The use of a silicone-based biomembrane for microaerobic H$_2$S removal from biogas

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ABSTRACT

A lab-scale bio-membrane unit was developed to improve H$_2$S removal from biogas through microaeration. Biomembrane separated biogas from air and consisted of a silicone tube covered by microaerobic biofilm. This setup allowed efficient H$_2$S removal while minimizing biogas contamination with oxygen and nitrogen. The transport and removal of H$_2$S, N$_2$, O$_2$, CH$_4$ and CO$_2$ through bare membrane, wet membrane and biomembrane was investigated. Membrane allowed the transfer of gases through it as long as there was enough driving force to induce it. H$_2$S concentration in biogas decreased much faster with the biomembrane. The permeation of gases through the membranes decreased in order: H$_2$S > CO$_2$ > CH$_4$ > O$_2$ > N$_2$. H$_2$S removal efficiency of more than 99% was observed during the continuous experiment. Light yellow deposits on the membrane indicated the possible elemental sulfur formation due to biological oxidation of H$_2$S. *Thiobacillus thioparus* was detected by FISH and PCR-DGGE.

1. Introduction

During anaerobic treatment of wastewater with high sulfate concentration, sulfate reducing bacteria (SRB) degrade sulfur-containing compounds to corrosive and toxic sulfide [1–3]. Its elevated concentration in both gaseous and liquid phase can cause many problems regarding health, environmental, operational and maintenance issues. Most of the commercial and well-established sulfide removal technologies used in full-scale applications rely on physico-chemical processes such as adsorption on activated carbon and absorption in alkaline solutions [3,4]. Although these processes are rapid and efficient, their large capital and operational costs (high pressures or temperatures), chemicals requirement and production of secondary pollutants are unfavorable, especially for medium-low productions [4–6]. Thus, the search for more economical methods has led to biological methods based on the biochemical oxidation of sulfide to elemental sulfur and sulfate by sulfur oxidizing bacteria (SOB) [5]. Biological methods impose lower operational costs with lower or no need for chemical addition; they require only oxygen [7,8]. Among the biological methods, microaeration (controlled dosing of small amount of air/oxygen into

anaerobic digesters) has recently gained growing attention for its high efficiency, reliability, simplicity and economic efficiency [9–12].

When H$_2$S concentration in biogas is too high (several thousands of ppm), microaeration may introduce too high amounts of nitrogen gas and/or may cause undesirable sulfur deposits in biogas pipes. Therefore, we introduced the novel concept of biomembrane, which serves as biofilm support and provides surface for sulfur precipitation thus avoiding its accumulation in the pipeline. Moreover, the separation of biogas and air decreases biogas contamination by nitrogen.

In the present paper, the efficiency of microaeration with silicone-based biomembrane for the removal of H$_2$S from biogas was tested, in batch as well as continuous system. Transport and removal of H$_2$S, N$_2$, O$_2$, CH$_4$ and CO$_2$ through the biomembrane was measured for three different setups: bare membrane, wet membrane and biofilm-covered membrane (biomembrane). The growth of SOB biofilm in biomembrane unit was observed and the presence of SOB was determined by FISH and PCR-DGGE analyses.
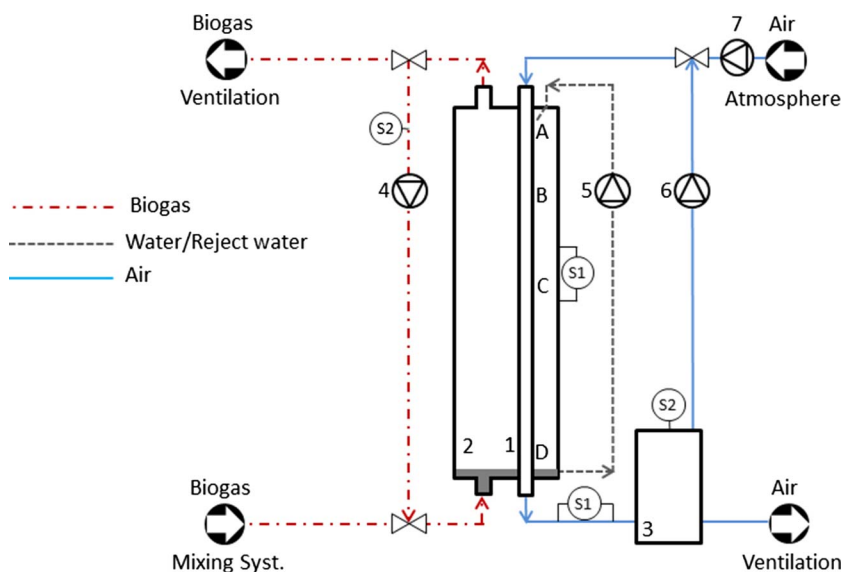
Fig. 1. The scheme of BMU. 1 – Biomembrane, 2 – reactor, 3 – air reservoir, 4 and 7 – pumps, S1 – H$_2$S sensors, S2 – gases sampling points, A–D – microbiological sampling points.

