was 2.4 g-S m^2 d^{-1}, based on internal membrane area. This value is in the same range of that reported by Pokorna-Krayzelova (Pokorna-Krayzelova *et al.*, 2017) when operating a silicone based biomembrane system for sulfide oxidation.

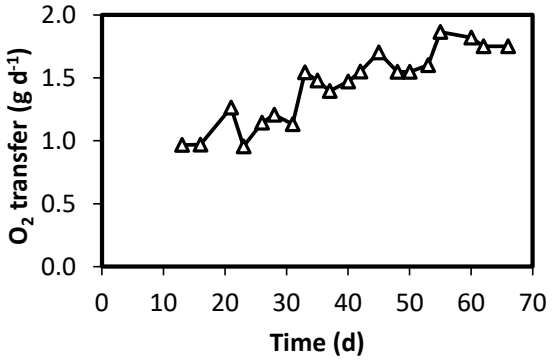

Figure 4.3. Oxygen mass transfer through the oxygenation membrane in the Reactor 2

Table 4.2. Sulfide loads leaving the system, during Reactor 2 operation. Averages over the indicated periods are presented.

Parameter	**Units**	**Days 1-10 (no micro-oxygenation)**	**Days 16-66 (with micro-oxygenation)**
Sulfide leaving the system in the gas phase	(g-S L^{-1} d^{-1})	0.0122	0.0067
Sulfide leaving the system in the liquid phase	(g-S L^{-1} d^{-1})	0.0585	0.0253
Sulfide leaving the system (Total)	**(g-S L^{-1} d^{-1})**	**0.0707**	**0.032**

During operation of Reactor 2, CO_2, CH_4 and H_2S were detected in the oxygen container (element 5 in Figure 1). Concentration of these species was determined before flushing the container with fresh oxygen, to include the effect of dilution when determining oxygen consumption. Since the liquid phases are most likely close to saturation with methane and carbon dioxide, back-transport of these compounds is expected to occur. Concentrations before oxygen flushing are presented in Figure 4, indicating that transfer of CO_2 and CH_4 were relevant, reaching at the end of operation values in the ranges 4-7% and 15-25%, respectively. H_2S was also detected, starting with values close to 1700 ppm, which decreased to 700 ppm by the end of operation period. It is worthy to notice that results shown in Figure 4.4 indicate that silicone membrane is not selective for oxygen. In fact, Pokorna-Krayzelova et al. (Pokorna-Krayzelova *et al*., 2017) showed that permeabilities of H_2S, CO_2 and CH_4 were higher than that of O_2, in silicon rubber membranes. Mass balance computed for CH_4 showed that the amount of methane transferred was relevant, between 0.3 and 0.5 L per day (volume in standard conditions). This represents that in average about 9% of all the methane produced left the system through the oxygen container. Research carried-out by Pokorna-Krayzelova et al. (Pokorna-Krayzelova *et al*., 2018) determined methane losses of 3.7% when operating a silicone biomembrane system for sulfide oxidation.

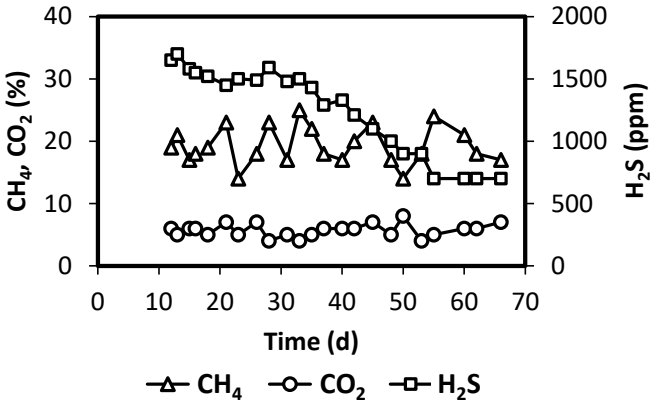

Figure 4.4 Concentration of sulfide in oxygen container for Reactor 2 system.

