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Optimization of arsenic removal from an acid mine drainage in an anaerobic membrane bioreactor

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ABSTRACT

This study aims at optimizing the arsenic removal performance of a sulfidogenic anaerobic MBR treating acid mine drainage (AMD). The feed sulfate concentration was kept constant at 2,000 mg/L and ethanol concentration was decreased steadily from 1,500 mg COD/L to 500 mg COD/L. Metal concentrations were kept at 75 or 150 mg/L Fe, 25 mg/L Cu, 5 mg/L Zn, 5 mg/L Co, 5 mg/L Mn, 2.5 mg/L Ni and 2.5 mg/L As. High sulfide concentration led to dissolution of orpiment (As_2S_3) and low As removal efficiency. Later, decrease of sulfide concentration in the bioreactor resulted in increasing As removal efficiency over 99% due to formation of orpiment and co-precipitation of As with amorphous iron precipitates. Flux was increased up to around 10 L/(m^2h) (LMH). It was concluded that heavy metals in the AMD behaved as a filtration aid and increased the sludge filterability, which was assessed by the regular analyses of supernatant filterability, specific resistance to filtration and capillary suction time.

1. Introduction

The mining of gold, copper and nickel containing minerals generates acid mine drainage (AMD), which causes long-term degradation of the environment. Arsenic is common in AMD and causes significant adverse effects on the fresh waters located downstream of mining sites (Fernandez-Rojo et al., 2017). Metal concentrations in AMD show great variations depending on the conditions and both As and the other metals may dissolve according to the reactions given below (Natarajan, 2008).

$$FeAsS + 7/2\,O_2 + H_2O \rightarrow Fe^{3+} + SO_4^{2-} + H_2AsO_4^- \tag{R1}$$

$$FeAsS + 13\,Fe^{3+} + 8\,H_2O \rightarrow 14\,Fe^{2+} + SO_4^{2-} + 13\,H^+ + H_3AsO_4 \tag{R2}$$

It is also well known that the presence of iron oxidizing acidophilic microorganisms increases the iron oxidation and metal release rates (Altun et al., 2014; Kaksonen and Puhakka, 2007). Hence, exposing sulfide-bearing materials to air and water is responsible for the AMD generation. Although this process may occur naturally, antropogenic mining activities promote the formation of AMD and hence environmental degradation (Akcil and Koldas, 2006).

The remediation options for AMD can be listed as neutralization, adsorption, ion exchange, membrane technologies, biological remediation, and electrochemical treatments (Park et al., 2019). However, the precipitation of metals with

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biogenically produced sulfide has many advantages including low solubility of metal-sulfide precipitates, a high degree of selectivity allowing separate metal recovery, low sludge volume and relatively low cost (Huisman et al., 2006). Treatment of AMD using sulfate reducing bacteria (SRB) requires external supplementation of organic matter as the AMD generally contains low organic matter concentration (Kaksonen and Puhakka, 2007; Sahinkaya et al., 2011). In the process, SRB use organic matter as electron source for the reduction of sulfate and generate sulfide (reactions 3 and 4), which precipitates heavy metals (reaction 5). Also, simultaneous generation of alkalinity increases pH (Huisman et al., 2006; Sahinkaya and Gungor, 2010). Sulfidogenic oxidation of ethanol, a good organic carbon source for SRB (Bekmezci et al., 2011; Nagpal et al., 2000), generates acetate as an intermediate, which may further be utilized as electron donor to generate bicarbonate and sulfide (Liu et al., 2018).

$$2C_2H_5OH + SO_4^{2-} \longrightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \tag{R3}$$

$$CH_3COO^- + SO_4^{2-} \longrightarrow 2HCO_3^- + HS^- \tag{R4}$$

Metals can precipitate according to the following reaction (reactions 5) (Kaksonen and Puhakka, 2007).

$$H_2S + M^{2+} \longrightarrow MS(s) + 2H^+ \tag{R5}$$

In addition to Fe, Zn, Ni, Co and Cu, AMD may also contain high concentrations of arsenic depending on the site and environmental conditions. In the Carnoules mine drainage, the total Fe and As concentrations were reported as 441–484 mg/L and 30–39 mg/L, respectively (Fernandez-Rojo et al., 2017). In another study, Fe^{2+} and As concentrations were reported as 790–1315 mg/L and 84–152 mg/L, respectively (Fernandez-Rojo et al., 2019). Hence, both Fe and As concentrations in the same mine site may show great variations. Arsenic concentration in the Solbec-Cupra tailing in Canada was reported as 10 mg/L (Kastyuchik et al., 2016).

Similar to other metals, As can be precipitated as orpiment (As_2S_3) with sulfide generated under sulfate reducing conditions (Altun et al., 2014; Battaglia-Brunet et al., 2012; Newman et al., 1997; Sahinkaya et al., 2015). Sulfate reduction bioprocess may be a good alternative for As containing AMD remediation as SRB may have tolerance to high As concentrations. Battaglia-Brunet et al. (2012) reported sulfate reduction in the presence 100 mg/L As(V) with glycerol and/or hydrogen as electron source even at pHs between 2.7 and 5. However, the stability of orpiment depends on pH and sulfide concentrations. Arsenic may be re-solubilized at high pHs and sulfide concentrations (Altun et al., 2014; Newman et al., 1997; Sahinkaya et al., 2015).