Table 1
Characterization of sludge and reject water.

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

n.a. – not available.

2. Materials and methods

2.1. Experimental set-up

The biomembrane unit (BMU) shown in Fig. 1 was designed to simulate the placement of the membrane into the headspace of an anaerobic reactor. The BMU consisted of a plexi-glass reactor and a membrane. The membrane was made from silicone rubber (poly-di-methyl siloxane, PDMS), the inner and outer diameters were 10 mm and 12 mm, respectively, the length was 0.9 m, and surface area was 0.034 m^2. Air reservoir was added to the air side to increase the air-to-biogas ratio. The volume of biogas and air side was 5.27 and 1.45 L, respectively, including all tubes and connections. The flow of gases was countercurrent. Biogas flowed bottom to top inside the reactor (at a flow rate of 16.2 L h^{-1}) and air flowed top to bottom inside the membrane (at a flow rate of 16.2 L h^{-1}). Due to its relatively constant composition, a synthetic biogas with a volumetric composition of 64.1% of methane, 35.5% of carbon dioxide and approximately 2.5–5 mg L^{-1} (0.2–0.4%) of hydrogen sulfide was used for all the experiments. This biogas was obtained by mixing these three gases from separate tanks to the desired composition using mass flow controllers controlled by a program developed in-house using National Instruments software LabVIEW 2012 running on Compact RIO system (National Instruments, US).

2.1.1. Batch experiments

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

The inoculum was taken from a mesophilic anaerobic stabilization tank of a municipal wastewater treatment plant (WWTP) in Česká Lípa (Czech Republic) while reject water was from the central municipal WWTP in Prague (Czech Republic). The characteristics are summarized in Table 1.

2.1.2. Continuous experiment

The continuous experiment was also studied with Setup III. Real biogas (average flow of 3.1 L d^{-1}) from lab-scale UASB reactor treating brewery wastewater (average H$_2$S concentration of 6.9 g m^{-3}) was connected to the biogas side of BMU. Air side was flushed with the fresh air at the beginning, closed and recirculated (at a flow rate of 16.2 L h^{-1}).

The concentration of H$_2$S and the composition of gases (CH$_4$, CO$_2$, N$_2$, and O$_2$) were measured regularly on both sides.

2.2. Calculation of permeability

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

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

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

(1)

where:

p_i: partial pressure of the gas in the side at the beginning of a particular time step (i), [atm],
V: volume [m^3],
R: ideal gas constant, [0.00008205 m^3 atm mol^{-1} K^{-1}],
T: ambient temperature, [298.15 K].

The moles transferred through the membrane (3) were calculated using Eq. (2).

$$n_{mi} = \frac{P*A}{x}*(p_{F_{i-1}} - p_{P_{i-1}})*t \qquad (2)$$

where:

P: permeability of the gas through the membrane, [mol m m^{-2} s^{-1} Pa^{-1}],
A: surface area of the membrane, [m^2],
x: thickness of the membrane, [m],
$p_{F_{i-1}}$: partial pressure of the gas on the feed side in the previous time step (i) [Pa]. (For O$_2$ or N$_2$ on the air side, for CH$_4$, CO$_2$ and H$_2$S on the biogas side),
$p_{P_{i-1}}$: partial pressure of the gas on the permeate side in the previous time step (i) [Pa]. (For O$_2$ or N$_2$ on the biogas side, for CH$_4$, CO$_2$ and H$_2$S on the air side),
t: duration of the time step [s].

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

2.3. Chemical analyses

Hydrogen sulfide concentration in the gas was measured using an online electrochemical gas sensor (Membrapor H$_2$S sensor type H$_2$S/S-10000-S). Other gases (CH$_4$, CO$_2$, N$_2$, and O$_2$) were measured by the GC Shimadzu 2014 equipped with a thermal conductivity detector (CH$_4$, CO$_2$, air) and by the GC 8000^{TOP} (Fisons Instruments, USA) equipped with a heat conductivity detector HWD 800 (O$_2$, N$_2$, CH$_4$). Analysis of COD, pH, solids, ammonia and sulfide were done according to the Standard Methods (American Public Health Association, 1997). The sulfur composition of sludge was assessed by Elemental Vario EL III (Elementar Analysensystem GmbH, Germany) and by X-ray fluorescence analysis using the ARL 9400 XP sequential WD-XRF spectrometer (THERMO ARL, Switzerland).

