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High rate sulfate reduction in a submerged anaerobic membrane bioreactor (SAMBaR) at high salinity

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Abstract

Sulfate reduction in salt rich wastewaters (50 g NaCl L^{-1} and 1 g $MgCl_2 \cdot 6H_2O$ L^{-1}; conductivity 60–70 mS cm^{-1}) was investigated in a 6 L submerged anaerobic membrane bioreactor (SAMBaR) and inoculated solely with the halotolerant sulfate reducing bacterium *Desulfobacter halotolerans*. The SAMBaR was fed with acetate and ethanol at organic loading rates up to 14 g COD L^{-1} day^{-1} in excess of sulfate (COD/SO_4^{2-} of 0.5) and operated at pH 7.2 ± 0.2 and a hydraulic retention time (HRT) from 8 to 36 h. A sulfate reduction rate up to 6.6 g SO_4^{2-} L^{-1} day^{-1} was achieved in the SAMBaR operating at a flux of 17.1 L m^{-2} h^{-1}, which resulted in a HRT of 9 h including the backflow of permeate used for backflushing. The fairly constant very high specific sulfate reduction rate of 5.5 g SO_4^{2-} g VSS^{-1} day^{-1} showed that the performance of the SAMBaR was limited by the low amount of biomass (0.85 g VSS L^{-1}) present in the reactor at the end of the experiment. It was shown that sulfate reducing submerged anaerobic membrane bioreactors can be operated over extended periods of time without chemical cleaning of the membranes at a certain fixed flux if this flux is substantially below the nominal critical flux determined experimentally (18–21 L m^{-2} h^{-1}). Intermittent operation as well as backflush of the membranes were shown to slow the fouling in the membranes. Frequent backflush (e.g. 1 min each 10 min) is the suggested operational strategy to minimize fouling in anaerobic MBRs.

Keywords: Sulfate reduction; High salinity; Submerged anaerobic membrane bioreactor; Halotolerant SRB; *Desulfobacter halotolerans*; Acetate; Ethanol

1. Introduction

Biomass retention is one of the most important aspects of modern anaerobic technology. Uncoupling of the hydraulic retention time (HRT) and cell retention time by self-aggregation (e.g. granular sludges) or biofilm formation is essential for the successful operation of conventional high rate anaerobic bioreactors [1,2]. Conventional anaerobic reactors, however, are less suited for the introduction of a particular metabolic capacity via the addition and retention of specialized microorganisms, as the added microorganisms mostly do not entrap or immobilize the granules or biofilms and are washed out from granular sludge or biofilm systems. The unsuccessful immobilization of specific strains into reactor biomass has been reported in fluidized bed [3], upflow anaerobic granular sludge bed (UASB) [4] and hybrid (UASB + packed bed) [5] reactor systems. A complete retention of all microorganisms in the bioreactor, including newly added bacterial species with a specific metabolic capacity, can be achieved in anaerobic membrane bioreactors. In addition, membrane bioreactors (MBR) are not dependent on granulation or biofilm formation, so that MBRs can also be operated with cell suspensions or flocs with poor settling characteristics. Thus, inoculation of the MBRs with a pure culture or a combination of known bacterial species can be performed without any risk of their washout. This is of particular interest for biological systems that depend on the retention of a large population of slow growing microorganisms that perform a specific metabolism, even at a very low HRT.

Anaerobic membrane bioreactors might offer advantages in terms of volumetric loading rates (resulting in a small foot-

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print reactor), effluent quality and process stability [6]. In practice, anaerobic biomass can be sensitive to high salinity environments. High salt concentrations are known to significantly reduce the treatment efficiency of methanogenic and sulfidogenic conventional mesophilic [7,8] and thermophilic [9–11] anaerobic bioreactors. Indeed, high osmolarity environments trigger rapid fluxes of cell water, causing a reduction in turgor and dehydration of the cytoplasm [12]. Thus, the successful operation of sulfate reducing bacteria (SRB)-based bioreactors operating at high salinity requires the retention of halophilic SRB in anaerobic reactors.

The ability of halophilic anaerobic microorganisms to degrade different organic substrates has been reviewed and appears that only a few easily degradable substrates such as simple sugars and amino acids can be fermented via dissimilatory sulfate reduction [13,14]. The upper limit of salinity at which dissimilatory sulfate reduction has been observed is 240 g NaCl L^{-1}, for the incomplete lactate, ethanol and pyruvate oxidizer *Desulfohalobium retbaense* [15]. The highest salinity for the complete oxidation via sulfate reduction reported so far is around 130 g NaCl L^{-1} for the acetate oxidizer *Desulfobacter halotolerans* [16]. The incorporation of such a halophilic SRB in a membrane bioreactor would greatly extend the application of desulfurization to wastewater treatment systems that can presently not be treated biologically.

The aim of this work was to assess the performance of a sulfate reducing submerged anaerobic membrane bioreactor (SAMBaR) fed with acetate and ethanol as the sole electron donors operated at high salinity (50 g NaCl L^{-1} and 1 g $MgCl_2{\cdot}6H_2O$ L^{-1}; conductivity 60–70 mS cm^{-1}) and inoculated with the pure culture *Desulfobacter halotolerans*. The major limitation to the use of membranes is the continuous reduction in permeate flux by membrane fouling and the operational costs associated with it [17]. The reduction in permeate flow is known to be the main factor in determining the economic feasibility of membrane processes [17]. Therefore, different operational procedures for the minimization of fouling were studied, including the determination of the critical flux and the assessment of the influence of flux stoppage and membrane backflush on the increase in transmembrane pressure (TMP).

2. Materials and methods

2.1. Continuous experiments

2.1.1. Experimental setup

A submerged anaerobic membrane bioreactor (SAMBaR) of 6 L (1 m high, internal diameter 10 cm) was operated during 92 days in order to study the feasibility of high rate sulfate reducing processes at high salinity (50 g NaCl L^{-1} and 1 g $MgCl_2{\cdot}6H_2O$ L^{-1} in the influent; 60–70 mS cm^{-1}). The SAMBaR (Fig. 1) was equipped with a set of five cylindrical polysulfone membranes (Triqua B.V., Wageningen, The Nethelands) with a total effective surface of 0.07 m^2 (Fig. 1). The mean pore size of 0.2 μm guaranteed the uncoupling of the hydraulic retention time (HRT) and the cell retention time. The SAMBaR was equipped with a double wall, through which water, heated in a thermostatic waterbath (Julabo, Seelbach, Germany), was recirculated to maintain the reactor temperature at $33 \pm 1\,^{\circ}C$. This temperature was selected because it is the optimum temperature for the growth of *Desulfobacter halotolerans* [16], used as reactor inoculum.

The pH in the reactor was maintained at 7.25 ± 0.2 (within the optima pH range for growth of *Desulfobacter halotolerans*, [16]) by means of an automatic pH control, adding HCl (1 M) when necessary (Fig. 2B). The pH was measured

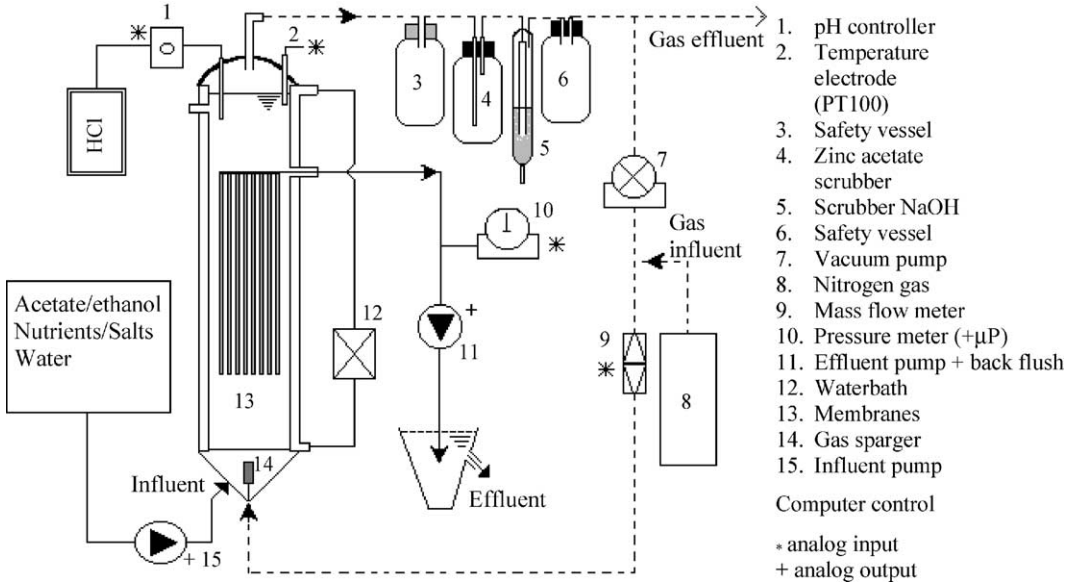

Fig. 1. Schematic representation of the submerged anaerobic membrane bioreactor (SAMBaR).

