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Sulfate reduction and filtration performances of an anaerobic membrane bioreactor (AnMBR)

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HIGHLIGHTS

- Sulfate reduction performance was over 90% in AnMBR.
- Long term filtration performance of the process was assessed.
- High molecular weight organics deposited on the membrane.
- Metal-sulfide precipitates were detected in the cake layer.
- *Desulfovibrio*-like bacteria dominated in the sulfidogenic AnMBR.

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ABSTRACT

Sulfate-reducing bioprocesses can be used in the treatment of sulfate-containing industrial effluents including mining, metallurgical and food industries. Although membrane bioreactors (MBRs) have been studied for anaerobic wastewater treatment, sulfidogenic anaerobic MBRs (AnMBRs) received little or no attention. The aim of this study was to evaluate the performance of sulfate-reducing AnMBR and investigate the membrane foulants and filtration characteristics. Sulfate reduction and COD oxidation efficiencies exceeded 90% when the reactor was fed with a synthetic sulfate-rich (2000 mg/L) wastewater with COD/sulfate ratio of 0.75. Long-term filtration performance was evaluated using several filterability tests. Deposition of high molecular weight soluble organics and S, Si, Fe, Cu, Na, and Mg were detected in the cake layer. The formation of metal-sulfide precipitates was the main reason for heavy metal deposition on the membrane surface. *Desulfovibrio*-like sulfate reducing bacteria was detected in the bioreactor. Results showed that sulfate-reducing AnMBR offers potential for real scale applications.

1. Introduction

Sulfate-rich wastewaters are discharged from various industries and these wastewaters must be treated to protect natural water bodies [1]. Within these industries, the mining sector is quite important as highly acidic, sulfate- and metal-containing acid mine drainage water (AMD) is generated due to exposure of metallic ores to air and water. Conventionally, hydroxide precipitation is the most commonly applied method for the treatment of metal-containing waters. Production of high quantities of sludge and high costs of the chemicals added are the main disadvantages of the method. Also, sulfate removal is only possible when Ca^{2+} containing chemicals, such as lime, are used for neutralization. However, stringent discharge legislations will dictate more efficient sulfate removal and the recovery of valuable metals from waters, which are possible with the use of active bioprocesses [2].

In the treatment of AMD and metal containing industrial wastewater, sulfate-reducing bioreactors are becoming an alternative to conventional chemical treatment [2,3]. With the supplementation of organic compounds, sulfate is biologically reduced to H_2S under anaerobic conditions. Moreover, produced bicarbonate increases the pH of the wastewater (Reaction 1) and metals precipitate as metal sulfides (Reaction 2), which are particularly important in the case of AMD treatment [3]. By this way, metals and sulfate are concomitantly removed and pH can be increased to neutral values in a single bioreactor. The precipitate formed according to Reaction 2 can be further used for metal recovery [4].

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$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^- \quad (1)$$

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+ \quad (2)$$

Although sulfidogenic AMD treatment is an efficient process, a proper organic matter addition is required both as a carbon and electron source for the process due to low organic matter (< 10 mg/L) content of AMD [4]. Sulfate reducing bacteria (SRB) can utilize a wide range of low molecular weight substrates such as ethanol, lactate and acetate. However, in the case of some industrial wastewaters which contain both high sulfate and organic matter, the process may not require any additional carbon supplementation. Although some SRB can oxidize organic substrates down to acetate, some can mineralize them to CO_2 [2]. It is well known that acetate may accumulate at high organic loadings and this may decrease sulfide and alkalinity production [5]. Although lactate is a good substrate for SRB, ethanol may be preferred due to its lower cost. When ethanol is used as carbon and electron source, acetate oxidation should take place for alkalinity production [3] (Reactions 3 and 4). In the first step, ethanol is oxidized to acetate (Reaction 3). Then, acetate is oxidized and alkalinity is produced (Reaction 4), which is the rate-limiting step [2]. Therefore, organic matter loading should be carefully adjusted in order to prevent acetate accumulation and alkalinity consumption.

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad (3)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (4)$$

Membrane bioreactor (MBR) technology, originally combining the activated sludge process with the membrane filtration process, has significant advantages due to complete physical retention of microorganisms with the use of micro- or ultra-filtration membranes (pore size: 0.05–0.4 μm) [6]. In the past two decades, remarkable progress has been achieved on the MBR technology and it has become an attractive option for the treatment and reuse of industrial and municipal wastewaters. Although membrane fouling is the major drawback of the MBR processes, great scientific improvement occurred in the last years, such as mitigating membrane fouling using quorum quenching [7] and controlling the extracellular polymeric substances [8].

The process has also been tested for anaerobic treatment of both domestic [9] and industrial effluents [10]. Sulfidogenic acetate oxidation is the rate-limiting step in sulfate reducing bioprocesses due to slow growth rates [2,3] and low attachment or granulation ability of acetate-oxidizing sulfate reducers [11]. Hence, the use of MBR process for sulfate reduction bioprocesses may have a significant advantage due to complete retention of biomass, which enables avoiding wash out of slow growing or non-granule forming microorganisms from bioreactor [12]. In the study of Vallero et al. [13] sulfate reduction in salt rich wastewater (conductivity 60–70 mS/cm) was investigated in a submerged in anaerobic membrane bioreactor inoculated with a halotolerant bacterium *Desulfobacter halotolerans*. The reactor was supplemented with ethanol or acetate in excess of sulfate as COD/sulfate was 0.5. The reactor gave high performance in terms of sulfate reduction rate, which reached up to 5.5 g SO_4^{-2}/(g.VSS.d). However, the reactor performance was limited by the low biomass amount. The study illustrated that the reactor could be operated over extended periods of time without chemical cleaning at a flux of around 17 L/m^2/hour (LMH). In another study, Tabak and Govind [14] studied the biotreatment of acid mine drainage using sulfate reducing MBR equipped with polypropylene hollow fibre membrane. The study indicated that the SRB membrane bioreactor can be applied in field-scale treatment of acid mine drainage, metals can be recovered and the treated water can be used in agricultural irrigation. Although several studies have been performed on the use of MBRs for methanogenic anaerobic wastewater treatment [15], little is available in the literature on the use of MBRs for sulfidogenic anaerobic treatment.