Biological sulfate reduction for AMD treatment has been extensively studied using various types of bioreactors, e.g. up flow and down flow fluidized bed bioreactors (Ozkaya et al., 2019), sludge blanket bioreactors (Sahinkaya et al., 2015) and continuously stirred tank bioreactors (Lopes et al., 2008; Sahinkaya, 2009). However, relatively few studies have been conducted using sulfidogenic membrane bioreactor (MBR) (Sahinkaya et al., 2018; Vallero et al., 2005; Yurtsever et al., 2016). In our previous study (Sahinkaya et al., 2018) in which sulfate reduction performance was studied in the absence of metal feeding, sulfate reduction efficiency of over 90% was attained in the sulfidogenic MBR. However, frequent membrane cleaning was required due to a dense cake layer accumulation on the membrane. In our subsequent study (Sahinkaya et al., 2019), the treatment of AMD was studied in a sulfidogenic MBR. Although high removals efficiencies for Mn, Cu, Zn, Fe, Ni and Co were observed, only partial As removal was observed, i.e. 41%–67%. Hence, in the present study, it was aimed to optimize the sulfidogenic MBR operation to maximize As removal and minimize the effluent sulfide concentration, which eliminates further processes to oxidize excess sulfide, by controlling organic matter dosage to the bioreactor. Also, the impacts of feed metal and COD concentrations on the filtration performance and the foulant characteristics were studied in detail. To the best knowledge of the authors, optimization of arsenic removal in a sulfate reducing MBR is being studied for the first time in the literature, which is a novel alternative for As-containing AMD treatment.

2. Materials and methods

2.1. The composition of synthetic AMD and Anaerobic Membrane Bioreactor (AnMBR)

Synthetic AMD (pH 3.5–4.0) was prepared considering a previous study conducted in a real active mine site (Sahinkaya et al., 2011) and provided in Table 1.

A plexiglass reactor used in our previous study (Sahinkaya et al., 2018) was also used in the present study. The total volume of the AnMBR was 6.6 L and the operating volume was around 3.5 ± 0.14 L. Double sided flat-sheet polyethersulfone (PES) ultrafiltration (0.02 μm pore average size) membrane modules were immersed in the AnMBR. The total active area of membrane at each module was 0.0072 m^2 and depending on the average flux intended, the number of the modules was adjusted. In the anaerobic MBRs, cake layer is the main cause of transmembrane pressure (TMP) increase (Dong et al., 2015) and proper physical cleaning of the membrane gains importance. In the study, in order to decrease the cake accumulation, 5 min filtration and 1 min resting cycle was used. Also, headspace of the bioreactor was circulated over the membrane (Table 2) both to mix the bioreactor and scour the cake layer. The AnMBR was operated at 35 ± 2 °C in a temperature controlled room.

The biomass in the bioreactor was already acclimated to AMD since the bioreactor was previously operated in the presence of metal under sulfate reducing conditions (Sahinkaya et al., 2019). The sulfate reducing community was dominated by *Desulfovibrio*-like bacteria (Sahinkaya et al., 2018).

Table 1
Synthetic acid mine drainage used in the study.

Component	Concentration	Metals	Concentration (mg/L)
Sulfate, mg/L	2000	Fe	75 or 150
Ethanol, mg COD/L	500–1500	Cu	25
Yeast extract, mg/L	50	Co	5
KH_2PO_4, mg/L	56	Mn	5
NH_4Cl, mg/L	110	Zn	5
Ascorbic acid, mg/L	11	Ni	2.5
pH	3.5–4.0	As(V)	2.5

Table 2
Operational conditions of the AnMBR.

Periods	Days	HRT (h)	Flux (LMH)	Gas scouring rate ($m^3/m^2/h$)	Feed COD (mg/L)	Feed Fe (mg/L)	Feed pH
1	0–14	26 ± 5	7.2 ± 0.0	4.8 ± 1.5	1500	75	4.0
2	15–48	18 ± 6	9.5 ± 1.8	7.7 ± 2.2	1500	75	4.0
3	49–61	12.5 ± 0	9.4 ± 0	7.3 ± 0	1500–1250	150	3.7
4	62–69	12.5 ± 0	9.4 ± 0	7.3 ± 0	1000	150	3.7
5	70–89	13 ± 0.2	9.13 ± 0.2	4.9 ± 2	750	150	3.6
6	90–95	24 ± 0	4.9 ± 0.0	4.2 ± 0	1500	–	7.0
7	96–103	46 ± 0	2.57 ± 0	4.2 ± 0	1500–1250	150	7–3.6
8	104–106	46 ± 0	2.57 ± 0	4.2 ± 0	1000	150	3.5
9	107–118	25 ± 2	4.8 ± 0.4	4.2 ± 0	750	150	3.6
10	119–131	24 ± 0	5 ± 0	4.2 ± 0	500	150	3.7

2.2. Operation of Anaerobic Membrane Bioreactor (AnMBR)

The operational conditions of the bioreactor are provided in Table 2. Between days 0 and 48, hydraulic retention time (HRT) of the AnMBR was around 1.0 d in period 1 and 0.75 d in the period 2. The concentrations of Fe and COD were kept at 75 mg/L and 1500 mg/L, respectively. Periods 1 and 2 differed in the flux, HRT and gas scouring rate applied. In the second period, flux increased from 7.2 to 9.5 LMH and gas scouring rate was simultaneously increased to 7.7 ± 2.2 $m^3/m^2/h$ not to increase the cake formation on the membrane. After second period, feed COD concentration was steadily decreased from 1500 mg/L down to 750 mg/L (Period 5). However, the MBR stability was lost due to mechanical problems in the period 6, then feed COD was adjusted back to 1500 mg/L and simultaneously feed pH was increased to 7.0 in order to recover the system performance. The reactor recovered quickly within 6 days and in the periods 7 and 8, when feed COD concentrations were decreased steadily at relatively high HRT of around 2.0 days. Lastly, HRT was decreased back to around 1.0 day with simultaneous decrease of feed COD to 500 mg/L at period 10. Hence, the performance of the AnMBR was assessed at low pHs, down to 3.5, and the impact of decreased sulfide concentration, controlled by feed COD, on As removal was investigated. Additionally, the impact of increasing Fe concentration on the As removal was investigated by doubling the influent Fe from 75 to 150 mg/L after 3rd period.