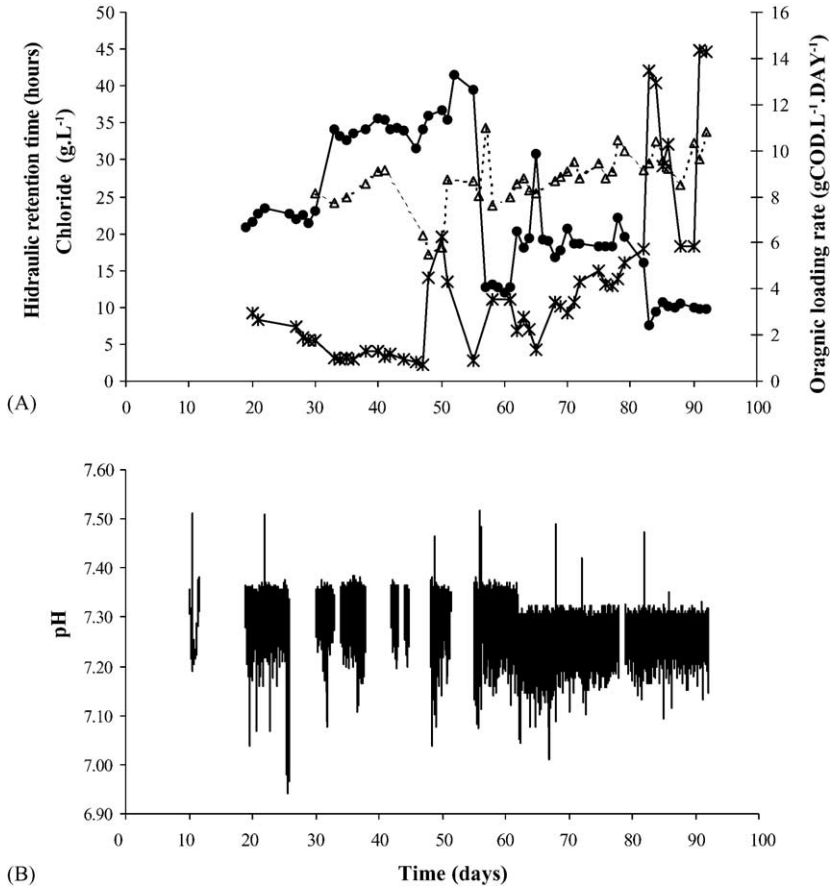

Fig. 2. Evolution of the (A) organic loading rate (∗), hydraulic retention time (●) and chloride concentration (△) and (B) pH applied to the SAMBaR.

with sulfide resistant Hamilton Flushtrode pH-electrodes (Hilkomij B.V., Rijswijk, The Netherlands) connected to an automatic pH controller with two changeable set points to adjust the pH (Elektronika Wageningen, The Netherlands). The pH electrodes were checked and calibrated three times per week.

Nitrogen gas was sparkled in the bottom of the SAMBaR (at a gas loading rate of 14 L $L_{reactor}^{-1}$ h^{-1}) in order to promote reactor mixing, to strip off the sulfide and to prevent the fast accumulation of foulants onto the membrane surface [17]. A vacuum pump was installed for the recirculation of the nitrogen gas. As sulfate reducing systems do not produce large volumes of biogas (sulfide is a quite soluble gas and was stripped out of the recycle gas flow, see Fig. 1), recirculation of the nitrogen gas was adopted. Four bottles were mounted in the recirculation gas line. The first bottle (1 L) was used for the collection of the reactor bulk that was eventually transported with the gas out of the SAMBaR. The second bottle (1 L) was filled with a zinc acetate solution to selectively retain the gaseous H_2S. The third bottle (0.5 L) was filled with a 1 M NaOH solution to remove carbon dioxide (CO_2) from the gas prior to its recirculation into the reactor. The fourth bottle (1 L) was used to avoid the alkaline solution to flow into the vacuum pump. The effluent gas was led through a waterlock placed between the vacuum pump and the fourth bottle. The scrubbed (H_2S- and CO_2-free) recirculation gas (thus essentially N_2) was finally combined with the influent N_2 gas and led into the reactor through a gas sparkler (Fig. 1). A mass flow meter was placed before the reactor inlet in order to determine the gas sparkling rate (Fig. 1).

The influent flow, consisting of substrate, micro- and macro-nutrients (diluted with demineralized water), was provided by means of a computer controlled peristaltic pump (Watson-Marlow 501 U, Falthmouth, Cornwall, UK). Effluent was generated by operating a computer controlled peristaltic pump (Watson Marlow 501 U) after the membrane module, thus regulating the flux over the membranes. The flow rate was measured by weighing the produced permeate on an electrical balance. A pressure transducer (Figs. 1–10, Farnell, BTE6000 series 0–10 V output, Germany) was placed in line between the membranes and the effluent peristaltic pump so that the pressure applied to the membranes was recorded. The transmembrane pressure (TMP) was calculated as the difference between the pressure reference value of 1.08 bar (sum of the atmospheric pressure and the height of the water column on top of the membrane) and the pressure reading of the pressure transducer. Sampling ports were placed in the influent and effluent tube systems in order to collect samples. Temperature, pH, TMP and gas flow signals were sent to a computer, where the data were recorded.

Table 1
Operational procedures applied to the membranes in order to minimize fouling during the operation of the SAMBaR

Operational modes	
Production/relaxation mode	Backflush mode
6 min production (pumps on—flux)	1 min backflush ($Q_{Bf} = 2 \times Q_P$)
2 min relaxation (pumps off—no flux)	2 min production (to compensate the flow backflushed to the SAMBaR)
2 min production (pumps on—flux)	1 min relaxation (pumps off—no flux)
2 min relaxation (pumps off—no flux)	Go to production/relaxation mode sequence
Verification of transmembrane pressure (TMP)	
If TMP < 0.15 bar → production/relaxation mode	
If TMP > 0.15 bar → backflush mode	

Q_{Bf}—flow rate of backflush; Q_P—flow rate of production.

2.1.2. Membrane operational modes

In order to minimize membrane fouling, two distinct operational procedures were applied in the SAMBaR, viz. relaxation/production mode or backflush mode (Table 1). The operational mode was selected depending on the TMP registered. If the TMP was higher than 0.15 bar, the membranes were backflushed with the permeate at a flow two times higher than that normally applied. Otherwise (TMP < 0.15) the reactor operated in the relaxation/production mode (Table 1). Fig. 3 shows a typical 3 h representation of the TMP in relation with the two operational procedures adopted to minimize fouling. Fig. 3 also illustrates the mathematical procedure (linear regression) to calculate the TMP increase rate (defined as dP/dt and proportional to the membrane fouling rate) in the membranes during the experiment.

Whenever a TMP of 0.4 bar was reached, an ex-situ chemical cleaning of the membranes was carried out (as recommended by the membrane supplier). The membrane set was removed from the SAMBaR and immersed in a 1 g L^{-1} hypochlorite (NaOCl) solution for 1 h, followed by another 1 h immersion in 3 g L^{-1} of citric acid ($C_6H_8O_7$) solution. During these immersions, the membranes were backflushed with the solutions at a flux of 5 L m^{-2} h^{-1}. Before placing the membrane back inside the reactor, the membranes were back-

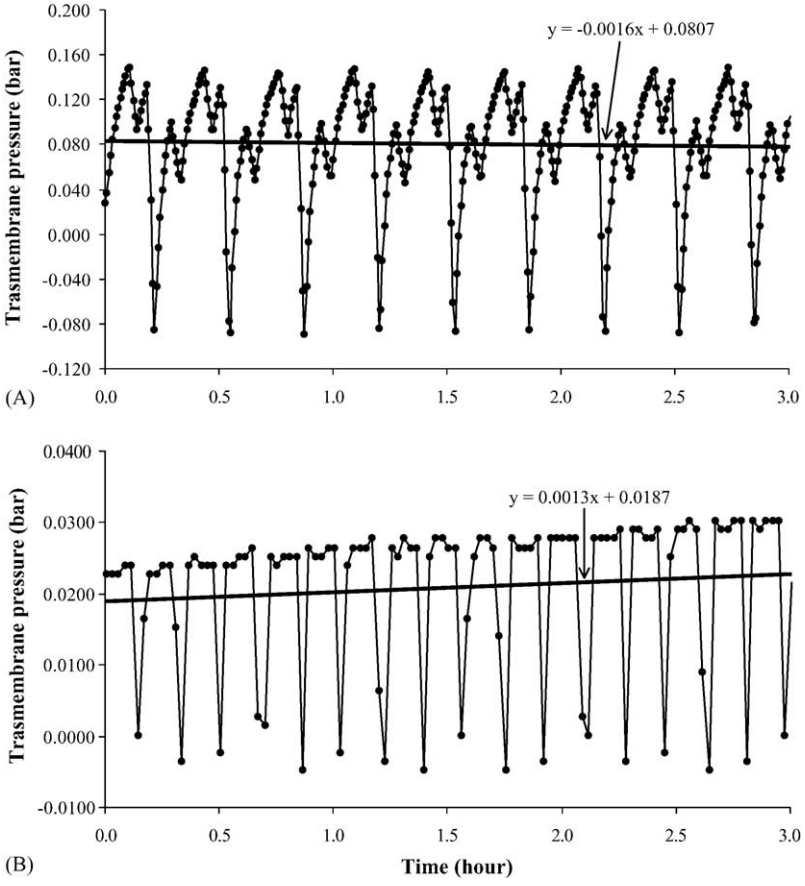

Fig. 3. Typical transmembrane pressure (TMP) variation in function of the SAMBaR operational mode. Note that the linear regression (trend line) allowed to calculate the TMP increase rate.

flushed (at a flux of 5 L m^{-2} h^{-1}) with demineralized water for 1 h in order to remove any residual chemical solution.