The aim of the present study is to evaluate the potential of AnMBR for sulfate reduction and sulfide generation together with investigating the filtration performance and characterizing the membrane foulants, which may help to apply the process in pilot scale first and then in full-scale. Although AMD may contain metals at high concentrations, this study was conducted in the absence of heavy metals in order to evaluate the process potential and membrane foulants under base conditions. Particularly, at the strictly sulfate-reducing conditions, membrane foulants are expected to be different from the previous studies due to the high concentration of sulfide.

2. Materials and methods

2.1. Anaerobic membrane bioreactor (AnMBR)

The AnMBR was made of plexiglass with dimensions (L × W × H) of 12 × 15 × 37 cm and has a total volume of 6.6 L with an active volume of 4 L (Fig. S1). The head space volume of the AnMBR was around 2.6 L. In the AnMBR, a flat sheet polyethersulfone (PES) ultrafiltration membrane with a pore size of 0.02 μm was used. In order to control the cake formation on the membrane, intermittent filtration (5 min suction and 1 min relaxation) was performed. Additionally, the reactor headspace was circulated on the membrane surface with a velocity of 1 m^3-gas/m^2.h by a gas pump in order to provide mixing in the reactor and scour the cake layer on the membrane. The reactor has a portable cover on which, level, pH, and oxidation-reduction potential (ORP) sensors, as well as sample, feeding and gas recirculation ports were placed.

At the start-up, the AnMBR was seeded with an active anaerobic sludge (MLSS: ~15 g/L) obtained from an anaerobic digester of a municipal wastewater treatment plant. The seed sludge was composed mainly of methanogenic microorganisms, however, sulfidogenic microorganisms rapidly multiplied and high sulfate removal was achieved at a short time, i.e. within 20 days. The main reasons for this observation were retaining of the microorganisms inside the reactor by the membrane and keeping an infinite sludge retention time (SRT). A double-sided membrane module was used and the total active membrane area was 0.01 m^2. The reactor was operated at 35 ± 2 °C in a temperature-controlled room.

2.2. Operation of the AnMBR

Sulfate-rich wastewaters including acid mine drainage water generally have low organic carbon. Therefore, external carbon supplementation is required as carbon and electron sources for SRB. Sulfate concentration in the AMD may show variation depending on site, weather conditions and many other factors. Depending on our previous study [16] conducted with real AMD, sulfate concentration in the present study was kept constant at 2000 mg/L. The performance of the bioreactor also highly depends on the selected carbon source. In this study, a relatively low-cost carbon source, ethanol, was selected and the COD/sulfate (mg/mg) ratio was adjusted to 0.75, which is slightly higher than the theoretical requirement (0.67 mg/mg) considering that some carbon source is also needed for biomass growth. The performance of AnMBR for COD oxidation, sulfate reduction and sulfide generation were evaluated under varying operational conditions. For this purpose sulfate, sulfide, COD, alkalinity, MLSS, MLVSS concentrations, ORP and pH were frequently measured throughout the reactor operation. Transmembrane pressure (TMP) and flux of the MBR were continuously monitored and recorded by online measurement system for the determination of membrane fouling and the requirement for external membrane cleaning.

AnMBR was operated at a flux of 2.09 ± 0.15 LMH during the first 53 days of operation. During this period, no TMP increase was observed and therefore membrane cleaning was not required. After the day 53, hydraulic retention time (HRT) was decreased from 1.97 ± 0.07 days to 1.09 ± 0.18 days and thereby flux increased to 3.90 ± 0.50 LMH (Fig. 1). To evaluate the treatment performance, the reactor was

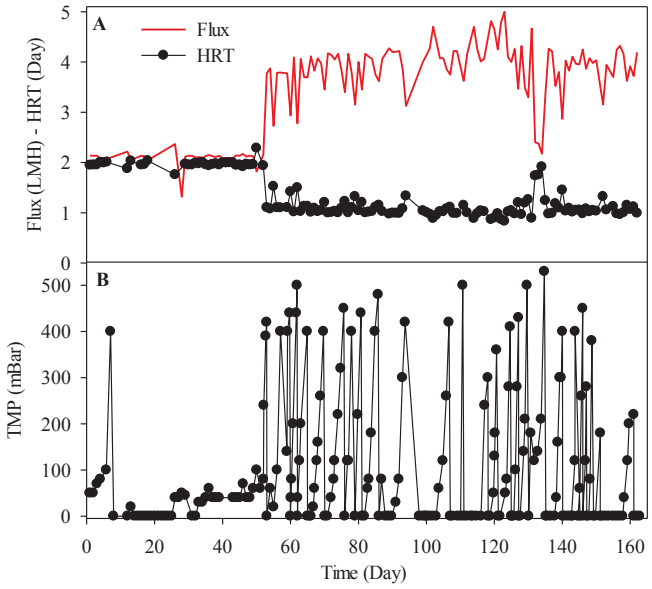

Fig. 1. Flux, HRT (A) and TMP (B) changes in the AnMBR.

Table 1
Components of the synthetic wastewater used throughout the study.

Component	Concentration
$MgSO_4{\cdot}7H_2O$	2563 mg/L
Na_2SO_4	1479 mg/L
Ethanol	1500 mg COD /L
Yeast extract	50 mg/L
KH_2PO_4	56 mg/L
NH_4Cl	110 mg/L
Ascorbic acid	11 mg/L

operated for the long period of time (165 days) and fed with synthetic wastewater containing 2000 mg/L sulfate and 1500 mg/L COD (Table 1).

Constant flux was achieved through the membrane by applying vacuum to permeate, and the pressure difference was monitored. The membrane was operated at a pressure between 10 and 350 mbar. The occurrence of higher pressure (> 400 mbar) showed that membrane was fouled and required physical and chemical cleaning. Physical cleaning was applied by taking the membrane out of the AnMBR and washing by scrubbing with a sponge under a tap. Following physical cleaning, chemical cleaning was applied by keeping the membrane in an oxidant (NaOCl, 0.1%) and then an acidic (sulfuric acid, pH 2) solutions for one hour. After chemical cleaning, membranes were washed with tap water and replaced into the reactor.