4.3.2. Sulfur balance

Figure 4.5 presents the contribution of the different sulfur species to the total sulfur exiting Reactor 2. Calculation was performed for the operation without and with oxygenation, *i.e.* days 1-10 and 16-66, respectively. The sum of total mass of sulfur species leaving the reactor during the studied periods were evaluated. Species considered where: sulfide in the biogas, sulfide in reactor effluent, sulfate in the reactor effluent, sulfite in the reactor effluent, sulfur contained in the biofilm formed inside the silicone membrane and sulfur lost through the membrane. These values are presented as a percentage of the sulfate load applied to Reactor 2 (0.165 g-S d^{-1} or 0.00526 mol-S d^{-1}). Then, a value of 100% in Figure 5 indicates that sulfur that left the system during the considered operation period matches the sulfur that entered the system.

Before oxygenation started (days 1-10), the measured sulfur species accounted for almost 100% of the applied sulfur load. Difference between entering and exiting sulfur was only 2%, result that supports the procedure used for mass balance determination. During non-oxygenated period, most of the sulfur left the system dissolved in the liquid phase (about 73%). Sulfide content of the biogas accounted for 17%. Distribution of dissolved sulfide species (HS^- and H_2S) is a strong function of pH, considering that pK_a is close to 7. Moreover,

this distribution will also affect sulfide equilibrium between liquid and gas phases, by determining the concentration of H_2S, the volatile sulfur form (Velasco *et al*., 2007).

Figure 5 shows a decrease in the sulfide leaving the system, both in the biogas and in the liquid phase, when micro oxygenation was applied. This is the result of the reduction of sulfide concentration in those phases (Figure 4.2, Table 4.2). The sulfur leaving the system due to transport through membrane was also considered (H_2S losses in Figure 4.5). It accounted for 2% of the total sulfur leaving the system. Sulfite and sulfate together contributed with 10% of the sulfur leaving the system. Sulfur present in the biofilm formed in the membrane lumen was also determined, representing slightly over 12%. Figure 4.5 shows a gap of close to 30% in the sulfur mass balance, when oxygenation was applied. This means that a large fraction of the incoming sulfur was not identified in the sulfur species tested. During the operation of a micro-aerated UASB reactor, Krayzelova et al. (Krayzelova *et al.*, 2014) observed that 33% of the applied sulfur left the system as elemental sulfur, suspended in the reactor effluent. During this research elemental sulfur was not determined in the liquid effluent of the UASB, which may explain the sulfur gap observed in Figure 4.5.

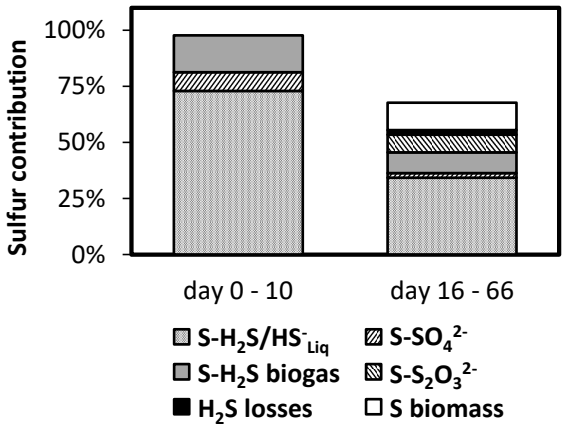

Figure 4.5. Cumulative contribution of different sulfur compounds to the sulfur leaving the Reactor 2 during the indicated periods. Values reported as a percentage of the mass of sulfur that entered the system (as sulfate in the feed).

4.4. DISCUSSION

Micro aeration has proven to be a simple, reliable and inexpensive way to control sulfide content of biogas (Krayzelova *et al*., 2017). Moreover, several studies have reported that small doses of oxygen may enhance anaerobic digestion by improving hydrolysis and/or acidification (Krayzelova et al., 2017¸ Nguyen and Khanal, 2018). However, depending on the reactor configuration, ensuring efficient provision of oxygen may not be a simple task. Most of available research has been focused on micro-aeration of anaerobic reactors for the treatment of solids or slurries. Little research has been dedicated to the application of micro-aeration in UASB reactors in particular, or granular reactors in general. Development of successful strategies to apply micro-aeration on granular reactors may extend benefits of sulfide removal by in-situ oxidation, to the anaerobic treatment of sulphate-rich wastewaters. Gas permeable membranes may be a way to achieve such goal.