2.4. Microbiological analyses

Samples for microbiological analyses were taken from sampling points A–D (Fig. 1) in two sets. First set of samples (A1–D1) was taken the second day of the biofilm growth experiment (E1, discussed in Section 3.1 biofilm growth), while the second set of samples (A2–D2) was taken at the end of the continuous experiment.

2.4.1. FISH analysis

Samples for fluorescence *in-situ* hybridization were processed according to Nielsen et al. [13]. Samples were fixed for both Gram negative and Gram positive cells except for samples A1 and B1 which were fixed for Gram negative only (low amount of biofilm). Samples fixed according to Gram positive procedure were used together with HGC probe, all other probes were applied to Gram negative fixed samples. After hybridization the cells were stained with DAPI staining (1 µg ml^{-1}, 15 min). Then Vectashield was applied and samples were analyzed on epifluorescence microscope Olympus BX51 under 400 × magnification. The specific probes used in this study are listed in Table 2. All of them were labeled with Cy3.

Table 2

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

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

FA: Concentration of formamide used in this study.

2.4.2. PCR-DGGE analysis

Total DNA was extracted from all samples using PowerSoil® DNA Isolation Kit (MOBIO Laboratories, USA). PCR was performed with general bacterial primers 341F-GC and 907R (Schäfer and Muyzer, 2001) from Sigma-Aldrich and FastStart™ High Fidelity PCR System, dNTPack (Roche). Mastermix per one reaction consisted of: 10 × Buffer without MgCl$_2$ 2.5 µl, MgCl$_2$ 1.7 µl, dNTP mix 1 µl, PCR water 16.2 µl, and polymerase 0.4 µl). Then 0.5 µl of each (25 µM) primer, 1 µl of BSA (Sigma-Aldrich) and 1.2 µl of extracted DNA were added. Cycling conditions were following: pre-denaturation for 5 min at 95 °C, followed by 34 cycles of denaturation for 30 s at 95 °C, annealing for 40 s at 57 °C, and extension for 40 s at 72 °C, and finished with extension for 30 min at 72 °C. DGGE was performed according to Schäfer and Muyzer [14] using Ingeny PhorU system (Ingeny, Leiden, NL). The denaturing gradient used was 30–60% and the electrophoresis run at 100 V for 16 h. After that the gel was stained with SYBR Green I staining solution for 1 h and chosen bands were excised with sterile scalpel. DNA was eluted in 40 µl of PCR H$_2$O for 24 h. Subsequently 1.2 µl of eluted DNA was used for re-PCR, which was done as described before with only differences in number of cycles (27 instead of 34) and using primer 341F without GC clamp. After PCR products confirmation by agarose electrophoresis they were purified with Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced at the Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague. The obtained sequences were processed with Chromas and classified using RDP database.