2.3. Analyses

Samples were filtered using cellulose acetate syringe filters with pore size of 0.45 μm before the measurements of COD, sulfate, and sulfide. COD was measured by closed reflux method according to the standard methods [17]. Before COD measurements, sample pH was decreased below 2.0 with concentrated H_2SO_4 and the sample was purged with N_2 gas for 5 min to remove H_2S from the sample. For sulfate measurements, a turbidimetric method utilizing $BaCl_2$ [17] and Dionex Ion Chromatograph (ICS-5000) equipped with IonPac AS9-HC Analytical Column were used. Sulfide was analyzed spectrometrically using a Hach DR/5000 Spectrophotometer. Mixed liquor Suspended solids (MLSS), volatile suspended solids (MLVSS) and alkalinity concentrations were also determined according to APHA standard methods [17]. Alkalinity was measured in unfiltered samples titrated with 0.1 N HCl until the endpoint of pH 4.5.

For SMP (soluble microbial products) analyses, the mixed liquor was centrifuged for 10 min at 4000 rpm and then the supernatant was filtered through a membrane with a pore size of 0.45 μm. The filtrate was used for measurement of SMP as protein and carbohydrate. Phenol–sulfuric acid [18] and Lowry [19] methods were used for the measurements of carbohydrate and protein, respectively. For EPS (extracellular polymeric substances) measurement, the remaining pellet from centrifugation was washed two times and suspended in saline water (0.5% NaCl). The mixed liquor was then subjected to heat treatment (80 °C for 1 h) and then centrifuged once more [20]. The supernatant was filtered through 0.45 μm pore size filter and protein and carbohydrate concentrations in the EPS were measured.

Filtration characteristics of the sulfate reducing anaerobic sludge were evaluated by viscosity, capillary suction time (CST), and supernatant filterability (SF) analyses. In addition, the characteristics of organic and inorganic membrane foulants affecting the filtration performance of the membrane bioreactor were determined. For this purpose, gel-permeation chromatography (GPC), fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), SEM coupled with energy dispersive spectroscopy (SEM-EDS) and inductively coupled plasma (ICP) analyses were performed on the membrane or membrane cake layer.

The viscosity of the sludge samples was calculated from the relationship between shear rate and shear stress using the Bingham plastic model [21]. CST was measured using Trion Capillary Suction Timer with standard filter papers provided by the Trion Electronics. The measurements were then normalized via dividing by SS concentrations [22]. Supernatant filterability (SF) was determined by a method adapted from Dereli et al. [22]. Sludge samples were first centrifuged at 4000g for 10 min and the supernatant was filtered through the polyethersulfone (PES) microfiltration membrane with a pore size of 0.45 μm in a dead-end filtration system under constant pressure (0.5–0.6 bar) and stirring conditions. Permeate was collected at least for 10 min and the first 5 min data was discarded. The remaining data for permeate flow was averaged as mL/min.

GPC and FT-IR analyses were used to characterize the organic foulants. Molecular weights of the soluble organic macromolecules in the supernatant, permeate and cake/gel layers samples were analyzed by GPC. Average molecular weights were estimated using an Agilent 1260 Infinity GPC instrument equipped with two PL Aquagel-OH Mixed-H columns using 0.02% (w/v) NaN_3 aqueous solution as the eluent at a flow rate of 1 mL/min at 30 °C. Molecular weights were calculated according to the calibration curve prepared using the polyethylene glycol standards within the molecular weight range of 106–1,500,000 Da. FT-IR was used for analysis of the organics on cake layer. For the FT-IR analyses, the cake layer on the fouled membrane was carefully scrapped off by a plastic razor blade. The collected cake layer or fouling layer was dried at 50 °C for 24 h before FT-IR measurements.

For the determination of inorganic membrane foulants, SEM-EDS, and ICP analyses were performed. The morphologies of the fouled membrane were directly observed using SEM (Philips-XL30SFEG) after coating with Au–Pd. The semi-quantitative elemental analyses of inorganics in the cake layer was performed by EDS coupled with SEM. Inorganic components in cake layer samples were also measured with ICP. The cake layer developed on the fouled membranes was scraped into 50 mL solution of 2000 mg/L citric acid and mixed gently for 2 h in order to extract inorganic components. The acid extracts were filtered through 0.45 μm pore size of syringe filters before the measurements of Al, Cu, Fe, P, Ca, Mg, K, Si, Na and S using Perkin Elmer Optima 7000 ICP-OES (Optical Emission Spectrophotometry).

For revealing dominant microbial community in the bioreactor, denaturing gradient gel electrophoresis (DGGE) analyses of 16S rRNA genes was applied on the samples taken from MBR on days 150 and 165. DNA of the bacteria in the sludge samples which were obtained

from MBRs at different time intervals were extracted with Soil Microbe DNA MiniPrep isolation kit (ZymoResearch) according to manufacturer's instructions. The crude bacterial DNA samples were amplified with BacV3f and 907r primers. PCR amplification and subsequent DNA monitoring procedures for bacterial community detection have previously been described in Sahinkaya et al. [23]. The D-CODE System (BioRAD, The Netherlands) was used for DGGE experiments according to the previous studies [23,24]. PCR products of 45μL were loaded onto 6% (W/V) polyacrylamide gels in TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3) containing 30–70% denaturant gradient (where 100% denaturant contains 7 M urea and 40% formamide). DGGE was run under 100 V and 60 °C conditions for 16 h and the gel was stained with SYBR Gold dye solution (100 μL/L in 1x TAE) for 30 min and then monitored with Vilber Lourmat Quantum St4 system.

Prominent bands were excised with a scalpel and placed in sterile tubes. 20 μL of nuclease-free sterile water was added to tubes and stored at 4 °C overnight for DNA elution. The PCR runs for the eluted DNA samples were repeated with same primers (BacV3f and 907r) without GC clamp for re-amplification of the bacterial DNA. The sequencing reactions of the purified PCR products were performed at REFGEN (Ankara, Turkey) and the similarity of DNA sequences were analyzed via the BLAST program in GenBank.