2.1.3. Critical flux determination

In this work, the flux-step method was used to determine the critical flux value [18]. The flux was stepwise increased for a fixed duration (10 min) for each increment (3 L m^{-2} h^{-1}), giving a relatively stable TMP at low flux but an ever-increasing rate of TMP increase at higher fluxes. This flux-step method yielded the highest flux for which TMP increase remains stable as the critical flux. The linear regression of the recorded TMP for each flux applied determined the rate of TMP increase. The TMP value was recorded in the computer each 30 s. The critical flux determination was carried out with a suspension (1.5 g VSS L^{-1}) of crushed anaerobic sludge. The test sludge was crushed in order simulate the *Desulfobacter halotolerans* reactor suspension (which takes long time for its growth and is thus ineffective for critical flux tests).

2.1.4. Inoculum

A culture of the mesophilic acetate oxidizing SRB *Desulfobacter halotolerans*, initially cultured in a defined medium [16] and subsequently subcultured in the medium described below, was used as the inoculum in this study. *Desulfobacter halotolerans* strain GSL-Ac1, kindly provided by Prof. Ingvorsen (Aarhus University, Denmark), was enriched from moderate hypersaline sediments in the southern arm of Great Salt Lake (UT, USA) and isolated in a synthetic medium containing 10% NaCl and 1% $MgSO_4 \cdot 7H_2O$ [16]. Strain GSL-Ac1 uses acetate, ethanol and pyruvate as electron donor and carbon source. It is able to reduce sulfate, sulfite and thiosulfate at high salinity (up to 13% NaCl and 4.5% $MgCl \cdot 6H_2O$), but grows optimally around 1–2% NaCl [16]. *Desulfobacter halotolerans* grows at a pH ranging from 6.2 to 8.1 (pH optimum, 6.2–7.4) and the maximum growth temperature is 37 °C (optimum between 32 and 34 °C).

2.1.5. Substrate and medium

Acetate (days 0–79) and ethanol (days 68–92) were supplied as the electron donor and carbon sources, providing an influent COD concentration between 1 and 5.9 g L^{-1}. Sulfate was added to the reactor as sodium sulfate at a COD/SO_4^{2-} of 0.5 (g COD per g SO_4^{2-}), so theoretically all substrate could be degraded via sulfate reduction. Sodium chloride (50 g NaCl L^{-1}) and magnesium chloride (1 g $MgCl_2 \cdot 6H_2O$ L^{-1}) were used as model compounds to increase the salinity of the wastewater. In addition, non-sterilized basal medium containing macro- and micro-nutrients were supplied to the influent at a ratio of 2.22 mL per g COD fed. Basal medium was prepared as described in Vallero et al. [10] and a trace element (4.5 mL L^{-1}) solution was prepared according to Zehnder et al. [19]. From day 68 onwards the basal medium was further supplied with a vitamin solution (50 mg L^{-1} biotin and 50 mg L^{-1} 4-aminobenzoate). Both the basal medium and substrate stock solutions were prepared using demineralized water.

Desulfobacter halotolerans was cultivated in autoclaved (30 min at 121 °C) mineral medium. This mineral medium differed from the basal medium supplied in the reactor in that it was further supplemented with a 1 mL vitamin solution according to Stams et al. [20] and buffered at pH 7.0 using 4 g L^{-1} $NaHCO_3$ and 1.6 bar of N_2/CO_2 (80/20%). An inoculum size of 5% (v/v) was used.

2.1.6. Experimental design

The SAMBaR was operated for 92 days at a high salinity of 50 g NaCl L^{-1} and 1 g $MgCl_2 \cdot 6H_2O$ L^{-1} (about 60–70 mS cm^{-1}). The SAMBaR was inoculated with 1 L (17% reactor volume) of a pure culture of *Desulfobacter halotolerans* growing in the exponential phase (resulting in a reactor VSS concentration of 0.018 g L^{-1}). The organic loading rate and the HRT of the SAMBaR varied as a function of the flux and the strategy applied for the maintenance of the membrane, viz. relaxation/production or backflush mode (Table 1 and Fig. 2A). The reactor was operated in batch mode (no effluent production) for 19 days till a drop in the redox potential to values around −240 mV and a significant sulfide production were observed. On day 19, the set of membranes was installed in the reactor (Fig. 1) and a flux (J) of 4.7 L m^{-2} h^{-1} was applied, corresponding to a HRT of about 24 h when operating in relaxation/production mode (Fig. 2A). Between days 25 and 26, the flux occasionally increased to 32 L m^{-2} h^{-1}, which caused the mechanical collapse of the membranes (due to the acute increase in the transmembrane pressure). New membranes were placed in the SAMBaR and the same flux of 4.7 L m^{-2} h^{-1} was applied till day 54 (when the membranes were chemically cleaned), resulting in a HRT of about 24 or 36 h when operating in, respectively, relaxation/production or backflush mode (Fig. 2A). On day 55, the flux was increased to 9.4 L m^{-2} h^{-1}, resulting in a HRT of about 12 or 18 h when operating in, respectively, relaxation/production or backflush mode. The membranes were chemically cleaned on day 82, before further increasing the flux to 17.1 L m^{-2} h^{-1}, resulting in a HRT of about 10 h when operating including backflush mode (Fig. 2A). On days 85 and 89, the membranes were mechanically cleaned by gentle displacement of the cake layer deposited on the membrane with a brush.

2.1.7. Analysis and chemicals

The gas composition was measured on a gas chromatograph (Hewlett-Packard HP 5890, Palo Alto, USA) according to Weijma et al. [21]. Liquid samples of 3 mL were frequently taken from the influent and reactor bulk for analysis. Volatile fatty acids (VFA) and alcohols were analyzed on a gas chromatograph (Hewlett-Packard HP 5890A, Palo Alto, USA) according to Weijma et al. [21]. Sulfide was measured according to Trüper and Schlegel [22]. Occasional samples (25 mL) were taken from the reactor bulk in order to determine the amount of volatile suspended solids (VSS) and total suspended solids (TSS) inside the SAMBaR, analyzed

according to standard methods [23]. The electrical conductivity (EC) or the reactor mixed liquor was measured using a standard EC meter (WTW LF 196, Weilheim, Germany). Sulfate was measured on a DX-600 ion chromatograph (IC) system (Dionex Corporation, Salt Lake City, USA). The specific sulfate elimination rate was calculated from the total amount of sulfate reduced divided by the concentration of VSS in the SAMBaR. The particle size distribution (PSD) was measured by laser diffraction analysis with an accuracy in the submicron (0.05 μm) range (Coulter LS230, Beckman Coulter, USA). A reactor sample was harvested on day 64 for microscope observations (Olympus BH-2). All chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany).

3. Results

3.1. Reactor performance

3.1.1. Reactor performance in batch mode (days 0–19)

During the start-up of the SAMBaR in batch mode (no membranes and no gas sparkling), the reactor acetate concentration decreased from 1000 to 270 mg COD L^{-1} in 12 days (Fig. 4A). The sulfate concentration decreased from 3200 to 1880 mg SO_4^{2-} L^{-1} in the same period (Fig. 5A), resulting in an increase of the sulfide concentration up to a maximum of 105 mg L^{-1} on day 17 (Fig. 5C).

3.1.2. Reactor performance when operating at flow-through conditions (days 20–92)

After switching to flow-through mode, an acetate removal efficiency of 80% was obtained on day 36 (Fig. 4A). During this period, the SAMBaR was operated in backflush mode at a flux of 4.7 L m^{-2} h^{-1}, corresponding to a HRT of about 34 h (Fig. 2A). The acetate removal efficiency dropped to about 60% between days 36 and 44 due to a lack of micronutrients in the feed (Fig. 4A). After the micro-nutrient supply was resumed on day 44, full acetate removal was achieved on day 47 (Fig. 4A). From day 47 to 50, the effluent acetate concentration increased due to an unintentional increase in the influent acetate concentration (Fig. 4A).