For the first 90 days of the operation, solid retention time (SRT) averaged 83 ± 5 days to eliminate high amount of metal accumulation in the bioreactor, and later sludge was not drawn from the bioreactor except for the required analyses.

The operation was stopped when TMP exceeded 0.5 bar for cleaning the membrane from foulants. The fouled membrane was firstly physically cleaned and then soaked successively in 1000 mg/L NaOCl and H_2SO_4 (pH 2) solutions for 1 h.

2.3. Sludge filterability

During the operation of the AnMBR, TMP, specific resistance to filtration (SRF), supernatant filterability (SF) and capillary suction time (CST) analyses were carried out. Also, sludge viscosity was measured. All the analyses were conducted according to our previous study (Sahinkaya et al., 2018).

2.4. Characterizing membrane foulants

Inorganic membrane foulants were characterized using inductively coupled plasma (ICP) and scanning electron microscopy coupled with energy dispersive spectroscopy (SEM-EDS) analyses. Heavy metals in the cake layer was measured with ICP-OES (Perkin Elmer Optima 7000) after extraction according to Sahinkaya et al. (2018).

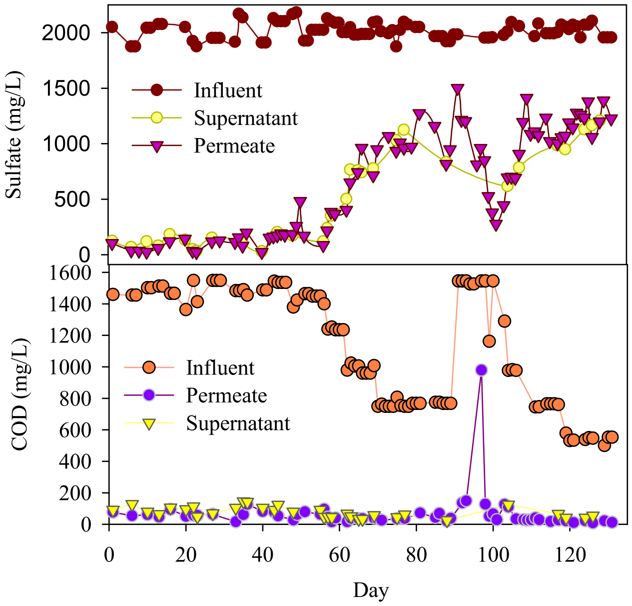


Fig. 1. Variations of sulfate and COD concentrations throughout the AnMBR operation.

2.5. Analytical methods

Concentrations of COD, sulfide and sulfate were measured after filtration through 0.45 μm pore-sized syringe filters. Sulfide interference in COD measurement was prevented by purging the sulfide after decreasing the sample pH below 2.0 with addition of H_2SO_4. The closed reflux method was used for COD measurement according to standard methods (APHA, 2005). $BaCl_2$ turbidimetric method was used for the measurement of sulfate (APHA, 2005). Sulfide concentration was spectrophotometrically measured (Hach-Lange DR5000) according to Cord-Ruwisch method (Cord-ruwisch, 1985). For the alkalinity measurement, 0.1-N HCl was used and pH was decreased until the endpoint of pH 4.5.

Perkin Elmer Optima 7000 ICP-OES (Optical Emission Spectrophotometry) was used for the measurement of Fe, Zn, Cu, Co, Ni, Mn and As after filtering the samples.

3. Results and discussion

3.1. COD and sulfate removal efficiencies

The variations of sulfate and COD concentrations were illustrated in Fig. 1. When the influent COD was 1500 mg/L, sulfate reduction and organic matter oxidation efficiencies averaged 94 ± 5% and 96 ± 2%, respectively. Hence, the presence of high concentrations of heavy metals and low pH did not result in a reduced process performance and quite high COD oxidation and sulfate reduction performances could be attained at COD/sulfate ratio of 0.75 that is slightly over than the theoretical value of 0.67 (reactions 3, 4).