3. Results and discussion

3.1. Sulfate-reduction efficiency of the AnMBR

AnMBR was operated at HRT of 1.97 ± 0.07 days during the first 53 days and then it was decreased to 1.09 ± 0.18 days (Fig. 1A). In the anaerobic membrane bioreactor, over 90% sulfate and COD removal efficiencies were reached in a short time although the AnMBR was seeded with a methanogenic sludge (Fig. 2A, B). The removal efficiencies decreased for about 25 days following the HRT decrease to 1.09 ± 0.18 days, however, sulfate and COD concentrations further decreased below 100 mg/L and 200 mg/L, respectively, and stayed low until the end of the operation. Under steady-state conditions, effluent sulfide concentrations were about 600–750 mg/L, close to the theoretically calculated values (Fig. 3A).

The high sulfate and COD removal efficiencies in the first 50 days showed that sulfate reducing conditions quickly prevailed in the AnMBR. Sulfate reducing bacteria (SRB), which were present in the

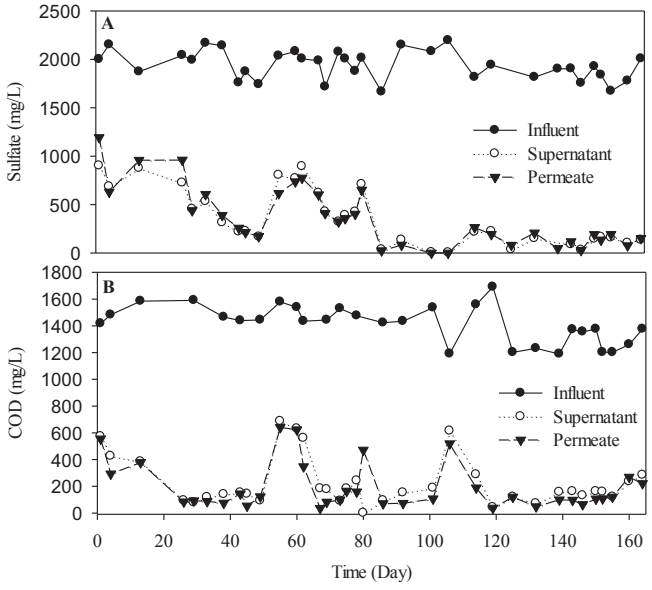

Fig. 2. Variations of sulfate (A) and COD (B) concentrations in the influent, permeate and supernatant.

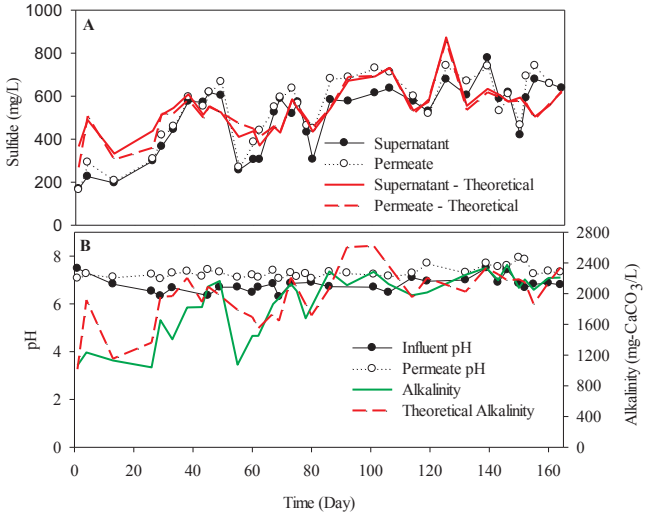

Fig. 3. Variations of sulfide (A), pH and alkalinity (B) throughout the AnMBR operation. Theoretical values were calculated based on Reaction 1.

anaerobic seed sludge, decreased sulfate concentrations to about 1200 mg/L with a removal efficiency of about 40%, and produced 200–300 mg/L sulfide even in the first days of operation. However, this sulfide production was lower than the theoretically expected values (Fig. 3A). Besides, about 60% of 1500 mg/L influent COD was removed in the first 10 days. During this period, the COD-removed/sulfate-reduced (w/w) ratio was around 0.95, which is much higher than the theoretical ratio (0.67) for sulfate reduction. This excess COD removal showed that methanogenic archaea in the anaerobic seed sludge were still active in this period. But since methanogenic archaea are more sensitive to the sulfide, SRB dominated in a short time. Sulfate removal efficiency reached above 90% at day 49, when COD removal efficiency was about 93%. At this period, after day 40, sulfide production also reached to the theoretical value of about 600 mg/L, calculated based on sulfate removal. The fluctuations in sulfide production throughout the operation were mainly due to oxygen intrusion during membrane cleanings.

Although sulfate and COD removal efficiencies both decreased to about 60% after HRT was decreased from about 2.0 days to about 1.0 day at the 53rd day, they then increased up to 95 ± 4% and 90 ± 10%, respectively after the 86th day of operation. In this period, effluent sulfate concentration decreased to below 50 mg/L and the COD-removed/sulfate-reduced (w/w) ratio decreased to 0.71, which is very close to the theoretical value of 0.67. This showed that COD removal was completely due to the sulfate-reducing bioprocess, and methanogens present in the seed sludge may have lost their activity. These findings showed that SRB could be selectively enriched in the AnMBR similar to other studies conducted with conventional bioreactors [4,5]. The sulfate removal performance in the present study (~95%) was also higher than our previous study (82% on average) in which synthetic textile dye wastewater treatment was studied in an AnMBR [25]. Under steady state conditions, sulfate and COD removal efficiencies were 94% and 92%, respectively. Accepting the required COD amount for each g of sulfate reduction as 0.67 g based on Reactions 3 and 4, it was calculated that around 91% of the electrons produced from COD oxidation was used for the sulfate reduction. The average effluent COD and sulfate concentrations under steady state conditions were 117 ± 70 mg/L and 124 ± 60 mg/L, respectively. Based on effluent COD and sulfate concentrations together with the electron mass balance, it can be concluded that acetate could be completely used for sulfate reduction since acetate-oxidizing SRB could be fully retained in the AnMBR. This is an important characteristic of the AnMBR as acetate oxidation is the rate limiting step in ethanol

oxidation by SRB [5]. Besides, acetate oxidation step is also important in terms of alkalinity generation, since it is produced only when acetate oxidation was achieved (see Reactions 3 and 4). Hence, alkalinity in the effluent increased with time during the operation and reached to 2000–2400 mg-$CaCO_3$/L, which was very close to the theoretically calculated alkalinity production (Fig. 3B). Thereby, influent pH ranging between 6.5 and 7.0, increased to about 7.5 in the effluent due to the alkalinity production by SRB (Fig. 3B).