The sulfate removal efficiency increased continuously till day 55, reaching a maximum sulfate removal efficiency of 85% on day 55 (Fig. 5A). Upon increasing the flux to 9.4 L m^{-2} h^{-1} (resulting in a HRT decrease from 39 to 12 h) on day 55, the sulfate removal efficiency dropped to around 20% (Fig. 5A). Note that this was an effect of decreasing the HRT, as the reactor kept working at a fairly constant sulfate elimination rate of around 1.5 g SO_4^{-2} L^{-1} day^{-1} till day 61 (Fig. 5B). After the flux was increased to 9.4 L m^{-2} h^{-1} on day 55, the acetate removal efficiency also decreased to 15% (Fig. 4A). In addition, neither biotin nor 4-aminobenzoate, essential vitamins required for the growth of *Desulfobacter halotolerans* [16], were added to the SAMBaR till day 68, which may have contributed to the performance deterioration of the reactor. Indeed, a remarkable increase in the sulfate elimination rate of the SAMBaR was observed after the addition of vitamins (biotin and 4-aminobenzoate), and the replacement of the substrate acetate by ethanol on day 68 (Fig. 5B), resulting in an increase in the sulfate removal efficiency from 7 to 68% on, respectively, days 65 and 82 (Fig. 4A).

The addition of ethanol and vitamins to the system not only boosted the sulfate removal efficiency, but also the acetate removal efficiency. Although apparently there was no acetate removal from day 72 onwards (Fig. 4A), the calculated amount of acetate present in the reactor mixed liquor, based on the stoichiometry of the ethanol oxidation to acetate (Eq. (1)), shows that there was a net removal of acetate till day 79 (Fig. 4A).

$$2C_2H_5OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad (1)$$

Complete ethanol removal occurred 10 days after its addition into the SAMBaR (Fig. 4B). The full removal of ethanol at a maximal concentration of 5950 mg COD L^{-1} on day 91 (Fig. 4B) at a flux of 17.1 L m^{2-} h^{-1} (HRT of 9.7 h; Fig. 2A) indicates that the reactor was operated at underloaded conditions. In addition, it shows that the biomass had a higher affinity for ethanol than for acetate (Fig. 4A versus Fig. 4B). Under these operational conditions, a maximal sulfate elimination rate of 6.60 g SO_4^{-2} L^{-1} day^{-1} was achieved on day 92 (Fig. 4B). The sulfate reduction is correlated to ethanol oxidation, as evidenced by the sharp drop in the sulfate elimination rate between days 86 and 91 (Fig. 5B), when the influent ethanol concentrations were much lower (Fig. 4B). This is confirmed by the results in Fig. 4C, which shows that the stoichiometry of ethanol utilization closely followed that of Eq. (1), with ∼0.5 mol sulfate reduced, and ∼1.0 mol acetate produced per mole of ethanol utilized.

Due to the high gas loading rate (to clean up the membranes), the sulfide concentration remained rather constant at concentrations around 80–100 mg L^{-1} during the whole experimental run (Fig. 5C). An exceptional sulfide peak manifested around day 47 (Fig. 4C), when the gas load in the SAMBaR was unintentionally very low.

3.2. Reactor biomass characteristics

3.2.1. Solids concentration and specific biomass activity

The TSS and VSS concentration in the mixed liquor present in the reactor could not be measured during the first days of SAMBaR operation, as this required a too big reactor liquid sample (due to the dilute nature of the freshly inoculated reactor mixed liquor at the beginning of the experiment) for the solids determination. The TSS and VSS concentrations increased from day 55 onwards till a maximal concentration of around 0.85 (±0.02) g VSS L^{-1} and 1.75 (±0.10) g TSS L^{-1} on day 91 (Fig. 6A). The VSS/TSS ratio remained fairly constant at around 0.4 (±0.09), except

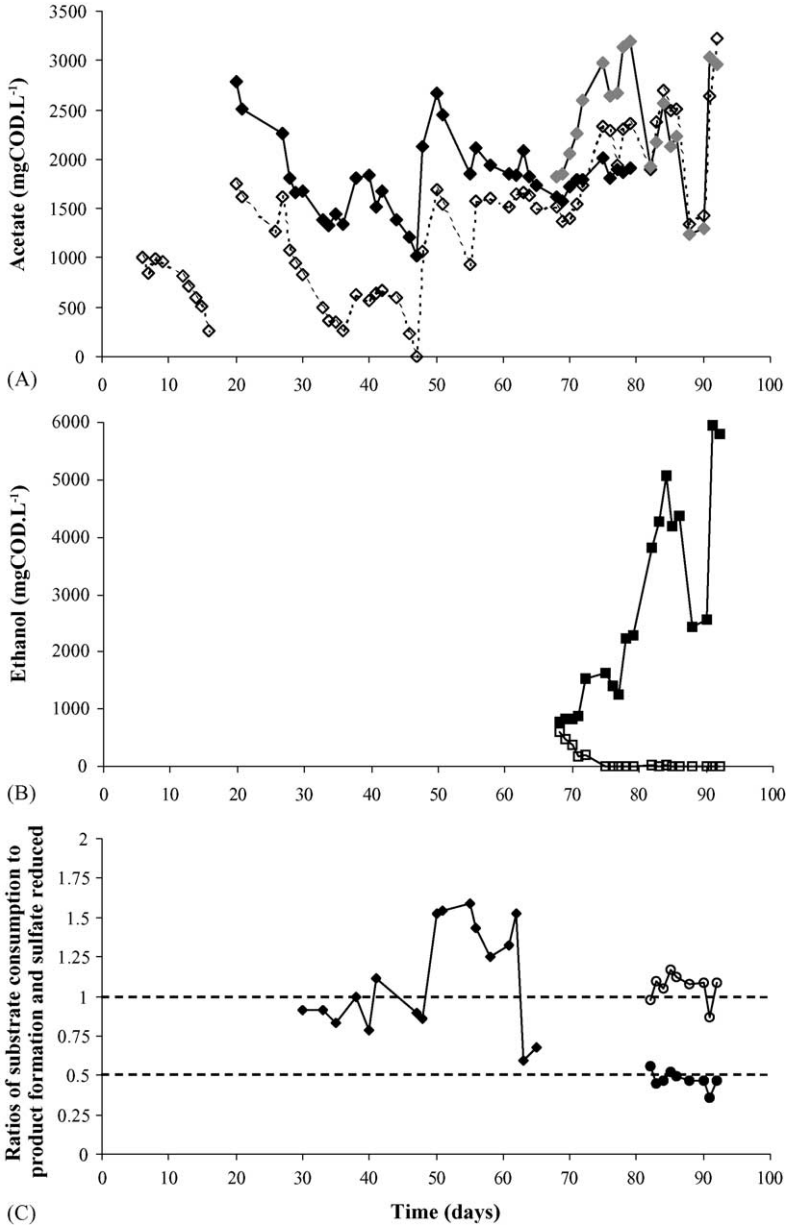

Fig. 4. Process performance of the SAMBaR. (A) Evolution of the acetate concentration in the influent (♦), effluent (◊) and calculated reactor acetate concentration based on the stoichiometry of incomplete ethanol oxidation (◆). (B) Evolution of the ethanol concentration in the influent (■) and effluent (□). (C) Evolution of the stoichiometrical molar ratio of acetate consumption to sulfate reduced (♦), acetate produced to ethanol consumed (◯) and sulfate reduced to ethanol consumed (●).

at the beginning of the experiment and on day 68, when the VSS/TSS ratio was equal to 0.10 (±0.01) and 0.27 (±0.02), respectively. The specific activity of the sludge was very high with values of 5.5 (±1.0) g SO_4^{2-} g VSS^{-1} day^{-1} between days 55 and 92 experiment (Fig. 6B), irrespective of the sulfate removal efficiency (Fig. 5A).

3.2.2. Particle size distribution

Particle size distribution measurements of the inoculum show that 90% of the particles were bigger than 38 μm and particles smaller than 0.2 μm, the size of the membrane pore, were absent (Fig. 7A). After 50 days of operation, 90% of the particles were bigger 70 μm, whereas only 0.31% of the particles were smaller than 1 μm (Fig. 7B) and only 0.0043% of the particles were smaller than 0.2 μm (Fig. 7B). The mean particle size of the inoculum and the SAMBaR mixed liquor on day 50 was, respectively, 370.8 and 463.2 μm, with no particles bigger than 2000 μm (the upper detection limit of the equipment). The SAMBaR sludge flocs contained many blackish spots, most probably metal precipitates. Surprisingly, the particle size distribution could not be measured anymore by laser diffraction on day 56, as a small fraction of the particles surpassed the upper detection limit of the equipment (2000 μm).