The configuration in which the membrane was placed in the sludge bed (Reactor 1) failed to provide a relevant decrease in sulfide concentration. Submerged membrane configuration may not be then a suitable configuration for an oxygenation system, considering the observed performance. Moreover, access to the interior of a full-scale reactor for installation or maintenance of such system would not be practical. In an external membrane module operation flow of phases can be better controlled, mass transport can be then enhanced, and easy access to the system is ensured.

Only few reports are available dealing with micro-aeration of UASB reactors. Zhou *et al*.(Zhou *et al.,* 2007) reported 20-30% of H_2S removal in a UASB treating evaporator condensate from a sulfite pulp mill. On the other hand, Krayzelova et al. (Krayzelova *et al*, 2017) achieved 73% of sulfide removal, when treating a synthetic wastewater. In both cases, UASB reactors were micro-aerated by injecting air directly in the reactor. In this research, Reactor 2 provided a 55% of sulfide removal. These values of sulfide removal are lower than those reported for mixed reactors with air injection in the headspace. Nevertheless, these

results may be considered promising experience that can lead to successful implementation of micro-oxygenation of granular reactors for wastewater treatment. On the other hand, membrane aeration could represent an effective way to provide

oxygen in a controlled way, and may provide conditions for the development of sulfide oxidation microorganisms, as observed by Camiloti *et al.,* (Camiloti *et al*., 2019) when operating an equivalent setup.

During Reactor 2 operation, oxygen consumption was largely higher than the stoichiometric requirement. Then it is inferred that O_2 permeability of the silicone membrane did not limit the efficiency of sulfide removal. Excess O_2 may have been used in the biofilm for substrate aerobic oxidation. Another potential route of oxygen consumption could be the establishment of a cycle of oxidation/reduction of sulfur, in the membrane module/reactor system. Sulfate reducing bacteria can use sulfate, thiosulfate or even sulfite as an electron acceptor in the process of dissimilatory sulfate reduction by SRB (Boopathy *et al.,* 1993; Balk *et al*., 2008; Suzuki *et al.,* 2010). So, there is a chance that production of oxidized sulfur products, generated by SOB, have triggered a new reduction process, causing a higher consumption of oxygen. Indeed, sulfate and sulfite concentrations in the liquid effluent of the UASB reactor increased during the operation period when membrane oxygenation took place. Control of oxygen dose to prevent under or over oxygenation is indeed a challenge that may be addressed by precise automatic process control (Nguyen and Khanal, 2010). Relevant levels of methane losses were identified during system operation. This may seriously jeopardize the sustainability of membrane assisted oxygenation systems for sulfide control. Methane losses are a result of mass transport through the membrane, since reactor concentration in the liquid phase is normally close to saturation. Then, development and/or selection of membrane materials are decisive to promote higher oxygen transfer rate for oxygen, and lower for methane. Moreover, detailed determination of the required membrane area is key, to provide conditions for the transfer of only the required oxygen, limiting excessive methane losses.

During this research, a tubular membrane was used, considering a single 2.2 m length, 5 mm internal diameter tube. Biomass growth within the tube caused membrane clogging before two months of operation. A configuration ensuring an easy access to the membrane may be more adequate, facilitating removal of excess biomass developed as biofilm. The modification of commercially available membrane modules may be a simple and affordable way to implement a micro-oxygenation module for sulfide oxidation.

4.5. CONCLUSIONS

The use of a membrane-based oxygenation system is an interesting alternative to provide conditions for sulfide oxidation, reducing the concentration of this compound both in liquid effluent as well as in biogas, in granular based anaerobic reactors, like UASB. The use of an external membrane module, connected to the reactor by the recirculation line, seems to be a convenient way to do so, since it would facilitate access and maintenance. In this research a membrane that was non-nonselective for sulfide was used, resulting in methane loses (about 9%). Membrane selection and operation to reduce methane loses is required to ensure sustainability of membrane-oxygenation of UASB reactors. Even though partial sulfide oxidation was observed in this research (55% removal), results are considered promising, since may lead to successful implementation of micro-oxygenation of granular reactors for wastewater treatment