The relatively fast enrichment of the SRB in the AnMBR can be attributed to maintaining the slow-growing SRB within the reactor by the membrane and keeping sludge age infinite. Cake layer formed on the membrane also improved treatment efficiency and resulted in slightly lower COD and sulfate concentrations in the permeate (Fig. 2B). This finding suggested that bacteria in the cake layer provided further biodegradation of organic matter and sulfate reduction, which was also observed in our previous study [25].

3.2. Variations of MLSS, MLVSS, SMP and EPS in the AnMBR

At the start of the operation, MLSS and MLVSS concentrations in the reactor were 15,500 mg/L and 6,400 mg/L, respectively. However, both of them decreased sharply during the first days of the reactor operation (Fig. 4A), since a part of biomass died upon exposure to high sulfide concentrations of 200–300 mg/L (Fig. 3A). After day 27, both parameters started to increase again due to the dominance and growth of SRB, which are resistant to high sulfide concentrations. MLSS and MLVSS concentrations in the reactor reached 17,200 mg/L and 7,200 mg/L, respectively, on day 100 (Fig. 4A). Because of a technical problem on day 112, a part of sludge was lost and MLSS decreased to about 13,000 mg/L. But, it started to increase again due to the successful growth of SRB in the reactor (Fig. 4A).

Our previous studies showed that SMP and EPS are important parameters for membrane fouling [24,25]. SMP concentrations in the reactor supernatant and permeate continuously increased until day 73 (Fig. 4B). SMP in permeate was 25–35% lower compared to SMP in supernatant due to rejection by the membrane and the cake layer. Similar findings were also observed both in aerobic [26] and anaerobic [27] MBRs. The decreased COD and sulfate concentrations during the filtration were due to biodegradation by the biofilm developed on the membrane. During the first days of reactor operation, COD-to-SMP conversion ratio was relatively high (about 15%) because of the high death rate of microorganisms other than SRB (Fig. 4A). As the sulfate reducing conditions prevailed and the reactor reached a stable operation, this ratio decreased to about 4.7% after day 73 when SMP became stable at around 70 mg COD/L. In a literature study, conversion of biodegraded COD to SMP in an anaerobic reactor operated at high SRT [28] was close to 5%, which is very similar to our finding.

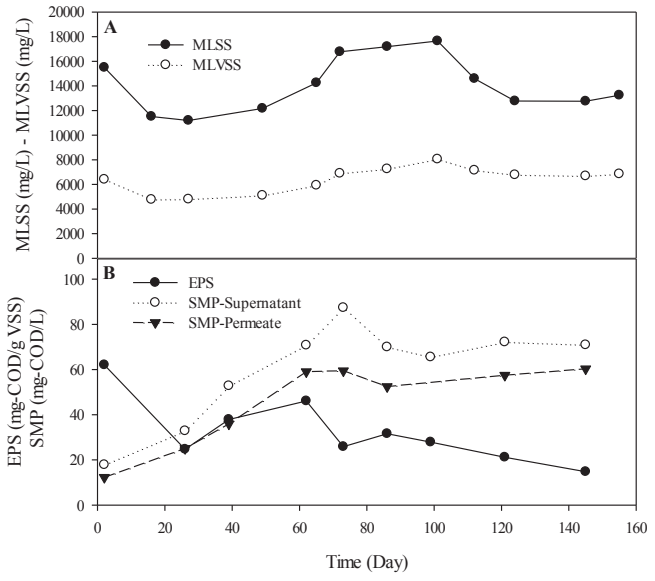

Fig. 4. Variations of MLSS and MLVSS (A) and SMP and EPS (B) in AnMBR.

EPS, on the other hand, decreased until the 25th day (Fig. 4B), which may be attributed to detachment of the loose-bound EPS from the microorganisms' surface and their further solubilization, which eventually increased SMP concentrations in the same period (Fig. 4B). SMP released to the supernatant as a result of detachment of loose EPS is expected to affect membrane fouling much more than the bound EPS. Therefore, the increase in SMP may be the main factor for the membrane fouling. After day 25, EPS increased again up to 40 mgCOD/gMLVSS until the HRT was decreased from 2 days to 1 day. Following the decrease in HRT, EPS continuously decreased until the end of reactor operation. In the study of Shariati et al. [29], it was also reported that the EPS decreased significantly when HRT was reduced from 24 to 16 h. EPS concentrations ranged between 20 and 40 mgCOD/gMLVSS and 2% of removed COD was converted to EPS on average.

3.3. Filtration efficiency of the AnMBR

AnMBR was operated at the flux of 2.09 ± 0.15 LMH during the first 53 days. At the start of operation pressure increased promptly, which should be due to using of unacclimated sludge under sulfate reduction conditions and high decay rate at the beginning of the operation. After chemical cleaning following this fast fouling, pressure did not increase to the level (~400 mbar) required for membrane cleaning at the flux of around 2 LMH for 53 days of operation (Fig. 1).

After HRT was decreased to 1.09 ± 0.18 days from 1.97 ± 0.07 days, the flux increased to 3.90 ± 0.50 LMH. During this period, frequent membrane fouling occurred due to dense cake layer formation. Pressure increase at increased flux (Fig. 1) may be explained by the local critical flux concept due to development of dense cake layer [24]. Therefore, chemical membrane cleaning was applied at 4-day intervals after day 60. After each chemical cleaning, pressure decreased to zero, but in a few days increased again to above 400 mbar requiring a new chemical cleaning (Fig. 1). The TMP increases in the sulfate-reducing AnMBR of the present study were much more frequent compared to the AnMBR treating textile wastewater in our previous study [25] although SMP concentrations in the supernatant were even lower in the present study. The main reason for this may be the use of an ultrafiltration membrane rather than a microfiltration membrane used in the previous study. The other potential reason was the higher sulfide concentration, which caused accumulation of higher metal concentrations in the cake layer.