3. Results

The content of nitrogen, oxygen, methane and carbon dioxide in biogas and air side in all three setups is given in Fig. 2. In general, the concentrations of the gases followed the same behavior in all setups. Only O$_2$ did not increase in the biogas side as it was consumed by SOB. Content of N$_2$ and O$_2$ in the air side (Fig. 2A and B, resp.) decreased with time, while increasing in the biogas side. Regarding CH$_4$ and CO$_2$ (Fig. 2C and D, resp.), their concentration gradually decreased in the biogas side, while increasing in the air side. Fig. 2E shows the
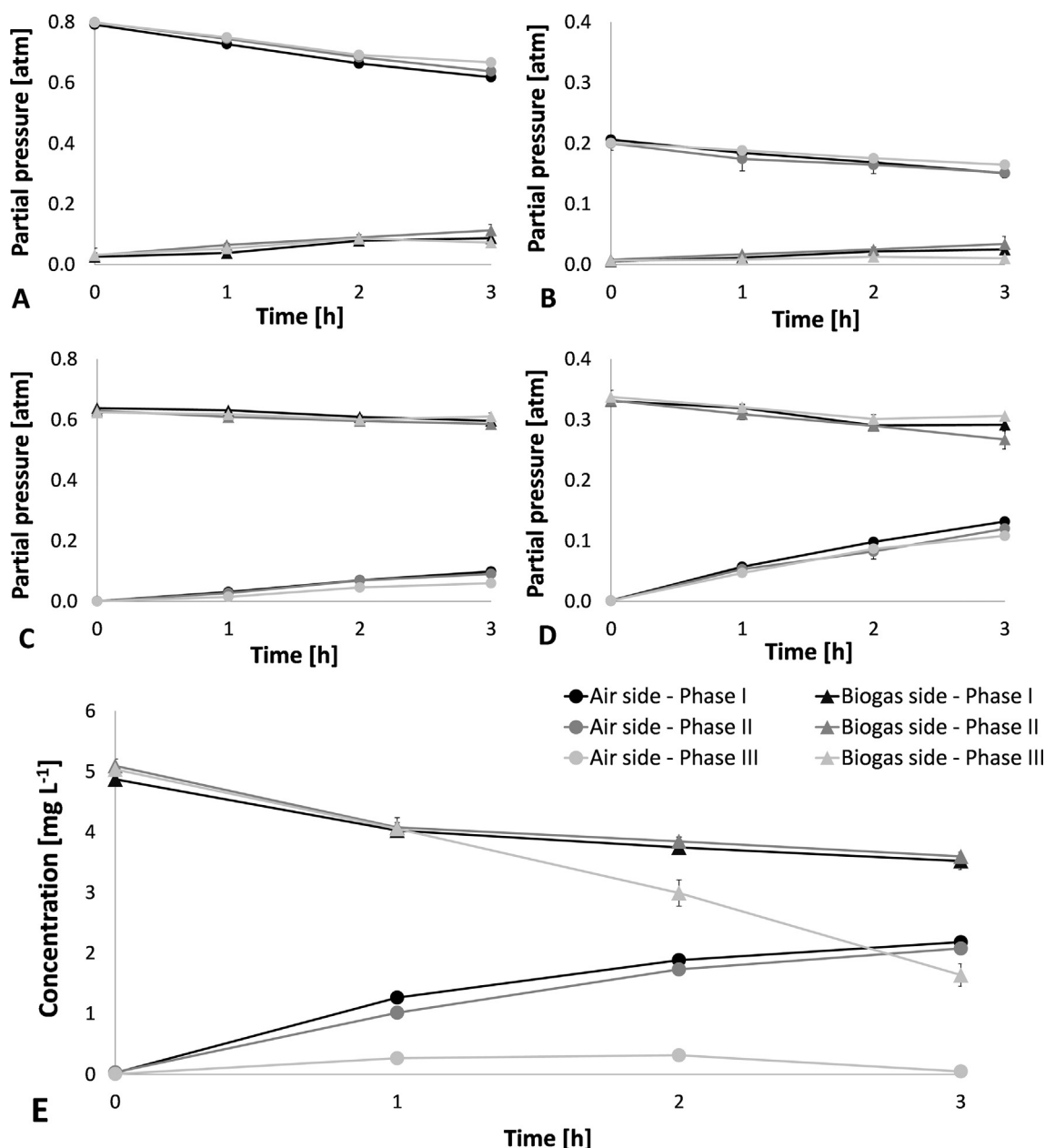
Fig. 2. The composition of gases in biogas and air side (Setup I, Setup II, and Setup III). A – nitrogen (in atm), B – oxygen (in atm), C – methane (in atm), D – carbon dioxide (in atm), E – hydrogen sulfide (in mg L^{-1}). Note the different scale.

concentration of hydrogen sulfide in biogas and air side in all three Setups. It decreased in the biogas side while increasing in the air side. Only in Setup III, the concentration in the air side increased to 0.32 mg L^{-1} and after two hour decreased close to zero. The experimental data for the H$_2$S concentration in the biogas side was approximated to a first order equation; a linear fit was applied to determine the rate at which it changed with time. The results obtained are −0.4; −0.5; and −1.2 mg L^{-1} h^{-1} for Setup I, II, and III, respectively. It is indisputable that the rate at which the concentration decreased in the biogas side was much faster in Setup III. The percentage of removal after 3 h of experiment was 55–70%. The presence of light yellow spots on the surface of the membrane appears to indicate that the H$_2$S in the biogas was oxidized to elemental sulfur by the biofilm.

Based on the model developed and the data collected, the permeability of each gas through the membrane was determined for the experiments performed in Setup I and Setup II (Table 3). It was not possible to fit the data of Setup III with the molar balance; therefore,

Table 3
Average permeability for each gas in Setup I and Setup II.

Case	Permeability [Barrer][a]				
	N$_2$	O$_2$	CH$_4$	CO$_2$	H$_2$S
Setup I	214 ± 11	501 ± 36	801 ± 8	2545 ± 35	3410 ± 339
Setup II	156 ± 47	486 ± 151	889 ± 99	2660 ± 14	3425 ± 64

[a] Barrer = 10^{-10} cm^3 (STP) cm cm^{-2} s^{-1} (cm Hg)$^{-1}$.

permeability values for Setup III were not calculated.