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CHAPTER V

General discussion and conclusions

5. GENERAL DISCUSSIONS AND CONCLUSIONS

5.1 GENERAL DISCUSSION

Microaeration in granular and supported biomass based reactors

Several authors to report the advantages of applying micro aeration in anaerobic digesters of slurry and municipal sludge. Some of the positive effects presented are: improving methane yield (Ruan *et al*., 2019; Li *et al*., 2019; Lim and Wang 2013; Johansen and Bakke 2006), improving the stability of anaerobic reactors during unbalanced conditions (Ramos and Fdz-Polanco 2013), and sulfide removal (Jenicek *et al*., 2010; Diaz *et al*., 2010, Diaz *et al*., 2011; Ramos *et al*., 2014; Ramos and Fdz-Polanco 2013; Fdz-Polanco *et al*., 2009; Kobayashi *et al*., 2012). The mechanisms described include increased enzymatic activity in the hydrolytic and acidogenic stage of the anaerobic process and consequently increased biogas production. In the case of sulfide removal, sulfide oxidizing biomass development was reported to be attached to the headspace wall and biogas-liquid interphase, enhanced by small oxygen dosage. Based on what has been described, the opportunity is presented to extend the benefits of micro-aeration to reactors based on granular biomass or adhered to support wastewater treatment. It should also be considered that there are few works carried out with this type of reactor. On the other hand, the granular biomass exposed to oxygen concentrations develops defense mechanisms, such as: increased enzymatic activity to reduce or eliminate reactive oxygenous agents, cellular aggregation with consuming bacteria, and aerotaxis (Dolla *et al*., 2006). Khan *et al*. (2011) studied the effect of aeration on the effluent quality, at 6 mg / L, in a UASB reactor, finding that the granule provides sufficient protection to the methanogenic consortium that avoided efficiency loss and/or anaerobic process performance. Similar mechanisms were reported for SRB biomass (González-Sanchez et al., 2005 and Kjeldsen et al., 2004).

In chapters 3 and 4, two configurations of anaerobic wastewater treatment reactors were evaluated. The first, based on biomass adhered to a structured bed and the second a UASB reactor based on granular biomass. In both cases it was possible to show that the dosage of small amounts of oxygen promoted the development of biofilms. In the reactor used in chapter 3 (ASFBR), the presence of oxidizing sulfide biomass in the biofilm was verified by molecular tests. Molecular tests were not carried out in the UASB reactor (used in chapter 4). However, the shape, color, and products formed in the oxidative process can confirm a vital role to the biomass developed due to the supply of oxygen assisted by the membrane. Other authors report comparable results in terms of the reduction of sulfur concentration, dissolved and in the biogas, in addition to the development of a biofilm with oxidizing sulfur activity (Camiloti *et al.,* 2018; Pokorna-Krayselova *et al*., 2017).

Membrane assisted microaeration

The microaeration strategy implemented during this thesis, by the use of permeable membranes, represents a feasible alternative to remove the sulfide produced during the anaerobic process. The use of membranes as an oxygen transfer mechanism was successful in the purpose of providing oxygen to the environment, promoting the activity of SOB.

As mentioned above, the presence of oxidizing sulfide biomass was verified in Chapter 3, and in the case of the UASB reactor, the biological pathway is suggested as an oxidation mechanism, generating elemental sulfur as the main product (see figure 5.1). This is insoluble, which makes it difficult for SRBs to further convert it then, sulfur can leave the system in the effluent from the reactor, or it may accumulate in the biofilm adhered to the membrane (Camiloti *et al*., 2015).

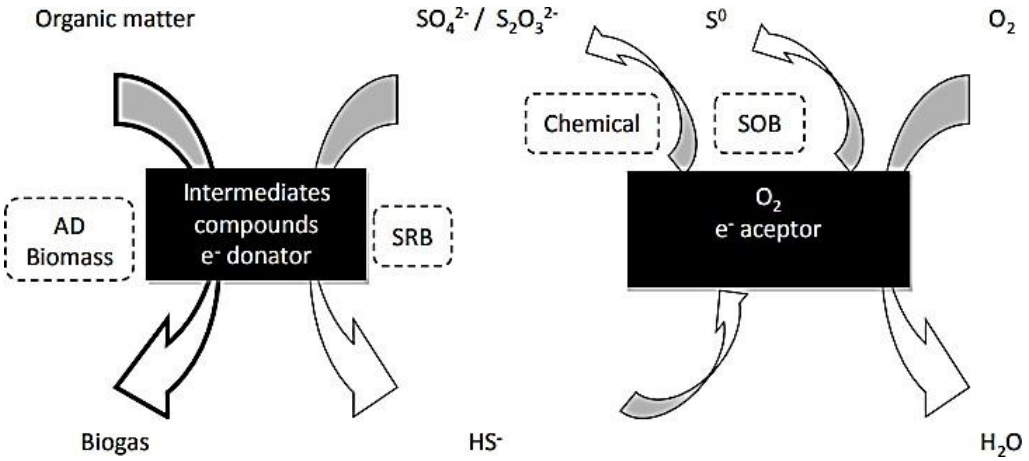

Figure 5.1 Sulfur cycle in the microaerobic system (own elaboration)