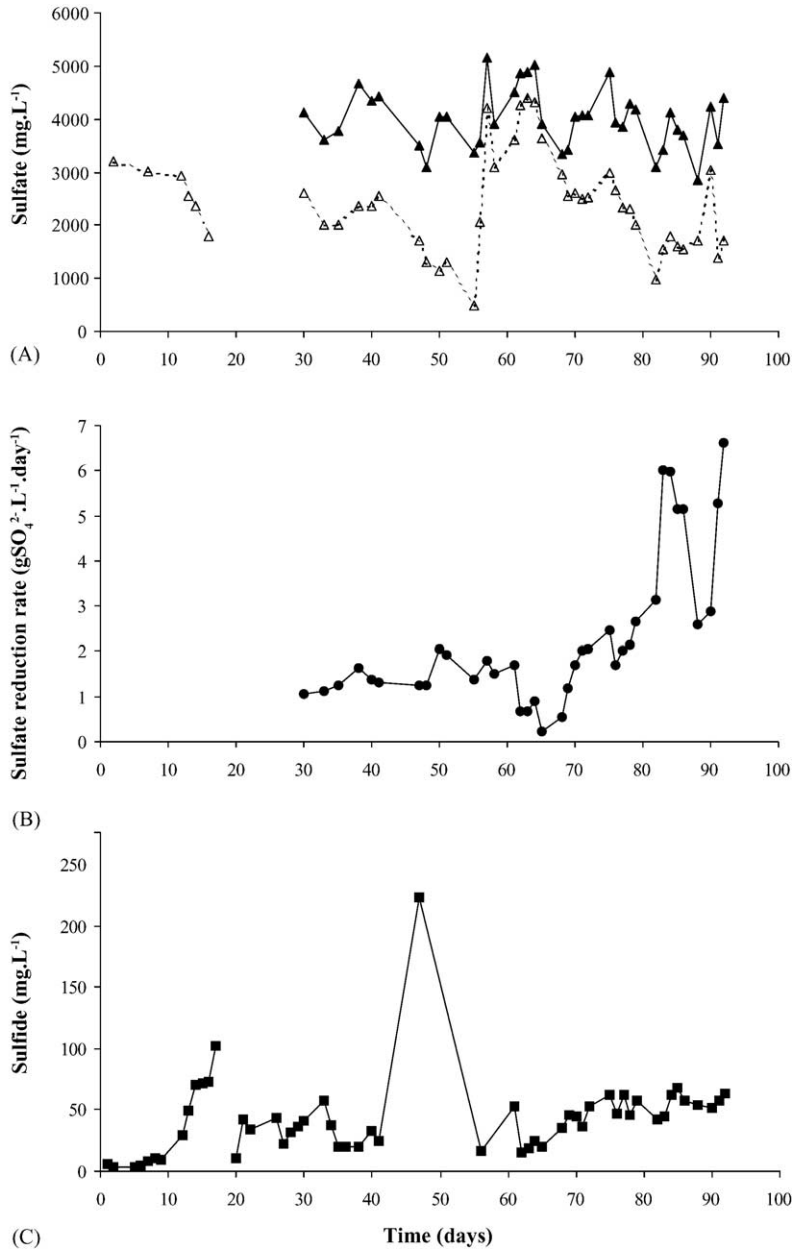

Fig. 5. Process performance of the SAMBaR. (A) Evolution of the sulfate concentration in the influent (▲) and effluent (△). (B) Evolution of the sulfate reduction rate (●). (C) Evolution of the sulfide concentration in the effluent (■).

3.2.3. Microscopic observations

Although the SAMBaR sludge contained bacteria other than *Desulfobacter halotolerans*, it still accounted for most of the microorganisms present (data not shown). In addition, many crystals, presumably metal sulfides, were present in the SAMBaR sludge.

3.3. Membrane operation and fouling experiments

3.3.1. Critical flux and TMP increase rate dependence on flux

No severe increase in the TMP was observed as a function of the stepwise increase of the flux (up to 80 L m^{-2} h^{-1}) when only basal medium was present in the SAMBaR (data not shown). It was also observed that each increase in the flux (3 L m^{-2} h^{-1}) produced an increase in the TMP (data not shown). In addition, a relatively low TMP (0.15 bar) was observed for the maximal applied flux of 80 L m^{-2} h^{-1} when only basal medium was added to the SAMBaR (data not shown).

According to Chen et al. [24], the critical flux is defined as the last flux step at which the TMP remains constant. A closer examination of the initial flux steps, however, reveals that the TMP never remains absolutely constant at any point during the test (Fig. 8A). Even a flux as low as 3 L m^{-2} h^{-1} produced a TMP increase rate (dP/dt) of 3.7 mbar day^{-1}

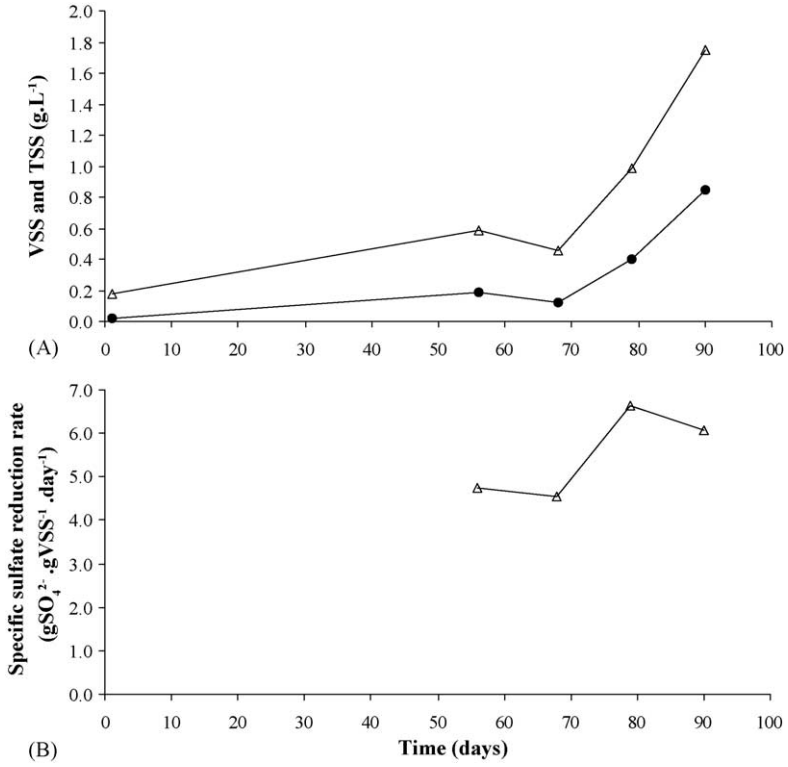

Fig. 6. Process performance of the SAMBaR. (A) Evolution of the total (△) and volatile (●) suspended solids in the reactor mixed liquor. (B) Evolution of the specific sulfate reduction rate (△).

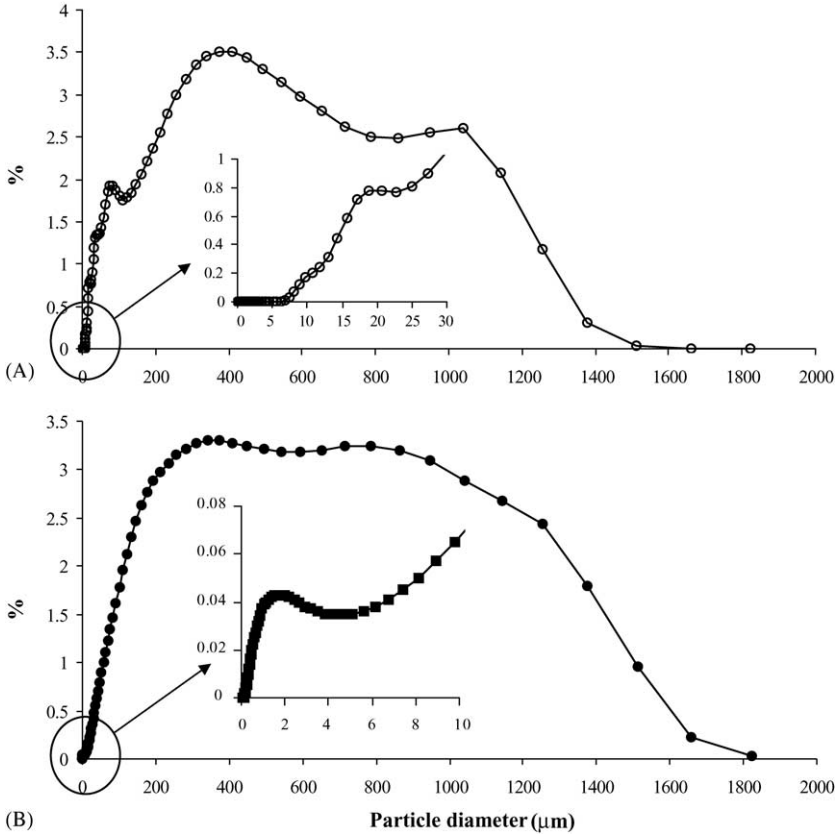

Fig. 7. Particle size distribution. (A) Reactor inoculum consisting of a pure culture of *Desulfobacter halotolerans*. (B) Reactor mixed liquor sampled on day 56.