3.1. Biofilm growth

In Setup III, repeated measurements (E1–E4) were done over 41 days to test the biofilm growth for H$_2$S removal from biogas. The results of H$_2$S concentration in biogas and air side is shown in Fig. 3. In
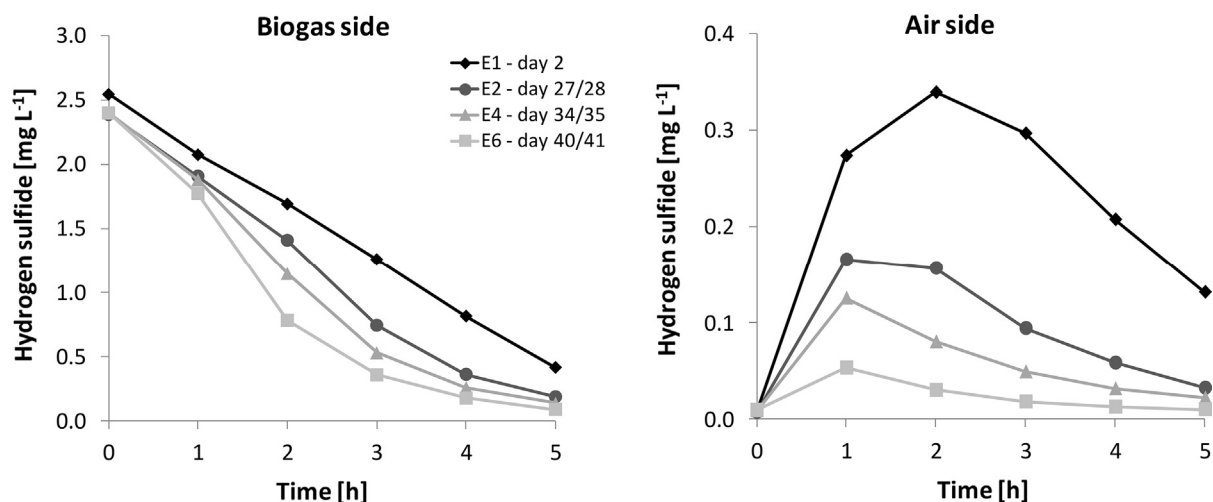
Fig. 3. The H_2S concentration in biogas and air side during biofilm growth experiments. Note the different scale.

Table 4
H_2S removal rate and CH_4/CO_2 ratio for biofilm growth experiments.

Exp. Number (-)	Day of experiment [d]	CH_4/CO_2 ratio at the beginning (-)	CH_4/CO_2 ratio at the end (-)	Specific H_2S removal rate [g m^{-2} d^{-1}]
E1	2	1.97	2.24	1.26
E2	27–28	1.94	2.11	1.57
E3	34–35	1.94	2.12	1.93
E4	40–41	1.92	2.08	2.16

the biogas side, H_2S concentration decreased from 2.5 mg L^{-1} to less than 0.5 mg L^{-1} in 5 h for E1 and in 4 h for E2–E4. In the air side, H_2S concentration firstly increased during the first 1–2 h (in E1 up to 0.35 mg L^{-1}) and then decreased (for E2–E4 to less than 0.1 mg L^{-1}). O_2 and N_2 concentrations in the biogas side increased by 1% and 10%, respectively. CH_4 and CO_2 concentrations in the air side increased by 3% and 10%; respectively (data not shown). Table 4 shows CH_4/CO_2 ratio at the beginning and at the end of the experiments as well as the specific H_2S removal rate. The CH_4/CO_2 ratio was close to 2 at the beginning of the experiments, while it increased to 2.1–2.2 at the end of the experiments. H_2S removal rate increased from 52.7 mg m^{-2} h^{-1} to 90.1 mg m^{-2} h^{-1} in 40 days.

3.2. Continuous experiment

The biogas from UASB reactor with high H_2S concentration (6.9 g m^{-3}) was continuously blown into the BMU in order to test the effectiveness of the biomembrane. The experiment took 15 days. The air side was closed and oxygen was decreasing over the time. Fig. 4A shows the total amount of removed hydrogen sulfide from UASB reactor and the amount of oxygen which stayed in the air side. Fig. 4B shows the concentration of hydrogen sulfide in UASB reactor, and biogas and air side of BMU.