The total membrane resistances before and after chemical cleanings averaged $3.82 \pm 0.64 \times 10^{13}$ 1/m and 0.185×10^{13} 1/m, respectively. The resistance of chemically cleaned membrane was close to that of a new membrane, 0.092×10^{13} 1/m. Hence, the membrane resistance decreased by 96%, after chemical cleaning.

3.4. Filterability and rheological properties of AnMBR sludge

The complex nature of the anaerobic sludge also affects membrane fouling and filtration characteristics. Therefore, filterability and rheological properties of sludge were investigated through the analyses of viscosity, CST, and SF (supernatant filterability). MLSS concentration significantly affects the rheological properties of sludge [21]. Besides, EPS content of the anaerobic sludge has a serious impact on sludge filterability [12]. Particularly, viscosity increases with increases in MLSS and EPS concentrations, and high viscosity affects the mixing of sludge in the reactor as well as shear stress on the cake layer during filtration [30]. The non-Newtonian characteristics of the sludge at high MLSS are important in the operation of MBRs. In our study, the anaerobic sludge viscosity was determined to be 3.64 ± 0.12 cP (Fig. S2).

This was much higher than a previous study on an anaerobic fluidized bed MBR, in which viscosities were 1.9 cP and 1.5 cP even at lower temperatures of 10 °C and 20 °C, respectively [31]. The high viscosity in our study can be attributed to the high MLSS and EPS concentrations, particularly due to infinite sludge age.

CST is another parameter used for evaluation of sludge in MBRs [25]. The CST of sludge samples apparently decreased from 205 s to 18 s between days 97–153 (Fig. S3). CST particularly decreased between 97 and 120 days and then became more stable with a smaller decrease. This profile is in accordance with MLSS as it is the main parameter affecting CST. On the other hand, the MLSS normalized CST also decreased from 14.18 L.s/gMLSS to 1.35 L.s/gMLSS during this period (Fig. S3) showing that MLSS should not be the only factor determining CST. The decrease in the normalized CST can be attributed to the decrease of EPS during this period (Fig. 4B). The direct relationship between CST and polymeric substances like EPS were also reported in the literature [32].

SF values tended to slightly decrease, which were about 0.87 mL/min, 0.66 mL/min, and 0.62 mL/min on days 31, 105 and 160, respectively.

3.5. Characterization of organic membrane foulants

For the characterization of organic foulants, GPC analyses were performed on samples taken from reactor, permeate and extracted cake layer. GPC analysis is based on the principle of rejection of high molecular weight compounds by the gel column, such that larger compounds leave the column first, and then the smaller compounds appear on the detector [12]. The calibration curve showing the relationship between molecular weight and elution time is shown in Fig. S4.

GPC analyses of samples that belonged to reactor supernatant, permeate, extracted cake layer and extracted EPS are shown in Fig. 5. The reactor supernatant and permeate samples gave similar three peaks between 15 and 20 min elution times (Fig. 5A). These 3 peaks at 16.5, 17 and 18.7 min refer to 8.8, 5.2 and 0.54 kDa, respectively. However, permeate peaks had smaller signal values compared to the samples obtained from the reactor. Although the molecules between 0.54 and 8.8 kDa are expected to pass through the 0.02 µm ultrafiltration membrane, some of them were filtered by the cake layer and did not appear in permeate. Three peaks referring to organic molecules larger than 1000 kDa were observed on samples taken from reactor. These peaks were not observed in permeate, since they were filtered by the membrane and cake layer. Such large molecules in the reactor supernatant are attributed to the release of loose-bound EPS from microbial flocs through the supernatant due to hydrodynamical forces related to reactor agitation and gas scouring of the membrane [33].

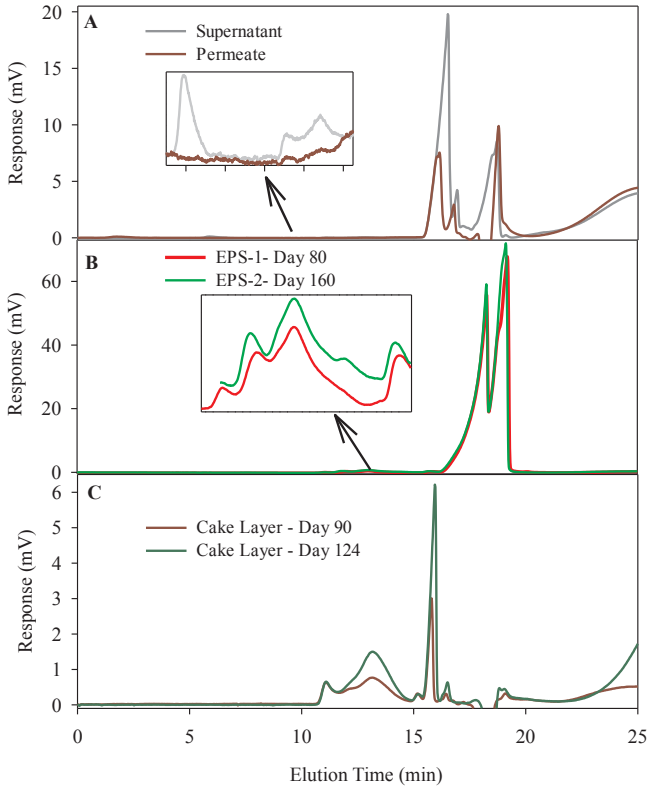

Fig. 5. GPC analyses of reactor supernatant and permeate samples (A), extracted EPS sample taken from the reactor (B) and extracted cake layer sample (C).