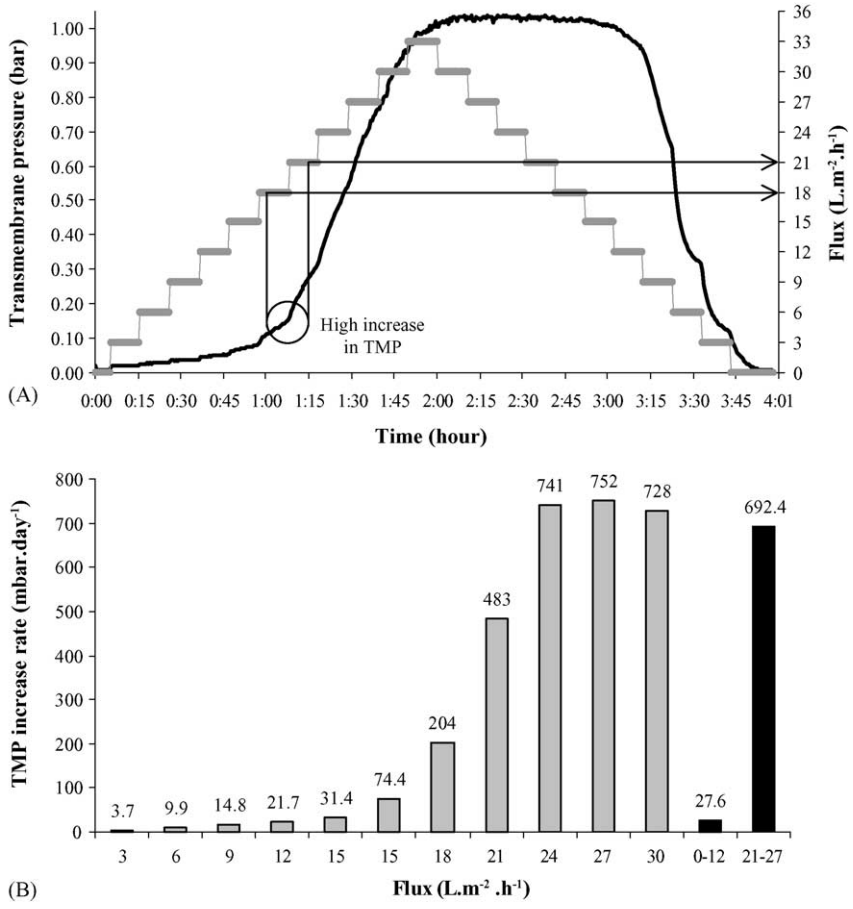

Fig. 8. Critical flux experiments. (A) Evolution of the transmembrane pressure in function of the applied flux. (B) Calculated TMP increase rate in function of the applied flux.

(Fig. 8B). Fluxes higher than 18–21 L m^{-2} h^{-1} caused a rapid increase in the TMP (Fig. 8A), resulting in very high dP/dts (Fig. 8B). As such, the value of the critical flux for the crushed sludge was determined to be between 18 and 21 L m^{-2} h^{-1}, corresponding to a TMP of about 0.16–0.18 bar (Fig. 8A). An overall dP/dt of 27.6 mbar day^{-1} was obtained for fluxes below 15 L m^{-2} h^{-1}, whereas a very high dP/dt of 692.4 mbar day^{-1} was obtained when operating at fluxes higher than 18 L m^{-2} h^{-1} (Fig. 8B). The maximal TMP of 1 bar was reached at a flux of 30 L m^{-2} h^{-1} and the TMP started to decrease only when the flux was diminished to 15 L m^{-2} h^{-1} (Fig. 8A).

3.3.2. Occurrence of membrane fouling in the SAMBaR

3.3.2.1. Flux of 4.7 L m^{-2} h^{-1}. Fig. 9A shows the full set of TMP values obtained from the operation of the SAMBaR. From day 19 till day 22 a constant flux of 4.7 L m^{-2} h^{-1} was applied to the reactor (Fig. 9B), resulting in a TMP increase rate (dP/dt) of about 13 mbar day^{-1} (Table 2). On day 22, however, a constant permeate flux (no relaxation or backflush) was imposed to the reactor, resulting in an immediate increase of the dP/dt to 137 mbar day^{-1} (Table 2). The relaxation/production operational mode was resumed on day 23. On this day, however, a low influent gas load (3 L L^{-1} h^{-1}) was imposed to the reactor, resulting in a dP/dt of around 92 mbar day^{-1} (Table 2).

The SAMBaR started to operate in backflush mode on day 25 (Fig. 9B). Due to improper input of information in the computer control, occasional fluxes of 32 L m^{-2} h^{-1} were imposed to the membranes during the 2 min reserved for the compensation of flow after backflushing the membranes (see Section 2). These occasional high fluxes caused an immediate increase in the TMP to values around 1 bar, which caused an irreversible mechanical collapse of the membrane (Fig. 9B). Note that this occasional flux of 32 L m^{-2} h^{-1} is higher than the critical flux of 18–21 L m^{-2} h^{-1} determined for crushed anaerobic sludge (Fig. 8A).

The SAMBaR operated in relaxation/production mode from day 26 to 32 (after replacing the membrane on day 26), resulting in a dP/dt of around 18.5 mbar day^{-1} (Table 2). The SAMBaR operated in backflush mode between days 33 and 54 (Fig. 9B). Surprisingly, the TMP diminished in the first days after switching to backflush mode, as indicated by the negative dP/dt of −15 mbar day^{-1} (Table 2). On day 48, however, the dP/dt started to increase again to values around 4.5 mbar day^{-1} (Table 2).

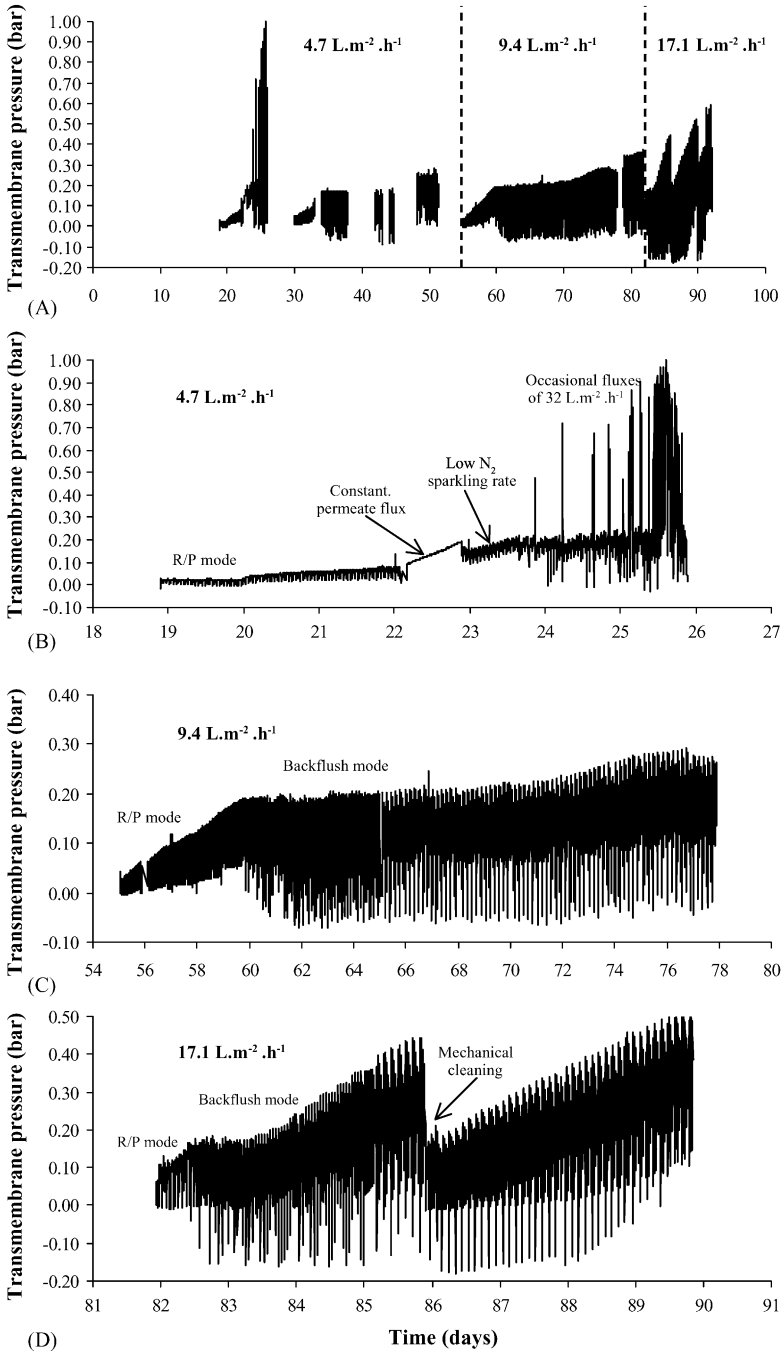

Fig. 9. Evolution of the transmembrane pressure. (A) TMP values during the whole experimental run. (B) TMP values between days 19 and 26 (flux of 4.7 L m^{-2} h^{-1}). (C) TMP values between days 54 and 78 (flux of 9.4 L m^{-2} h^{-1}). (D) TMP values between days 82 and 90 (flux of 17.1 L m^{-2} h^{-1}). Note the differences in both the transmembrane pressure and time scales.