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

3.3. Microbiological analyses

3.3.1. FISH analysis

The summary of FISH results using oligonucleotide probes with various specificity is showed in Table 5. Preliminary screening with less specific probes showed the dominance of Bacteria. No signal was detected with the probe specific for Archaea in all samples. Therefore PCR–DGGE was performed only with primers specific for Bacteria. Probes targeting the major classes of Proteobacteria (ALFmix, BET42a, GAM42a and DELTAmix) gave a positive signal for samples A1–D1. Furthermore *Thiobacillus thioparus* (positive TBD121 and negative TBD1419) was detected in these samples as the only known SOB. FISH analysis showed a significant decrease in diversity during the experiment as the only positive signal for samples A2–D2 was gained with probe HGC (Actinobacteria). Representative FISH pictures are shown in Fig. 5.

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

3.3.2. PCR-DGGE analysis

Bacterial DGGE profiles of two sets of samples are depicted in Fig. 6. The bands which were successfully sequenced are assigned with numbers and the putative affiliation from RDP database is shown. There are significant differences between first (A1–D1) and second (A2–D2) set of samples as was also observed with FISH (Table 5). Samples taken at the beginning of the experiment show much more diversity than those from the end of the experiment. As for the bacteria of sulfur cycle, sequence number 6 showed the closest similarity (99.4 %) with *Thiobacillus thioparus*. This SOB was detected only in the samples from the first set, which is in accordance with the results of FISH.

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

4. Discussion

This paper shows the ability of biomembrane to effectively remove H_2S from biogas while restricting biogas contamination with nitrogen. Biomembrane was used in BMU for both, batch and continuous experiments. While in batch experiments (E4) the specific H_2S removal was as high as 2.16 g H_2S m^{-2} d^{-1}, the maximum specific H_2S removal
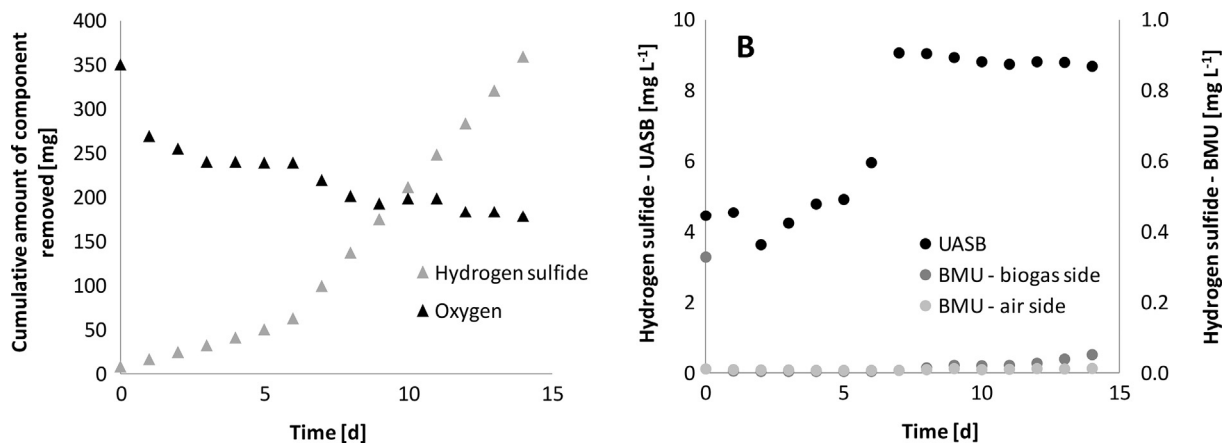
Fig. 4. The results of continuous process. A – The removed amount of H_2S and O_2. B – The concentration of H_2S in UASB and BMU.

Table 5
Results of FISH analysis; ALFmix = ALF1B + ALF968, DELTAmix = DELTA495a–c + competitors. nd = not determined. Samples A1–D1 were taken the second day of the biofilm growth experiment (E1), while samples A2–D2 were taken at the end of continuous experiment.

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

was only 1.10 g H_2S m^{-2} d^{-1} in the continuous experiment. This shows that the potential of the BMU was not fully used because the UASB reactor connected to the BMU did not produce enough biogas with H_2S. However, H_2S was completely removed from the biogas with the H_2S removal efficiency of more than 99% during the continuous experiment.

4.1. Biofilm growth in the biomembrane unit

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

Few studies comparable to this research have been published so far. Camiloti et al. [15] used External Silicone Membrane Reactor (ESMR) to remove sulfide from wastewater. Silicone membrane was connected to the continuously stirred tank reactor and wastewater was re-circulated through the membrane. Oxygen was dosed solely through the membrane wall and it successfully (chemically and biochemically) oxidized sulfide to elemental sulfur and sulfate proving the applicability of membrane for desulfurization. In that case, membrane served as a barrier between air and the wastewater and sulfide was removed in the liquid phase. In the present paper, biomembrane served as a barrier between air and the biogas with a thin biofilm layer on the membrane surface at the biogas side for H_2S removal in the gas phase.