The main advantages of using membranes for transferring oxygen in anaerobic reactors are:

- Effectiveness to transfer oxygen and to promote sulfide removal. Such situation prevents biomass inhibition events due to presence of the pollutant. A similar result was reported by Camilot *et al.* (2015).
- Mainly dissolved oxygen enters the reactor. Pokorna-Krayzelova *et al.* (2017) indicate that the use of a membrane prevents the contamination of the biogas, in addition to the biogas dilution effect as a product of the direct application of air inside the reactor.
- Biogenic sulfur may be recovered from the process, which has the potential to be further purified for its use in agriculture (Fuentes-Lara *et al*., 2019) or other applications.
- The technological alternatives show high operational and initial cost, also of complexity of overall process is increased.
- The oxygen transfer mechanism through the use of a silicone membrane allows the dosing of dissolved oxygen, in this sense avoiding contaminating the biogas with oxygen and thus generating an explosive atmosphere.

Although the assisted membrane microaeration is an attractive alternative for sulfide removal, there are disadvantages and challenges to address:

- Silicone membranes were used in the experiments carried out in this study. These membranes have a limited transfer capacity, since they are not designed for this purpose.
- Methane and carbon dioxide were detected to permeate the membrane in such a way that there is a loss of biogas through the system, which helps transfer oxygen.
- It is necessary to develop a strategy to avoid blockages in the membrane. A clogging could imply a significant decrease of oxygen transfer capacity.

Chemical and/or biological sulfide oxidation in microaerobic systems

Work has been carried out progressively increasing the theoretical demand for oxygen, in terms of the stoichiometric quantity to oxidize one mole of H_2S to form elemental sulfur. Wu *et al*., 2016, using microaireation in laboratory scale anaerobic digesters treating rice straw like substrate, reported low efficiencies of desulfuration with amounts of oxygen near theoretical oxygen demand.

The end products of sulfide oxidation are certainly affected by the availability of dissolved oxygen (DO). Roosta *et al.*, 2011 indicates that at DO concentrations below 0.1 mg/L the main product of the sulfide oxidation reaction is elemental sulfur, and the reaction proceeds to sulfate when the DO concentration exceeds 0.5 mg/L. Other authors show the same results (Jensen *et al.*, 1995, Van de Zee *et al.*, 2007 and Cirne *et al*., 2008). This study found that when oxygen consumption ranges between 6 and 11 fold of oxygen stoichiometric demand, the end-products of oxidation were mainly elemental sulfur and minor amounts of sulfate and thiosulfate.

The contribution of the chemical pathway in microaerobic systems is not fully elucidated. In the abiotic tests, it was determined that the oxidation proceeds to oxidized species such as sulfate and thiosulfate, without detecting the formation of elemental sulfur. This situation allows us to infer that an important role could be assigned to the chemical pathway when a super stoichiometric dose of DO is provided in the speciation observed in various studies. In contrast, the biological pathway predominates under oxygen limitations and generates mainly elemental sulfur as a product of oxidation.

The antecedents allow inferring that both processes, chemical and biological, coexist. It is necessary to develop a dosage mechanism that favors the formation of elemental sulfur, since being a non-soluble product makes it difficult to iterate a reducing sulfate process.

Membrane permeability and methane loss

Currently there are reactor applications that use silicone membrane and its adhered biofilm for the generation of high value chemicals (Gross *et al*., 2007; Li *et al*., 2006; Setyawati *et al*., 2009). However, the use of small doses of oxygen in anaerobic reactors has not been widely studied. The use of commercial silicone membranes implies that their intrinsic characteristics like permeability, selectivity, and solubility must be considered.