3.3.2.2. Flux of 9.4 L m^{-2} h^{-1}. The reactor operated in relaxation/production mode from day 55 to 60, resulting in a dP/dt of around 27.8 mbar day^{-1} (Table 2). As for when operating at a flux of 4.3 L m^{-2} h^{-1}, the TMP diminished in the first days after the operation of the SAMBaR switched to backflush mode on day 61 (Fig. 9C), as indicated by the negative values of the dP/dt of −50 mbar day^{-1} (Table 2). On day 62, however, the dP/dt started to increase again, to values around 5.1 and 10.3 mbar day^{-1} between days 62 and 70 and between days 71 and 81, respectively (Table 2).

3.3.2.3. Flux of 17.1 L m^{-2} h^{-1}. The reactor operated in relaxation/production for only 1 day and experienced a high dP/dt of 129 mbar day^{-1} (Table 2). Again, the TMP dropped after the operation of the SAMBaR switched to backflush mode on day 83 (Fig. 9D), as indicated by the negative values of the dP/dt (−41.2 mbar day^{-1}; Table 2). The dP/dt started to

Table 2
TMP increase rates (dP/dt; mbar day^{-1}) according to the flux and the different operational procedures to minimise fouling

$J = 4.7$ L m^{-2} h^{-1}			$J = 9.4$ L m^{-2} h^{-1}				$J = 17.1$ L m^{-2} h^{-1}			
Production/relaxation mode		Backflush mode/chemical cleaning[a]	Production/relaxation mode		Backflush mode/chemical cleaning[a]		Production/relaxation mode		Backflush mode/chemical cleaning	Backflush mode/mechanical cleaning[b]
Day	dP/dt	Day	dP/dt		Day	dP/dt	Day	dP/dt	Day	Day	dP/dt
10	13	33–38	−15.3		55–60	27.8					
22	137[c]	48–54	4.5								
23	92[d]										
26–32	18.5										
					61	−50.5	82	129	83	−41.2	86–89	70.2
					62–70	5.1			84	39	90–92	104.3
					71–81	10.3			85	75.4		

J—flux; R/P—relaxation/production.
[a] Membranes chemically cleaned on days 54 and 81.
[b] Membranes mechanically cleaned on days 85 and 89.
[c] Constant permeate flux.
[d] Low N_2 gas sparkled in the reactor (20 L h^{-1}).

increase again on day 84 up to 75.4 mbar day^{-1} (Table 2). On days 86 and 90, the membranes were mechanically cleaned by gentle displacement of the membrane cake with a brush (Fig. 9D). The dP/dts measured on day 86 and 90 were equal to, respectively, 70.2 and 104.3 mbar day^{-1} (Table 2).

4. Discussion

4.1. Reactor performance

This paper clearly shows that high rate sulfate reduction at salinities of 50 g NaCl L^{-1} and 1 g $MgCl_2{\cdot}6H_2O$ L^{-1} (60–70 mS cm^{-1}) can be achieved by using a submerged anaerobic membrane bioreactor (SAMBaR) inoculated with a pure culture of the halophilic SRB *Desulfobacter halotolerans* using acetate or ethanol as electron donors. The high salt tolerance reported in this paper has significant practical implications as it enables the direct treatment of sulfate rich brines without prior dilution, thus enabling the direct application of SRB based bioreactors in closed cycles. It is worth mentioning that the substrate spectrum of *Desulfobacter halotolerans* is broader than merely sulfate, and also includes sulfite and thiosulfate. Thus, it can also be adopted in processes where these compounds are dominant, e.g. in scrubbed waters from flue gas desulfurization systems and in photographic effluents, respectively.

The maximal sulfate reduction rate of 6.6 g SO_4^{2-} L^{-1} day^{-1} (at a flux of 17.1 L m^{-2} h^{-1} and a HRT of 9.7 h) found in this work (Fig. 5B) is comparable to sulfate reduction rates reported for ethanol-fed immobilized biomass reactors operated at low salinity. The highest sulfate reduction rate reported so far in ethanol-fed sulfidogenic reactors is 9.9 g SO_4^{2-} L^{-1} day^{-1} for an ethanol-fed mesophilic expanded granular sludge bed (EGSB) reactor (HRT = 5–6 h) operated at low salinity [25]. Nagpal et al. [3] obtained sulfate elimination rates up to 6.33 g SO_4^{2-} L^{-1} day^{-1} in an ethanol-fed recirculating CSTR vessel and fluidized bed reactor operated at a HRT of 5.1 h and inoculated with a mixed culture of SRB (*Desulfovibrio desulfuricans* and *Desulfobacter postgatei*) immobilized on porous glass beads. According to a mathematical model the low volume of the bed relative to the total liquid volume of the system ($V_{bed}/V_{total} = 0.074$) was the limiting factor in the sulfate elimination rate of the fluidized bed reactor [3].

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Kalyuzhnyi et al. [26] achieved a sulfate reduction rate of 6 g SO_4^{2-} L^{-1} day^{-1} in an ethanol-fed UASB reactor operated at a HRT of 20 h and the system was found to be limited by sulfide toxicity (180 mg L^{-1} undissociated H_2S) of acetotrophic SRB. Such concentrations of undissociated H_2S are known to inhibit acetotrophic SRB [27]. In the present work, sulfide toxicity hardly could occur as the sparkling of the reactor mixed liquor with N_2 to minimize membrane fouling provided an excellent H_2S stripping, thus avoiding the build up of sulfide in the reactor mixed liquor (Fig. 5C). Results ob-

Table 3
Maximal specific sulfate reduction rate from biomass of anaerobic sulfidogenic bioreactors

Reactor concept	T (°C)	pH	HRT (h)	Substrate	Specific sulfate reduction rate ($g\ SO_4^{2-}\ g\ VSS^{-1}\ day^{-1}$)	Reference
CSTR vessel + fluidized bed reactor	rt	6.9	5.1	Ethanol	6.0–18.9[a]	[3]
EGSB	30–35	7.8–8.3	5–6	Ethanol	0.95[b]	[25]
EGSB	65	7.5	3	Methanol	1.22[c]	[31]
UASB	32 ± 1	8.3	2	Acetate	0.64[d]	[32]
Gas lift (with pumice stones)	55	7.0	4.5	H_2/CO_2 (80:20)	3.75[e]	[33]
Gas lift (with pumice stones)	35	7.0	2.25–4.5	H_2/CO_2 (80:20)	4.2[f]	[33]
SAMBaR	33 ± 1	7.2 ± 0.1	9.5	Ethanol	6.64	This work

rt—room temperature.
[a] The specific rate was calculated from the reported 0.07 g – 0.22 g SO_4^{2-} g protein^{-1} h^{-1} and the reported ratio of 0.278 g protein per g biomass dry weight.
[b] The specific rate was calculated from the final concentration of 10.5 g VSS L^{-1} and the maximal sulfate reduction rate (9.9 g SO_4^{2-} L^{-1} day^{-1}) reported by the authors.
[c] The specific rate was calculated from the final concentration of 9–10 g VSS L^{-1} in the reactor and the maximal sulfate reduction rate (11 g SO_4^{2-} L^{-1} day^{-1}) reported by the authors.
[d] The specific rate was calculated from the initial concentration of 21.7 g VSS L^{-1} (assuming no growth or loss of biomass) and the maximal sulfate reduction rate (14 g SO_4^{2-} L^{-1} day^{-1}) reported by the authors.
[e] The specific rate was calculated from the reactor mixed liquor concentration of 1.2 g VSS L^{-1} and the maximal sulfate reduction rate (7.5 g SO_4^{2-} L^{-1} day^{-1}) reported by the authors.
[f] The specific sulfate elimination rate was calculated from the reported 1.4 g S g biomass^{-1} day^{-1} in van Houten et al. (1997). We assume 1 g biomass equal to 1 g VSS.

tained by de Smul et al. [25] show that a remarkable increase in the sulfate removal rate can be achieved in an ethanol-fed EGSB reactor after stripping sulfide with N_2 and by controlling the reactor pH above 7.75. Prevention of H_2S toxicity is particularly important in bioreactors using cell suspensions as H_2S can cause acute toxicity to SRB without any recovery [28]. The stripping effect of the gas sparkling is thus of paramount importance and circumvents the need of other H_2S removal methods, as e.g. extractive membranes [29] or the formation of iron sulfide precipitates [30].

The maximal specific sulfate elimination rate of 6.64 g SO_4^{2-} g VSS^{-1} day^{-1} found in this work (Fig. 6B) is significantly higher than those obtained in any previous investigations in sulfidogenic bioreactor configurations, either using ethanol or different electron donors (Table 3). A possible explanation for this difference is that only part of the biomass in the granules or (thick) biofilms participate in the sulfate reduction process when the reactor configuration relies on granules or (thick) biofilms, as in UASB, EGSB, fixed or fluidized bed reactors.