With decreasing the feed COD concentration, sulfate reduction performance decreased due to COD limiting conditions. When the feed COD was 1000, 750 and 500 mg/L, the corresponding sulfate reduction performances were 66 ± 10%, 49 ± 6%, 40 ± 6%, respectively. Hence, decreasing COD/sulfate ratio from 0.75 to 0.25 significantly decreased sulfate reduction. Another significant point is that for the feed COD concentrations of 1000 mg/L and 750 mg/L, the reactor performances were evaluated twice, i.e. periods 4 and 8, and periods 5 and 9, respectively. Although HRT values were different, the sulfate reduction performances were quite similar, e.g. in period 4 and 8, sulfate reduction performances were 66 ± 10% and 66 ± 1%, respectively. Similarly, sulfate reduction performances in the periods 5 and 9 were 49 ± 6% and 47 ± 6%, respectively. Throughout the study, COD oxidation performance averaged 95 ± 2%. Dependence of the sulfate reduction performance on COD/sulfate ratio is also illustrated in Fig. 2. Sulfate reduction performance decreased almost linearly after decreasing COD/sulfate ratio below the theoretical value of 0.67. A COD/sulfate ratio of 0.67 is required to completely oxidize ethanol to CO_2 according to reactions 3 and 4. However, biomass growth over the organic compound was disregarded in these reactions, which means higher COD is required in reality. Hence, in the periods 1 and 2, COD/sulfate ratio was kept close to 0.75. In our previous study (Sahinkaya et al., 2018), the required COD for sulfate reduction was calculated as 0.71 g/g.

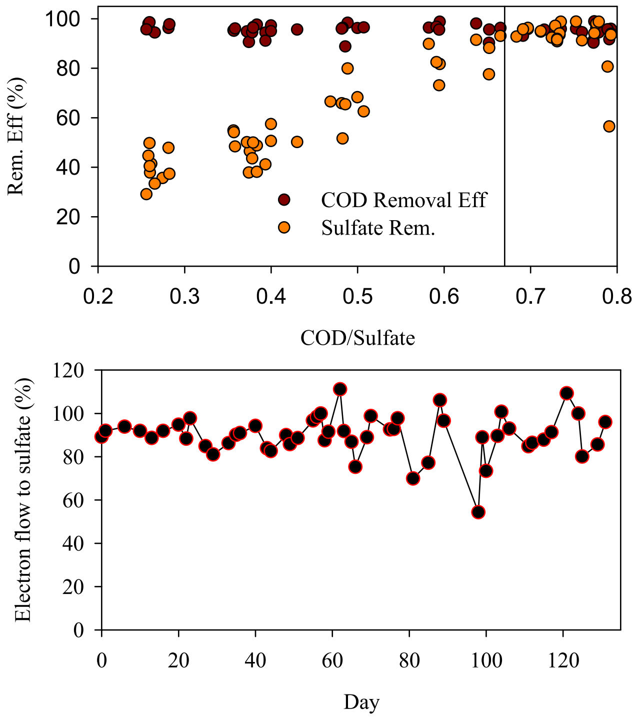


Fig. 2. Variations of sulfate and COD removal efficiency at varying COD/sulfate ratios (upper figure) and the electron flow from COD oxidation to sulfate (lower figure). The solid line in the upper figure illustrates the theoretical COD/sulfate ratio of 0.67.

The electrons released from oxidation of organics are used in sulfate reduction, theoretically corresponding to the use of 0.67 mg COD for each mg of sulfate reduced (Sahinkaya et al., 2018, 2013). Percent electron flow from ethanol to sulfate reduction was calculated according to Eq. (1). Where i and p stand for influent and permeate.

$$e - \text{flow}(\%) = 100 \times [0.67(SO_{4,i} - SO_{4,p})/(COD_i - COD_p)] \tag{1}$$

In this study, 90 ± 9% of the electron flow was towards sulfate reduction (Fig. 2). Similarly, in the absence of metals with sulfidogenic AnMBR, the electron flow to sulfate reduction was 91% (Sahinkaya et al., 2018). It seems that in the AnMBRs sulfate reducing bacteria were retained in the system, especially the slow growing acetate oxidizing sulfate reducers, which led to increased use of COD for sulfate reduction. In another study, with up-flow fluidized bed bioreactor (FBR) (Sahinkaya et al., 2007), the value was only 70%. Similarly, Kaksonen et al. found that 76% of the electrons generated from oxidation of ethanol was used for the sulfate reduction in a sulfate-reducing metal-precipitating fluidized-bed reactor (Kaksonen et al., 2004). Hence, MBRs may have advantage of keeping slow growing and non-biofilm forming suspended bacteria in the bioreactor.

3.2. Sulfide and alkalinity generations

Reduction of sulfate produces sulfide and alkalinity according to reactions 3 and 4. The variations of sulfide and alkalinity are illustrated in Fig. 3. In addition to measured values, theoretically calculated sulfide and alkalinity concentrations were presented considering the reactions 3, 4 and 5. Theoretical sulfide generation was calculated considering that one mole sulfide is required to precipitate one mole metal according to reaction 5. As expected, sulfide concentration decreased as the COD concentration in the feed was decreased. When COD/sulfate ratio was 0.75, around 95% sulfate reduction was observed and the theoretical and measured sulfide concentrations 563 ± 45 mg/L and 601 ± 60 mg/L, respectively. A difference of around 10% between the measured and calculated values was observed which was assumed acceptable considering the complex biological and chemical processes in the bioreactor. In the last period, when feed COD was decreased to 500 mg/L, permeate sulfide decreased substantially to 20–30 mg/L. Especially at low feed COD concentrations, the discrepancy between theoretical and the calculated values increased, which may be due to higher amount of sulfide consumption for metal precipitation at low sulfide concentrations.