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

The contamination of biogas with nitrogen and oxygen is one of the disadvantages of direct microaeration, where oxygen or air is blown directly into the gas or liquid phase of an anaerobic reactor [16]. Indeed, even small dilution of biogas may complicate its further use in cogeneration unit [17,18]. Nitrogen dilution of biogas in this study was 15% in E1 but it decreased to less than 6% in E4. Since the membrane was not totally covered with biofilm, even lower nitrogen dilution can be expected after complete membrane coverage with biofilm is achieved.

The amount of oxygen in biogas during the direct microaeration can
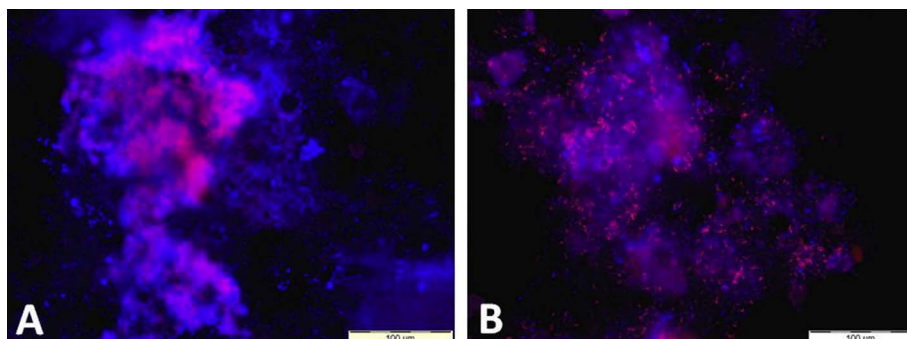

Fig. 5. FISH signal of Cy3-labeled probes (pink) and DAPI stain (blue). A – Sample D1, probe TBD121, B – Sample D2, probe HGC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

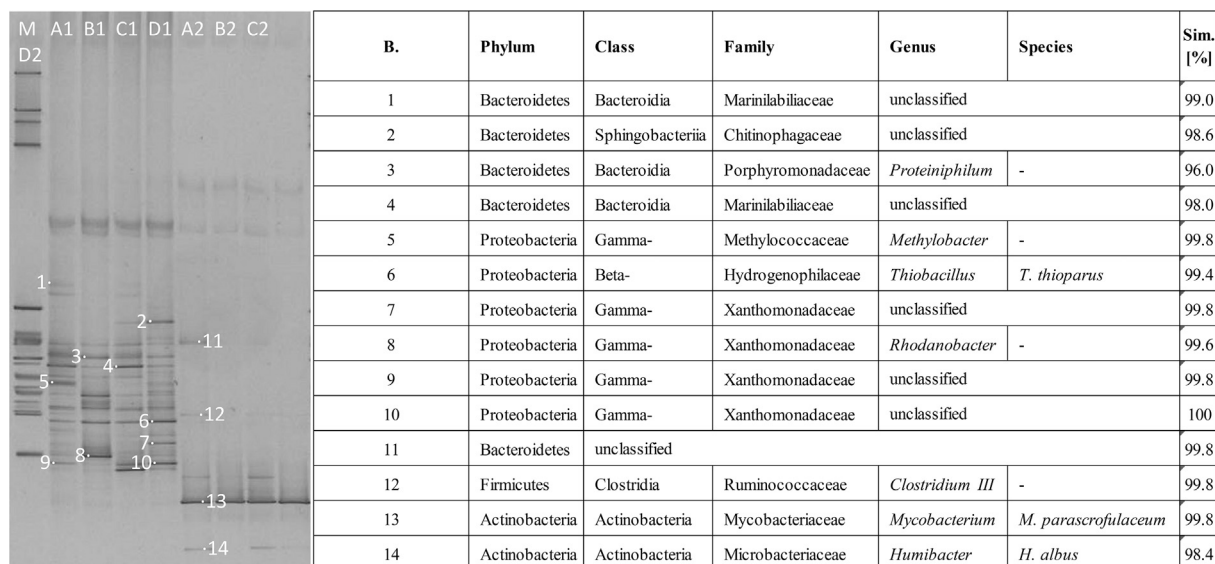| B.	Phylum	Class	Family	Genus	Species	Sim. [%]
1	Bacteroidetes	Bacteroidia	Marinilabiliaceae	unclassified		99.0
2	Bacteroidetes	Sphingobacteriia	Chitinophagaceae	unclassified		98.6
3	Bacteroidetes	Bacteroidia	Porphyromonadaceae	*Proteiniphilum*	-	96.0
4	Bacteroidetes	Bacteroidia	Marinilabiliaceae	unclassified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