GPC analyses were also conducted on the EPS samples extracted from the reactor biomass on 80th and 160th days of reactor operation (Fig. 5B). Both measurements gave similar peaks showing that molecular weights of EPS did not change by time during the reactor operation. Also, the peaks were similar to those obtained on reactor supernatant samples. Particularly the peaks appeared at 11th and 11.8th minutes showing the presence of organic molecules larger than 1000 kDa, which also verified that high molecular weight organics in the supernatant were the released EPS, assigned as high molecular weight SMP. These organics are considered to be highly responsible for membrane fouling. GPC analyses on cake layer obtained at 90th and 124th days are shown in Fig. 5C. Organic matter characteristics of the cake layer seemed not to change significantly between these two samplings. GPC analyses showed the presence of high molecular weight organics (> 1000 kDa) in the cake layer as well as in EPS matrix extracted from the sludge. The highest peak for the cake samples were at 16.5 min referring to organic molecules of about 8.8 kDa molecular weight. Although these are relatively small molecules which were also observed in permeate (Fig. 5A), they were also kept inside the cake layer. This supported the secondary dynamic membrane effect of the cake layer developed on the membrane. Also in the AnMBRs, the cake layer was generally much thicker compared to the aerobic MBRs, which may increase the capture potential of low molecular organics by cake layer [25]. Also the cake layer in the AnMBRs was less porous compared to that in aerobic MBRs due to higher compression of the cake layer as a results of required higher vacuum to take the permeate (see Section 3.6). The peaks between 10 and 15 min in the cake layer (Fig. 5C) referring to larger molecules were not observed in permeate. The filtration of these molecules by the membrane produces a much more clean water, but decreases the porosity of cake layer, increases the membrane resistance and finally TMP to a level required chemical cleaning.

FT-IR analysis of the membrane with cake layer (Fig. S5) also supported GPC findings. Polysaccharide, protein and lipid structures were determined in the cake layer, which were supposed to be responsible for the membrane fouling. The peak obtained at 1068 cm^{-1} pointed out the presence of polysaccharides and polysaccharide-like organic compounds [34]. Another peak at 1250 cm^{-1} was due to C-O bonds in carboxyl and carboxylate groups or Amide III protein structure [20]. The peaks at 1400, 1530 and 1630 cm^{-1} showed the presence of amide groups, which are secondary protein groups [35]. The two peaks between 2900 and 3000 cm^{-1} were related to cellulosic lipid structure of the EPS in several studies [20,35]. The last two peaks at 3264 and 3685 were due to O–H bonds in hydroxyl functional groups.

3.6. Characterization of inorganic membrane foulants

In order to be able to interpret the filtration characteristics of AnMBR, the membrane foulants and their sources should be determined. Inorganics play a significant role in the fouling by precipitating on or into the membrane or by bridging organic molecules on the membrane surface. The important inorganic foulants are $CaCO_3$, $CaSO_4$, $Ca_3(PO_4)_2$, $CaHPO_4$, SiO_2, and Al_2O_3. On elemental base, the inorganic components of the cake layer may involve high

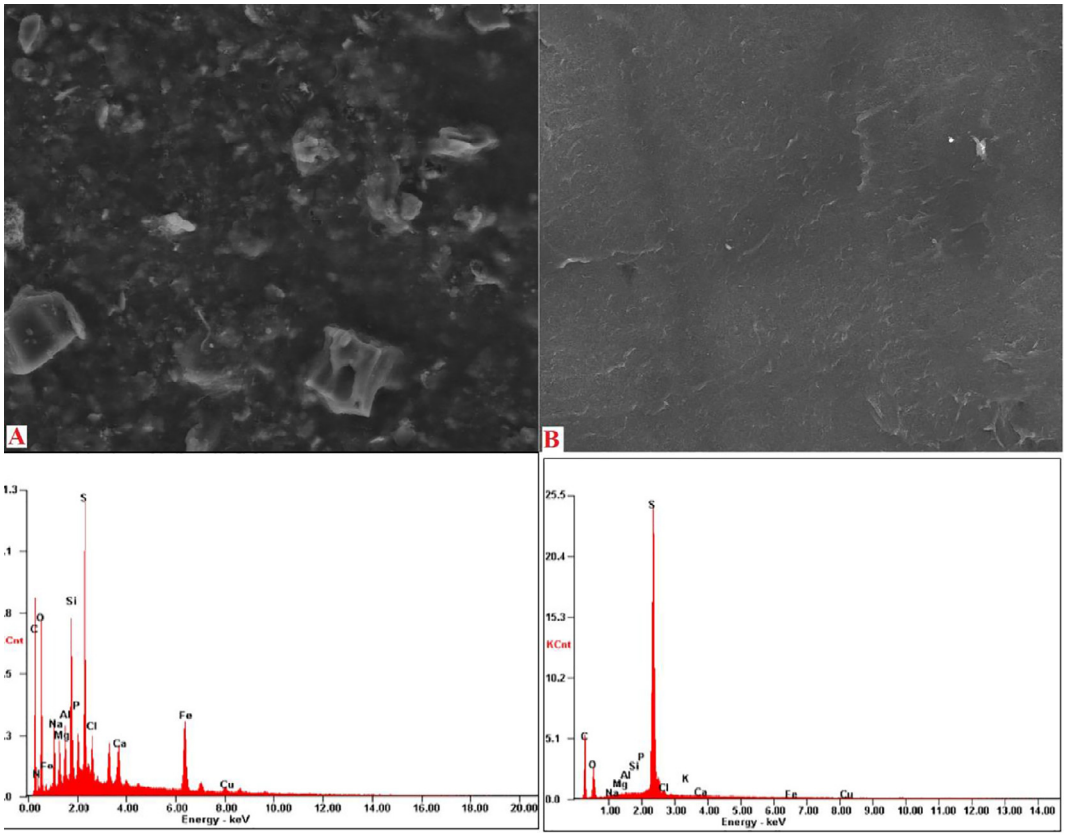

Fig. 6. SEM images (2500x) and SEM-EDS results of fouled membrane including the cake layer (A) and chemically cleaned membrane (B).

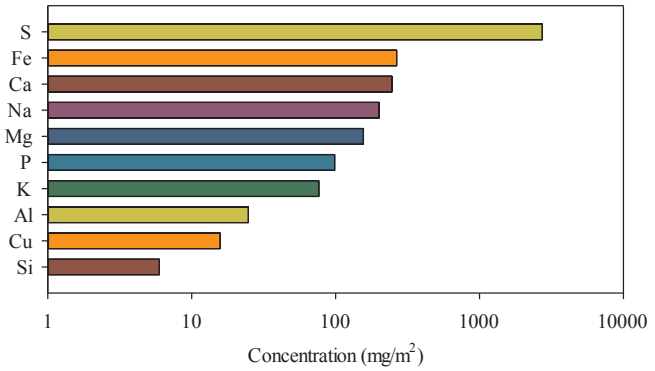

Fig. 7. Concentration of inorganic components in the cake layer obtained from AnMBR.

concentrations of Mg, Al, Fe, Ca and Si [36]. Particularly, under sulfate reducing conditions, the generated sulfide forms metal-sulfide precipitates. Also, the increase of pH during the sulfate reduction (Reaction 4) increases the precipitation of the metal-sulfides and metal-oxides on the membrane surface. Therefore, determination of inorganic foulants is important. For this purpose, SEM images were obtained from the membrane cake layer and inorganic foulants on the membrane cake were quantified by EDS. In addition, ICP analyses were performed on liquid samples extracted from cake layer in order to analyze the inorganic foulants.