Similar to sulfide, alkalinity concentrations decreased with decreasing feed COD concentrations. When the feed COD concentration was 1500 mg/L, corresponding to COD/sulfate ratio of 0.75, permeate alkalinity averaged 1902 ± 229 mg/L, which was quite close to the theoretical value of 1984 ± 114 mg/L. When COD/sulfate ratio was adjusted as

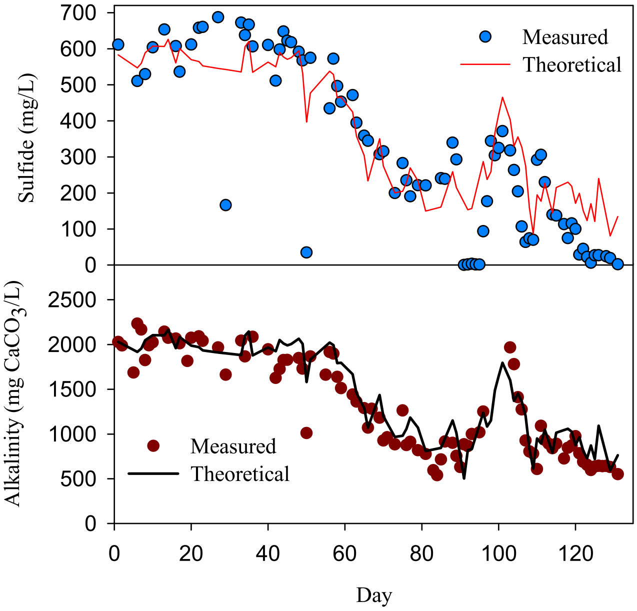


Fig. 3. Variations of sulfide and alkalinity concentrations throughout the operation. Theoretical values were calculated according to reactions 3, 4 and 5.

0.25 (the last period), permeate alkalinity averaged 693 ± 123 mg $CaCO_3$/L and the theoretical alkalinity production was computed as 791 ± 115 mg $CaCO_3$/L (Fig. 3). The difference between the measured and calculated values may originate from the alkalinity consumption for AMD neutralization. As a result of the alkalinity generation, the permeate pH averaged 7.3 ± 0.3. Hence, acidic wastewater was neutralized with the alkalinity generation without external alkalinity supplementation.

3.3. *Metal removal*

According to reaction 5, metals form insoluble sulfide precipitates in the bioreactor. The average feed, supernatant and permeate metal concentrations are provided in Table 3. Arsenic concentration is not given in Table 3 as it was discussed in more detail later. Except for Mn and As, higher than 99% removals were observed for the metals. The solubility product of MnS and $Mn(OH)_2$ are relatively high (Sahinkaya et al., 2011), which caused increased Mn concentrations in permeate. Hence, sulfide generated by sulfate reduction formed insoluble sulfide-salts with the metals present in the synthetic AMD. The permeate concentrations were generally quite close to the measurement limits and increasing feed Fe concentration from 75 to 150 mg/L did not adversely affect Fe removal performance or effluent quality.

In the study, feed COD concentration was decreased from 1500 mg/L down to 500 mg/L in order to decrease effluent sulfide concentration and evaluate its impact on As removal efficiency (Fig. 4). Decrease in sulfide concentration led to simultaneous decrease in As concentration. When the effluent sulfide concentration was around 600 mg/L, permeate As concentration averaged around 1.5 mg/L corresponding to 40% removal efficiency. When the sulfide concentration decreased to around 200 mg/L and 50 mg/L, permeate As concentrations decreased to 0.51 ± 0.21 mg/L (80% removal efficiency) and 0.016 ± 0.0058 mg/L (99.3% removal efficiency), respectively. Hence, As concentration in the permeate decreased to quite low levels when the sulfide concentration was reduced below 50 mg/L (1.5 mmol). At higher sulfide concentrations, As concentration in permeate increased almost linearly with increasing sulfide concentration in the bioreactor (Fig. 4). The reason of observing high permeate As concentration was dissolution of As_2S_3 precipitates at high sulfide concentrations according to reaction 6 as discussed in previous studies. Newman et al. (1997) compared three different bacteria for their As precipitating ability under sulfate reducing conditions. They compared *D. auripigmentum* to MIT-13, which is an As(V) reducer, and to *D. propionicus*, which is a sulfate reducer. Within these bacteria, As_2S_3 precipitation was only observed in *D. auripigmentum* cultures when As^{3+} concentration was 1 mM and sulfide concentrations were between 0.01 and 0.1 mM. When sulfide concentration was above 1 mM, AsS_3 precipitate was dissolved. In our study, similar results (Fig. 4) were observed as low As concentrations were reached when the permeate sulfide concentrations were lower than around 1.5 mM. Also, permeate As concentration increased with increasing sulfide concentration in the bioreactor (Fig. 4). Similarly, Battaglia-Brunet et al. (2012) studied As precipitation in a sulfidogenic fixed-film bioreactor. When the reactor was fed with glyserol, high rate As removal was obtained due to orpiment (As_2S_3) generation. When glyserol was replaced with hydrogen, sulfide generation increased and orpiment precipitates dissolved. In our previous fixed bed column bioreactor study (Altun et al., 2014), As precipitation rate increased appreciably when As containing