Table 3 shows that high specific sulfate reduction rates only were achieved for hydrogen-fed gas lift reactors. The poor aggregation of SRB on pumice stones, used as inorganic carrier, in these gas lift reactor, resulted in the formation of thinner biofilms and therefore, an overall more active biomass [33]. Nagpal et al. [3] also noticed the substrate diffusion limitations in biofilms and the presence of dead/inactive (inert) biomass in the sludge of an ethanol-fed fluidized bed reactor. This was based on the differences between the specific sulfate reduction rates found in batch growth experiments (0.15–1.34 g SO_4^{2-} g protein^{-1} day^{-1}) compared to those found in the reactor (0.07 g –0.22 g SO_4^{2-} g protein^{-1} day^{-1}). In the present work, the absence of inorganic carrier induced the growth of small bioparticles (Fig. 7B), conceivably diminishing the substrate limitation transport phenomena as reported for aggregates bigger than 0.5 mm [33]. Thus, bioreactors systems that apply the concept of suspended growth offer the advantage that they cultivate biomass with very high specific sulfate reduction rates. Remains to be answered, however, what will be the type of biomass (and specific sulfate reduction activity) that develops in long reactor runs.

The observed fairly constant specific sulfate reduction rate of 5.5 g SO_4^{2-} g VSS^{-1} day^{-1} shows that the performance of the reactor was limited by the low amount of biomass (0.85 g VSS L^{-1}; Fig. 5A) present in the reactor. It is well known that membrane bioreactors can be operated at much higher solid concentrations and mixed liquor suspended solids (MLSS) for aerobic membrane bioreactors typically range from 3 to 31 g L^{-1} [34]. The low biomass concentration in the SAMBaR, which was never bled during the experiment, is due to the very low growth rate of *Desulfobacter halotolerans*, equal to about 36 h with acetate as the substrate at 5% salinity (Ingvorsen, pers. commun.). As such, it can be expected that the capacity of the reactor will increase further by allowing the biomass to grow to higher VSS concentrations in the reactor. Fundamental research on the metabolic properties of *Desulfobacter halotolerans*, such as on the identification of growth limiting step, is suggested to increase the growth rate of the biomass in the bioreactor. In addition, alternative process operation strategies can be imposed to increase the biomass concentration. For instance, Paulo et al. [35] developed a pH controlled system that allows SRB to grow continuously at their near-maximum μ_{max} based on a limiting substrate dosing strategy. Adoption of this substrate dosing regime to a *Desulfobacter halotolerans* inoculated SAMBaR would enable to develop and maintain much higher biomass concentrations in a short period of time.

The sulfate elimination rate in principle can also be increased by the reduction of the HRT, but this investigation

shows that increasing the flux to values close to or beyond the nominal critical flux is highly detrimental to the operation of the membranes (Fig. 9B). However, taking into account that a membrane surface area to reactor volume of only $0.011\,m^{-2}\,L^{-1}$ was available in the experimental rig, this can be improved by adjusting the reactor design. This can be achieved by constructing a new experimental rig equipped with a high membrane surface area to reactor volume, thus enabling a decrease of the HRT while operating the SAMBaR at very low fluxes.

4.2. Metabolic characteristics of the sludge

Ethanol was incompletely oxidized by *Desulfobacter halotolerans*, and the stoichiometry of ethanol utilization followed closely that of Eq. (1), with about 0.5 mol sulfate reduced and 1 mol of acetate produced per mol of ethanol utilized (Fig. 4C). The higher affinity of the biomass for ethanol found in the present work contrasts with the findings of Brandt and Ingvorsen [16] who found that, rather than ethanol, acetate is the preferential substrate for *Desulfobacter halotolerans*. When grown on ethanol, cell yields were only 30% of acetate grown cultures, but intense sulfide production is reported when using ethanol as the substrate [16]. As such, in case a full COD removal is also required in the sulfate reducing ethanol-fed SAMBaR reactor, it must be taken into account that the acetate oxidation is the rate-limiting step and therefore the rate of acetate degradation will define the design of the ethanol-fed sulfate reducing reactor. A similar observation with respect to the big importance of the acetate degradation rate on the reactor performance has been reported for methanol-fed thermophilic [10,11,21] and VFA-fed mesophilic [36] reactors.

4.3. Operational strategies

The present investigation shows that anaerobic membrane bioreactors can be operated over extended periods of time at a fixed flux provided that this flux is substantially below the nominal critical flux determined experimentally ($18–21\,L\,m^{-2}\,h^{-1}$). Interestingly, the critical flux ($18–21\,L\,m^{-2}\,h^{-1}$) obtained with crushed sludge (Fig. 8A and B) coincided with the flux ($17.1\,L\,m^{-2}\,h^{-1}$) where a rapid increase in the transmembrane pressure occurred in the reactor (Fig. 9D). This indicates that crushed sludge can be used to assess experimentally the critical flux when no biomass suspension is available for the tests.

It must, however, be taken into account that even below the nominal critical flux the transmembrane pressure tends to rise slowly (Fig. 9B and C). Operating membrane bioreactors at fluxes higher than the critical flux must be avoided at any price, otherwise the TMP will than raise dramatically, resulting in a collapse of the membrane (Fig. 9B). Turbulence induced by the sparkling of nitrogen gas is beneficial for the operation of the membranes for extended periods of time. According to Chang et al. [17], the injection of coarse gas in membrane bioreactors keeps the solids in suspension and scours the membrane surface, suppressing fouling. Indeed, the results of this work show that a 4–5 times increase in the TMP increase rate when the SAMBaR was not mixed with nitrogen gas (Table 2 and Fig. 9B). The constant permeate flux on day 22 resulted in the TMP increase rate within 6–11 times compared to operating the SAMBaR in relaxation/production or backflush mode, respectively (Table 2 and Fig. 9B). Mechanical cleaning of the membranes by gentle displacement of the cake layer seemed not so effective for the recovery of the permeability of the membranes (as compared to chemical cleaning), as intense membrane fouling occurred right after the mechanical cleaning (Fig. 9D and Table 2). The intermittent operation mode [37] as well as the backflush operation mode of the membranes [38] has been reported to slow the fouling rate in membrane filtration of biomass. This work shows that it is attractive to operate anaerobic membrane bioreactors with the occasional backflush of the membranes. If backflush is adopted as the operational strategy to minimize fouling at a flux of $4.7\,L\,m^{-2}\,h^{-1}$, chemical cleaning of the membranes will be required only at about 106 days (adopting a TMP increase rate of $4.5\,mbar\,day^{-1}$; Table 2).

Future research is required to further optimize the system both with respect to the required time as well as the frequency of the backflush operation. In addition, the optimization of the gas loading rate as well as the improvement of reactor design is required. This will improve the contact of the coarse bubble gas (which cause the scour of the membrane) with the set of membranes, thus further reducing the membrane fouling. In addition, the growth of small bioparticles in MBRs, as observed in the current work (Fig. 7B), may lead to reduced membrane fouling, as bigger particles conceivably do not obstruct membrane pores [34]. The observed modifications of the particle size distribution (Fig. 7A versus Fig. 7B), however, cannot be correlated to a lower dP/dt during the experiment, as different fluxes were applied (Table 2) when samples were harvested for particle size distribution tests. The dP/dt values measured for each flux applied during the critical flux experiments are not equivalent to the values found for the long-term operation of the SAMBaR (Fig. 8B versus Table 2). However, the dP/dt values obtained in the critical flux test indicate at what flux fouling in the SAMBaR starts to become severe. As such, the flux-step method is a valuable tool to determine the operational conditions for the operation of membrane bioreactors.

5. Conclusions

(1) High rate sulfate reduction ($6.6\,g\,SO_4^{2-}\,L^{-1}\,day^{-1}$ at a HRT of 9 h) at salinities of $50\,g\,NaCl\,L^{-1}$ and $1\,g\,MgCl_2\,L^{-1}$ ($60–70\,mS\,cm^{-1}$) can be achieved in a submerged anaerobic membrane bioreactor (SAMBaR) inoculated with a pure culture of the halophilic SRB *Desulfobacter halotolerans* using either acetate or ethanol as electron donor.

(2) The rather constant very high specific sulfate reduction rate of 5.5 g SO_4^{2-} g VSS^{-1} day^{-1} found indicate that the performance of the reactor was limited by the low amount of biomass (0.85 g VSS L^{-1}) present in the SAMBaR.
(3) Sulfate reducing submerged anaerobic membrane bioreactors can be operated over extended periods of time without chemical cleaning of the membranes at a certain fixed flux provided that this flux remains well below the nominal critical flux determined experimentally (18–21 L m^{-2} h^{-1}).
(4) Intermittent operation as well as backflush of the membranes slow down the fouling of the membranes. Frequent backflush (e.g. 1 min each 10 min) is the suggested operational strategy to minimize fouling in anaerobic MBRs.

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