SEM images of fouled and chemically cleaned membranes obtained from anaerobic membrane bioreactor are illustrated in Fig. 6. The dense and nonporous cake layer (Fig. 6A) is supposed to be the main reason for the increases in membrane pressure and membrane resistance, which were discussed in Section 3.3. After chemical cleaning, the cake layer was totally removed and a smooth membrane was obtained (Fig. 6B). But, small stains of inorganic precipitates were still present on the membrane even after chemical cleaning.

The SEM-EDS results also confirmed deposition of inorganic substances on the cake layer (Fig. 6). Mainly, S, Si, Fe, Na, Mg, P and Ca were detected, and their weight fractions were about 6.7%, 4.8%, 4.4%, 3.3%, 2%, 1.3% and 1.1%, respectively (Fig. S6). Fe obtained in the cake layer was due to the formation of insoluble FeS by the reaction between Fe in the tap water and sulfide produced by SRB. The high sulfur in cake layer was supposed to be due to the membrane structure. C, O, and N observed on cake layer were due to bacteria and EPS inside the cake layer in addition to the C and O presence in membrane structure (Fig. S6). After chemical cleaning, the remained C, O and S peaks were due to their presence in the membrane structure, but N peak disappeared completely (Fig. 6). SEM-EDS results showed that inorganic foulants were mostly removed upon chemical cleaning (Figs. 6 and S6).

Al, Cu, Fe, P, Ca, Mg, K, Si, Na and S concentrations in the cake layer were also determined by ICP after extraction at acidic conditions (Fig. 7). The results obtained with ICP measurements were in good agreement with the SEM-EDS results. The concentrations of these inorganic constituents were calculated based on their mass per membrane surface area. High S, Fe, Ca, Na, Mg and P accumulations on the membrane were determined. Particularly S (2,371 mg/m^2) and Fe (265 mg/m^2) were very high showing the precipitation of FeS on the cake layer. Also, high Ca (246 mg/m^2) and P (100 mg/m^2) may be due to precipitation of hydroxyapatite ($Ca_5(PO_4)_3OH$) on the cake layer. ICP results showed that Si was very low in cake layer (8 mg/m^2) although Si was determined to be at a weight percentage of 4.8% on the fouled membrane including cake layer (Fig. S6). In addition, Na and Mg were measured above 200 and 100 mg/m^2, respectively and K was about 80 mg/m^2. On the other hand, metals like Al and Cu were between 15 and 30 mg/m^2.

Table 2
The gene sequencing results of DGGE bands.

Band No	Identic Species	Accession No.	Similarity (%)	Phylogenic phylum
1	*Geobacter daltonii*	NR_074916.1	96	Proteobacteria
2	*Salana multivorans*	NR_025495.1	92	Actinobacteria
4	*Desulfovibrio sulfodismutans*	NR_026480.1	97	Proteobacteria

3.7. Bacterial community profiling by DGGE

For revealing the dominant microbial community in the AnMBR, DGGE of 16S rRNA genes was applied (Fig. S7). BLAST search of gene sequences was performed in GenBank databases for searching out the similarity of our clones to the reference strains. Regarding the BLAST search, the microbial community of the AnMBR seems to be quite stable.

Four different bands were identified in gels and gene sequencing results for three bands were provided in Table 2. The dominant bacterial phyla in the community are the members of Proteobacteria phylum, which plays a significant role in sulfate reduction and can be found in most of the sulfate-rich microbial environments. Providing that sulfate is available in the environment, the bacteria can utilize a diverse range of substrates, containing lactate, pyruvate, glycerol, ethanol, methanol, and H_2, as electron donors [3]. *Geobacter* genus from Proteobacteria phylum abounds in anoxic environments and responsible for metal reducing under organic carbon augmented conditions [37]. The phylum is also reported as dominant in many studies concerning biofouled MBR membranes. Various studies performed by using different types of wastewater (municipal, paper mill, molasses, etc.) revealed that α, β, γ, δ and λ classes of Proteobacteria play an active role in biofilm formation on the surface of membrane and cause biofouling [25]. In our study, δ-Proteobacteria was detected in AnMBR which mainly utilized ethanol as a substrate during sulfate-reduction.

Besides, the other phylum observed in AnMBR is Actinobacteria, which can utilize various substrates as carbon and energy sources and also can be responsible for membrane fouling [38]. During AnMBR treatment, membrane fouling is affected by process conditions and sludge characteristics. Even though the same microbial community causing biofouling exists in the same bioreactor, the dominance inside the community can be notably affected by operational conditions, which results in different fouling trends [24,38].

Desulfovibrio sulfodismutans was detected as the dominant sulfate-reducer in the sludge. The presence of this specie was also reported previously in a sulfate reducing lab-scale bioreactor [39] and in a full scale anaerobic bioreactor treating paper mill wastewater [40].

Overall, the sulfidogenic AnMBR showed high performance for sulfate reduction, COD oxidation, sulfide, and alkalinity generation. Also, the process has acceptable filtration characteristics. Therefore, the process should be further tested in the presence of heavy metals simulating acid mine drainage water.

4. Conclusions

Sulfate and COD removal efficiencies of 95 ± 4% and 90 ± 10%, respectively, could be achieved with the AnMBR process at a COD/sulfate (mg/mg) ratio of 0.75. The reactor was successfully operated without membrane fouling at 2 LMH, but frequent membrane fouling was observed at 4 LMH due to dense cake layer formation. Viscosity, CST, and SF tests showed acceptable filterability of the sulfate-reducing sludge in the process. High molecular weight soluble organics and EPS accumulation as well as deposition of inorganic substances (S, Si, Fe, Na, and Mg) on the cake layer may have contributed to membrane fouling.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2018.05.001.

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