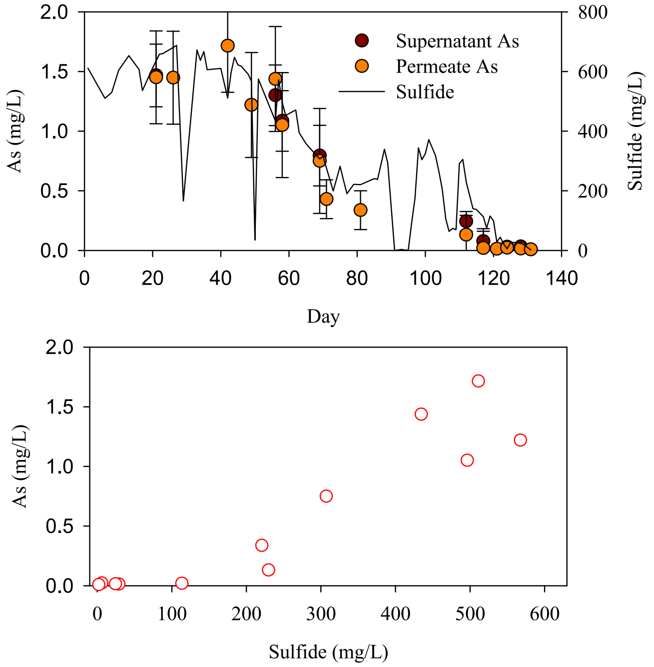


Fig. 4. Relationship between permeate sulfide and As concentrations.

Table 3
Feed, supernatant and permeate concentrations of the metals.

	Influent (mg/L)	Supernatant (mg/L)	Permeate (mg/L)	Removal Eff. (%)
Fe	75 or 150	0.09 ± 0.09	0.02 ± 0.06	99.98 ± 0.0
Cu	25	0.0375 ± 0.022	0.013 ± 0.015	99.94 ± 0.06
Zn	5	0.01275 ± 0.03	0.01 ± 0.02	99.8 ± 0.5
Co	5	0.011 ± 0.013	0.00 ± 0.00	99.98 ± 0.09
Ni	2.5	0.009 ± 0.027	0.0032 ± 0.016	99.87 ± 0.41
Mn	5	0.43 ± 0.34	1.4 ± 1.37	72 ± 27

feed was supplemented with 100 or 200 mg/L Fe. The removal efficiency further increased when the effluent sulfide was decreased below 46 mg/L (1.4 mM). Therefore, in order to achieve high As removals, sulfide concentration should be high enough to precipitate As, but should be low enough not to promote orpiment dissolution (Newman et al., 1997).

$$3/2\mathrm{As_2S_3}\ (\text{amorphous}) + 3/2\mathrm{H_2S} \rightarrow \mathrm{H_2As_3S_6^-} + \mathrm{H^+} \qquad \text{(R6)}$$

Precipitation of metals in the bioreactor increased the MLSS concentration, especially after changing the SRT from around 83 days to infinite (Fig. 5). Until day 90, MLSS concentration did not show significant change and averaged 24 ± 4 g/L, then with the increase of SRT to infinite MLSS concentration increased almost linearly to around 42 g/L until day 112 and then stabilized and averaged 42.1 ± 0.2 g/L. Similarly, MLVSS concentrations averaged 3.75 ± 1.10 g/L and 6.63 ± 0.82 g/L at SRT 83 days (until day 90) and infinite (after day 90), respectively. Hence, increasing SRT significantly increased MLSS and MLVSS concentrations.

Precipitation of metals with sulfide has many advantages compared to hydroxide precipitation; lower metal concentrations and better filterability of the generated sludge (Huisman et al., 2006; Kaksonen and Puhakka, 2007). The sludge volume may be 6–10 times lower compared to hydroxide precipitation and the generated sludge may be used for metal recovery (Huisman et al., 2006). Metal-sulfide sludge is also more stable and easier to manage.

3.4. Filtration performance

The variations of flux and TMP throughout the operation are provided in Fig. 5. For the first 90 days, flux averaged 9.0 ± 1.4 LMH and then flux averaged around 4.9 ± 0.3 LMH (excluding days 96–106). For the first 90 days, membranes were chemically cleaned three times. It seems that MBR can be operated at around 10 LMH with regular chemical cleaning. When the flux was decreased to around 5 LMH, TMPs stabilized at a quite lower level and cleaning was not required for around 50 days.