Fig. 6. DGGE profile of bacterial community in two sets of samples. Left: Numbers with dots indicate successfully sequenced excised bands. M = marker. The table: Identification of DNA sequences obtained from DGGE by RDP. B. = B and, Sim. = Similarity.

reach up to 4% [11,19,20]. In this paper, the amount of oxygen in biogas side reached 2% in E1, but after 40 days of biofilm growth it was less than 1%. Compared to the direct microaeration, biomembrane with adapted and active SOB biofilm can prevent oxygen contamination of biogas due to microaeration.

In the present manuscript, the air was pumped inside of the membrane with biogas being outside. Another option would have been the biogas inside of the membrane with air around it. However, this would probably cause clogging of the membrane with biofilm and precipitated elemental sulfur.

4.2. Bacterial diversity in the biofilm

The only known SOB detected in the biofilm by both FISH and PCR-DGGE was *Thiobacillus thioparus*. However, this species was found only on second day (E1). This set of samples showed also positive signal with the probe specific for genus *Paracoccus*, which includes species capable of sulfur oxidation [21]. In samples from day 56, genus *Mycobacterium* (phylum Actinobacteria) was detected as dominant (Fig. 5). Recently, strains of *Mycobacterium* capable of S^0 oxidation were isolated from deteriorated sandstone [22]. The suggestion that *Mycobacterium* might be part of S-cycle processes in the reactor is supported by the presence of massive S^0 deposits. *Mycobacteria* were also found in other reactors removing H_2S under similar conditions [23].

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

4.3. Permeability of the membrane for different biogas components

The permeability order of the components in Setup I and II was $H_2S > CO_2 > CH_4 > O_2 > N_2$ with H_2S being the fastest component to move through the membrane and N_2 the slowest. The permeation order of $H_2S > CO_2 > CH_4$ coincides with the findings reported by Kraftschik et al. [24]. Moreover, permeability values for N_2, O_2, CH_4 and CO_2 in PMDS reported in the literature (Table 6) follow the same permeation order ($CO_2 > CH_4 > O_2 > N_2$), with the exception of Tremblay et al. [25] who reported a higher permeation for N_2 than for CH_4.

As shown in Table 6, the values reported for the permeability of gases through silicone rubber vary greatly from one researcher to

Table 6
The comparison of N_2, O_2, CH_4, and CO_2 permeability [Barrer] in PMDS membranes.

Reference		N_2	O_2	CH_4	CO_2	H_2S
Merkel et al. (2000)		400	800	1200	3800	–
Javaid (2005)		460	–	1452	–	–
Tremblay et al. (2006)		180	–	90	1300	–
Basu et al. (2010)		250	500	800	2700	–
This study	Setup I	210	500	800	2550	3410
	Setup II	160	490	890	2660	3430

another. It has been reported that transport properties of a membrane can change depending on whether the experiment was carried out with a pure gas or a mixture of two or more gases [26]. This fact could have caused the difference observed between the permeability values reported in previous works and the ones obtained in this one. Calculations in the present paper were done based on the behavior of a mixture of gases, while in previous papers the values were most often calculated from experiments with pure gases.

4.4. Further challenges

Many researchers have identified biofilm control as the most challenging aspect of operating applications using biomembrane. Excessive biofilm growth will not only cause non-uniform flow distribution and channeling, but also the inhibition of substrate or gas diffusion, eventually deteriorating the system performance [27]. To determine the effect of a thicker biofilm layer or sulfur accumulation on the membrane on the transfer of gases must be examined. The control of the elemental sulfur deposition on the membrane and sulfur harvesting is another research challenge.

5. Conclusions

The ability of biomembrane unit to remove H_2S from biogas has been shown:

- In batch experiments, specific H_2S removal was 2.16 g m^{-2} d^{-1}.
- In continuous experiment, specific H_2S removal rate reached 0.98 g m^{-2} d^{-1} on average with H_2S removal efficiency of more than 99%. Methane losses accounted for 7%.
- Methane losses and nitrogen and oxygen biogas contamination

decrease with increasing membrane coverage with biofilm.
- Light yellow deposits on the membrane indicated elemental sulfur formation.
- *Thiobacillus thioparus* was identified by FISH and PCR-DGGE in all four biofilm samples taken at the beginning of biofilm growth experiment).
- Gases permeation through membrane decreased in order: $H_2S > CO_2 > CH_4 > O_2 > N_2$.

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