In the case of the UASB reactor coupled to the external oxygen transfer chamber, the recirculated flow rate that passed through the tubular membrane was 45 mL/min. Considering this flow rate and the solubility of methane in water (at 20°C), the theoretical maximum daily flow rate of dissolved methane that circulated was 1503.35 mg/d.

Methane losses were found in ranges that had been reported for anaerobic water treatment systems, about 9% (Velasco *et al*., 2018). Given the high solubility of methane in water, this hydrocarbon leaves the rector in the effluent and then is desorbed into the atmosphere. However, it is important to develop membranes with greater selectivity that prevent methane transfer in the opposite direction to oxygen diffusion.

Configuration of reactors for the implementation of membrane-assisted microaeration

One of the differences regarding the effectiveness of the use of membranes in UASB reactors was that in the configuration of the external oxygen transfer chamber, an environment with high turbulence was present on both sides of the membrane, while in the configuration of the membrane submerged in the sludge blanket there was only high turbulence inside the membrane. An external camera also has the advantage of easy access, in the event that cleaning or maintenance is required.

The studies included in this thesis, as well as others works (Camiloti *et al*., 2018; Pokorna-Krayzelov *et al*., 2018) report the growth of a biofilm on the membrane. In addition, the molecular analysis demonstrated the presence of oxidant sulfide biomass, therefore the activity of such biomass is essential for sulfide oxidation. Consequently, the membrane serves the dual purpose of providing dissolved oxygen to create a microaerobic environment and as a support for the biofilm.

The conditions for a membrane-based oxygen transfer mechanism to become a cost-efficient alternative to remove hydrogen sulfide in the anaerobic process would have to develop characteristics such as: oxygen selectivity and the prevention of methane and CO_2 diffusion. Another required condition is a high specific surface area, given that the transfer area is fundamental in the transfer process, and also, a modular design and/or simple coupling to the recirculation line of the reactor (see Figure 5.2 and supplementary material).

There is nowadays a developed membrane industry with highly efficient products. In this sense, frame and plate membrane systems could be used for optimizing the required volume and compete with end-of-pipe sulfide removal technologies currently on the market. With the development of a membrane module having the characteristics aforementioned, it should also implement a control system for oxygen dosage.

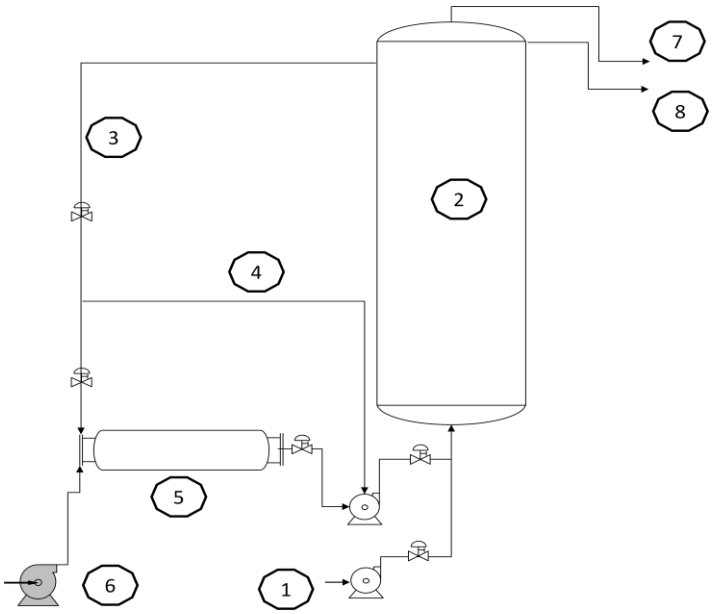

Figure 5.2 Membrane module coupled to recirculation diagram: 1: Feed, 2: Anaerobic reactor, 3: Recirculation line, 4: Recirculation by pass line, 5: Membrane Module, 6: Blower; 7: Biogas line and 8: Effluent

5.2. CONCLUDING REMARKS

Based on the results of this work, the following conclusions can be proposed:

- Sulfide oxidation was determined to occur in the presence of water. Under abiotic conditions, the reaction products were sulfate and thiosulfate. In the micro-aerobic process apparently both chemical and biological oxidative mechanisms converge.
- The implementation of the membranes, in the case of the structured fixed bed reactor, allowed the development of a biofilm, in which thanks to molecular techniques, sulfur oxidizing microorganisms (SOB) were found. In the case of the UASB reactor, the alternative of an oxygen transfer chamber, coupled to the reactor recirculation, was effective for sulfide removal; in this configuration a sulfur oxidation promoting biofilm was also formed.