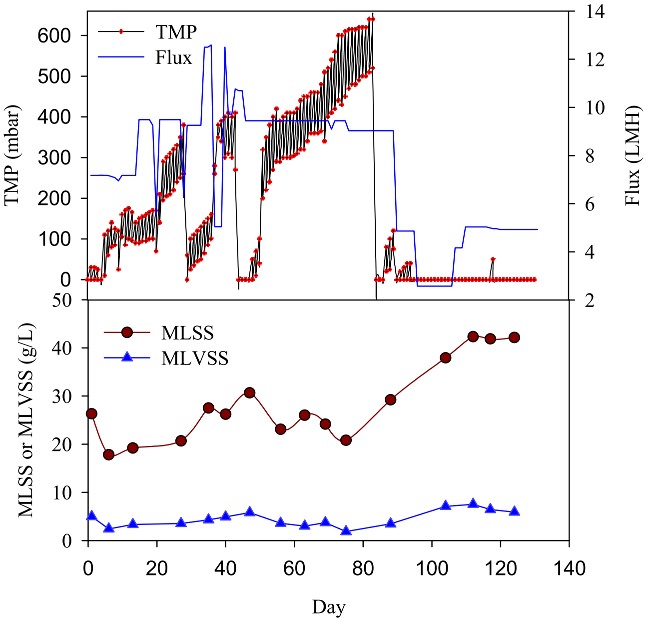
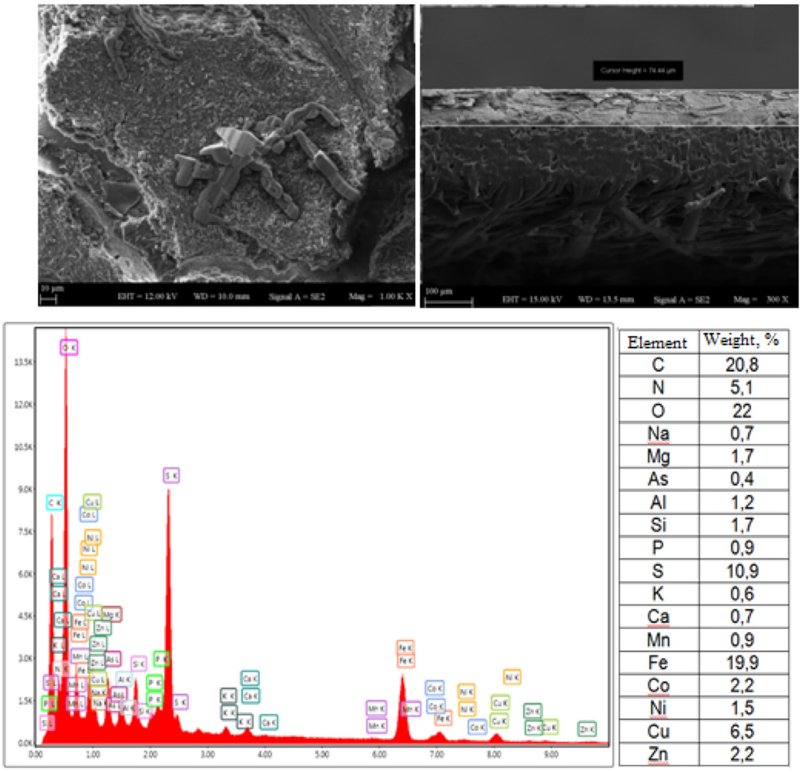


Fig. 5. Variations of TMP, flux (upper figure) and MLSS, MLVSS concentrations (bottom figure) throughout the operation.

Considering that the reactor was operated under anaerobic conditions and the MLSS concentrations reached to over 40 g/L, the observed flux values were quite high considering the literature values. In our previous study (Sahinkaya et al., 2018), a sulfidogenic MBR was operated at a flux of 2 LMH with very limited cleaning requirement, which, however, required very frequent membrane cleaning when the flux was increased to 4 LMH. Observing high flux values in the present study should be due to the removal of organics such as SMP and EPS from supernatant with the precipitating metals. In the synthetic AMD, Fe concentration was 75 or 150 mg/L and high SRT caused accumulation of metal-sulfide particles in the AnMBR. Hence, metals behaved as coagulants and swept out the colloidal materials from the mixed liquor, which caused high operational fluxes and low membrane fouling. In the study of Wei et al. (2014), municipal wastewater treatment under anaerobic conditions with MBR was studied. The sustainable flux was 6 LMH and a more rapid TMP increase was reported at 10–12.5 LMHs. In another study, Jeong et al. (2018) compared the filtration performances of ceramic and polymeric membranes for the treatment of municipal wastewater under anaerobic conditions. Although initial fluxes in both AnMBR were adjusted as 5 LMH, flux values were decreased in both reactors. In ceramic membrane integrated AnMBR, flux was maintained at around 3 LMH, whereas flux significantly reduced down to 2 LMH in the polymeric AnMBR. Jensen et al. (2015) studied treatment of slaughterhouse wastewater treatment under anaerobic conditions with MBR. Although critical flux was 9 LMH, sustainable permeate flux was between 3 and 7 LMH. Hence, relatively higher fluxes observed in our study, even in the presence of high metal concentrations, may present the possibility for a process development for further pilot and real scale applications.

During the operation of the MBR, regular sludge samples were taken for the measurement of CST and specific CST (data not shown). No clear dependence of CST or sCST on the operational conditions, e.g. HRT, SRT or Fe concentration, was observed and the values averaged 33 ± 10 s and 1.2 ± 0.27 Ls/g MLSS, respectively. Dereli et al. (2014) studied impact of SRT on sludge filterability in an AnMBR treating high strength wastewater. They reported increase of CST from 951 s to 2414 s with increasing SRT from 20 days to 50 days. Similarly, normalized CST increased from 61 to 86 Ls/g MLSS with increasing SRT. Huang et al. (2013) studied the performance of AnMBR for the treatment of domestic wastewater. Similar to the study of Dereli et al. (2014), CST increased from 282 to 532 s with increasing SRT from 30 to 90 days. CST is an easy way of measuring sludge filterability and short CSTs observed in our study indicated that sustainable filtration can be achieved for the treatment of AMD using AnMBR.

Specific resistance to filtration (SRF) and supernatant filterability (SF) are other parameters that can be used to assess sludge filterability. Throughout the reactor operation SRF and SF averaged $2.1 \pm 1.2 \times 10^{13}$ m/kg and 1.64 ± 1.0 mL/min, respectively. In a previous study, textile wastewater was treated in sequential anaerobic and aerobic MBRs (Yurtsever et al., 2016). SRF values for anaerobic and the following aerobic MBRs were 8.0×10^{14} and 1.8×10^{14} m/kg, respectively. Hence, the presence of metals at high concentrations in the AMD seems to improve the sludge filterability. Yurtsever et al. (2016) also reported SF values as 0.33 and 1.87 mL/min, respectively, for anaerobic and aerobic MBRs. In the present study, the observed SF (1.64 ± 1.0 mL/min) is very close to the one observed for the aerobic one. Hence, the presence of heavy metals in the solution may also decreased colloidal particle concentrations in the supernatant, which improved



Element	Weight, %
C	20,8
N	5,1
O	22
Na	0,7
Mg	1,7
As	0,4
Al	1,2
Si	1,7
P	0,9
S	10,9
K	0,6
Ca	0,7
Mn	0,9
Fe	19,9
Co	2,2
Ni	1,5
Cu	6,5
Zn	2,2

Fig. 6. SEM images and SEM-EDS analyses of the developed cake layer on membrane surface.

supernatant filterability. Improving filterability and decreasing membrane fouling with the addition of iron salts have been also reported in other studies (Wu and Huang, 2008; Zhang et al., 2008).

Sludge viscosity is also another parameter affecting sludge filterability (Hasar et al., 2004) and energy required for sludge mixing. Viscosity is especially important in our study as the accumulation of heavy metals significantly increased sludge viscosity. Throughout the study, Bingham plastic viscosity averaged 6.0 ± 0.7 cP. In our previous study (Sahinkaya et al., 2018), viscosity averaged 3.64 ± 0.12 cP for the sludge samples obtained from sulfate reducing AnMBR receiving no heavy metals in the feed. Hence, the presence of heavy metals significantly increased sludge viscosity as expected. In the study of Akram and Stuckey (2008), the improvement of flux in an AnMBR using powdered activated carbon (1.67 g/L) was observed where the viscosity was measured as 1.32 cP. Further increasing PAC concentration to 3.4 g/L resulted in a steep increase in viscosity to 7 cP and later to 14 cP.

3.5. Metal accumulation in the cake layer

The presence of heavy metals at high concentrations in wastewaters, especially Fe, can increase the cake accumulation on the membrane due to increased MLSS concentrations (Fig. 5). On the other hand, the presence of heavy metals may increase the permeability of the cake layer, which can be postulated from comparison of sludge filtration characteristics of the present study with those of the previous study conducted in the absence of heavy metals (Sahinkaya et al., 2018). Ji et al. (2008) reported that the permeability of the cake layer increased with polymeric ferric sulfate addition due to deposition of larger and looser flocs.

SEM-EDS analyses of the cake layer were conducted to determine the inorganic foulants (Fig. 6). As expected, Fe, S, Cu and Zn contents in the sludge were quite high. The thickness of the developed cake layer was around 75 µm. The cake layer was also scrubbed, metals were extracted and measured using ICP (Fig. 7). Similar to SEM-EDS results, Fe concentration was quite high and reached around 1.6 g/m^2. Unlike SEM-EDS results, Ca^{2+} and Mg^{2+} concentrations were higher than 600 mg/m^2, which were the second elements with the highest concentration in the cake layer. Observing relatively lower concentrations in SEM-EDS results may be related with the positions of the Ca^{2+} and Mg^{2+} elements as the SEM-EDS detects the metals deposited on upper layer of the thick cake layer. P precipitation was also observed in the cake layer, which may have precipitated with Ca^{2+}.

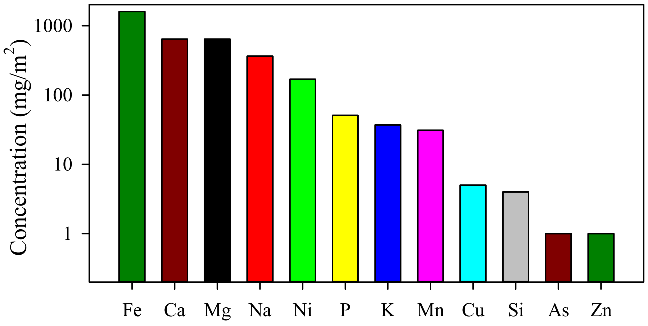


Fig. 7. Concentration of inorganics in the cake layer.

4. Conclusions

Optimization of As precipitation in a sulfidogenic MBR through controlling sulfide concentration by decreasing COD/sulfate ratio was studied. COD/sulfate ratio was decreased from 0.75 till 0.25 by decreasing feed COD concentration. Under COD limiting conditions, COD oxidation efficiency was higher than 95%, whereas sulfate reduction efficiency decreased from around 95% to 40% at COD/sulfate of 0.75 and 0.25, respectively, which caused sulfide concentration to decrease from 600 mg/L to 20–40 mg/L, respectively. At COD/sulfate ratio of 0.25, COD was just high enough to precipitate all the metals and to produce enough alkalinity to increase feed pH from around 3.5 to neutral values for the studied conditions. At high sulfide concentrations, orpiment (As_2S_3) started to be dissolved, whereas removal efficiency of As was above 99% at low sulfide concentrations ($<$50 mg/L). Hence, optimizing the sulfide concentrations improved both As removal and eliminated or decreased further sulfide treatment requirement, i.e. aerobic oxidation to elemental sulfur or sulfate. High metal in the feed increased sludge filterability in the MBR, which may be due to behaving of metal precipitates as filtration aids and decreasing colloidal particles and generating a more permeable cake layer. Study illustrated that As-containing AMD can be successfully treated with sulfidogenic AnMBR by optimizing sulfide concentrations in the bioreactor.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ece Yigit: Investigation, Formal analysis. **Adem Yurtsever:** Investigation, Formal analysis. **Senem Teksoy Basaran:** Writing - original draft, Validation, Visualization. **Erkan Sahinkaya:** Conceptualization, Supervision, Validation, Writing - review & editing.

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