- In the two reactor configurations tested, ASFBR and UASB, it was found that organic matter removals and biogas production were not significantly affected, and showing high sulfur removal rates.
- An oxygen consumption higher than that required to oxidize sulfide was observed. An explanation can be found in the sulfate-reducing biomass present in the biofilm adhered to the membrane, which could generate iterations of the sulfur cycle (successive oxidations and reductions) in addition to the use of dissolved oxygen in other metabolic processes.

Results indicate that micro aeration assisted by tubular membranes is a feasible alternative for sulfide removal, through the formation of sulfide oxidizing bacteria biofilm that grows on the wall of the membrane.

5.5 RECOMENDATIONS FOR FUTURE RESEARCH

Regarding the use of membranes for the purpose of creating microaerobic environments, it is necessary to address the following aspects:

- The opportunity to develop higher performance membranes in terms of transfer capacity is presented. Another challenge is the development of selective membranes, which only allow the transfer of oxygen.
- It is necessary to develop a membrane cleaning strategy that allows addressing the clogging problem.
- Moreover, it is necessary to continue studying the preferential mechanisms of oxidation, since super-stoichiometric oxygen consumption was observed.
- There is the opportunity to test products such as polymers and flocculant, allowing separate elemental sulfur from the effluent.

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5.7 SUPLEMENTARY MATERIAL

Figure 5.3 Isometric view of anaerobic reactor with membrane module coupled to recirculation line at real scale

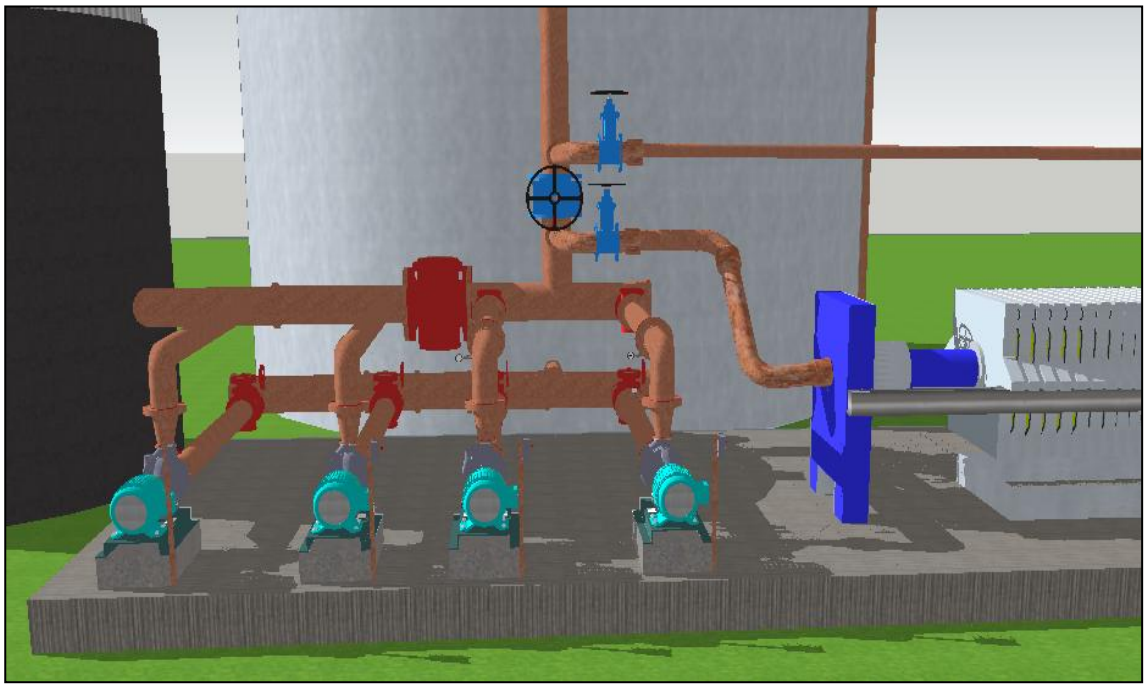

Figure 5.4 Recirculation system coupled to membrane module