Performance of sulfidogenic anaerobic baffled reactor (ABR) treating acidic and zinc-containing wastewater

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ABSTRACT

The applicability of anaerobic baffled reactor (ABR) was investigated for the treatment of acidic (pH 4.5–7.0) wastewater containing sulfate (1000–2000 mg/L) and Zn (65–200 mg/L) at 35 °C. The ABR consisted of four equal stages and lactate was supplemented (COD/SO4 = 0.67) as carbon and energy source for sulfate reducing bacteria (SRB). The robustness of the system was studied by decreasing pH and increasing Zn, COD, and sulfate loadings. Sulfate-reduction efficiency quickly increased during the startup period and reached 80% within 45 days. Decreasing feed pH, increasing feed sulfate and Zn concentrations did not adversely affect system performance as sulfate reduction and COD removal efficiencies were within 62–90% and 80–95%, respectively. Although feed pH was steadily decreased from 7.0 to 4.5, effluent pH was always within 6.8–7.5. Over 95% Zn removal was attained throughout the study due to formation of Zn-sulfide precipitate.

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1. Introduction

The exploitation of sulfide minerals results in oxidation of exposed iron and sulfur compounds, and thus, in the production of acidic metal and sulfate-containing wastewaters (e.g. acid mine drainage water (AMD)) (Nagpal et al., 2000a,b; García et al., 2001). Conventionally, hydroxide precipitation is the most commonly applied method for the treatment of metal containing waters. The production of high quantities of sludge is the main disadvantage of the method. Also, sulfate removal is only possible when Ca²⁺ containing chemicals, such as lime, are used for neutralization. However, stringent discharge legislations will dictate more efficient sulfate removal and recovery of valuable metals from waters, which are possible with the use of active bioreactor processes (Kaksonen and Puhakka, 2007).

In the treatment of AMD and metal containing industrial wastewater, sulfate-reducing bioreactors are becoming an alternative to conventional chemical treatment (Kaksonen and Puhakka, 2007; Liamleam and Annachhatre, 2007; Hoa et al., 2007; Costa et al., 2007). With the supplementation of organic compounds, sulfate is microbiologically reduced to H₂S under anaerobic conditions and heavy metals form stable precipitates with produced H₂S. Moreover, produced bicarbonate increases the pH of the wastewater (Eqs. (1) and (2)). This way, metals and sulfate are concomitantly removed and pH can be increased to neutral values in a single reactor (Eqs. (1) and (2)). The precipitate can be used for metal recovery (Kaksonen et al., 2003).

\[ \text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- \]  
(1)

\[ \text{H}_2\text{S} + \text{M}^{2+} \rightarrow \text{MS} + 2\text{H}^+ \]  
(2)

In the literature, several studies have shown that sulfate reducing suspended (Moosa et al., 2002, 2005; Sahinkaya, 2008) and attached growth (Steed et al., 2000; Kaksonen et al., 2003; Sahinkaya et al., 2007; Hoa et al., 2007) bioprocesses can be effectively used for AMD treatment. However, it is well known that with the biofilm type reactors higher removal rates at short hydraulic retention time (HRT) can be achieved compared to suspended growth reactors. For example Kaksonen et al. (2003), showed that at 35 °C fluidized-bed reactor (FBR) treatment of metal-containing wastewater results in almost complete precipitation of Zn and Fe at loading rates of over 600 and 300 mg/L d, respectively. However, accumulation of metal precipitates within biofilm reactors makes metal recovery difficult (Sahinkaya, 2008).

The anaerobic baffled reactor (ABR) is a modification of up-flow anaerobic sludge blanket (UASB) reactor and it is a staged reactor where biomass retention is enhanced by forcing the water flow through several compartments (Kaksonen and Puhakka, 2007). In ABRs, the over and underflow of liquid reduces bacterial washout, which enables it to retain active biological solids without the use of any fixed media. The other significant advantage of ABR is that partial separation of bacteria in different compartments occurs, which prevents most of the biomass to expose adverse environmental

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conditions, such as low pH and high metal concentrations (Uyanik et al., 2002a,b; Vossoughi et al., 2003). Vossoughi et al. (2003) studied the performance of ABR at COD/SO$_4^{2-}$ ratios of 16.7–6. They reported that methanogenic archeae (MA) and sulfate reducing bacteria (SRB) can coexist in the same reactor and COD removal efficiency slightly increased with increasing sulfate concentration. The maximum sulfate-reduction efficiency was 86–97% and at high sulfate concentrations, the conversion efficiency in the first compartment was low and most of the sulfate was reduced in the following compartments. Although several studies have shown that ABRs are very effective in anaerobic wastewater treatment and biomass granulation (Uyanik et al., 2002a,b; Sallis and Uyanik 2003; She et al., 2006), few studies (Barber and Stuckey, 2000; Vossoughi et al., 2003) have explored its sulfate-reduction potential. Also, the potential of ABRs for the biotreatment of sulfate and metal-containing wastewaters has not been studied (Kaksonen and Puhakka, 2007).

The dissolved organic carbon content of metal-containing wastewater is very low and usually <10 mg/L (Johnson, 2000). Therefore, addition of a suitable carbon source and electron donor for sulfate reduction is necessary to promote biogenic H$_2$S production. SRB utilize several low molecular weight substrates, such as lactate, formate, acetate, ethanol and hydrogen. Some SRB oxidize organic substrates completely to CO$_2$, while others incompletely to acetate (Widdel, 1988). It is well known that lactate is a good substrate for most SRB and it can be used in bioreactor applications for the treatment of sulfate and metal-containing wastewaters (e.g. AMD) (Kaksonen et al., 2003).

Hence, this study aims at evaluating lactate-fed ABR potential for the biotreatment of acidic, Zn- and sulfate-containing synthetic AMD. To our knowledge, this is the first study on AMD treatment using a sulfidogenic ABR.

### 2. Methods

#### 2.1. Bioreactor

A laboratory scale ABR was inoculated with an effluent of a full scale anaerobic digester located in Gaziantep, Turkey. Before inoculation, the sludge was sieved to remove coarse materials. The ABR was 20 cm wide, 80 cm long, 20 cm deep and constructed from glass, with a working volume of 20 L. Reactor was divided into four equal 5 L compartments by vertical baffles, each compartment having down-comer and riser regions created by further vertical baffle. The lower parts of down-comer baffles were angled at 45° in order to direct the flow evenly through the riser (Uyanik et al., 2002a,b). This produced effective mixing and contact between the feed and sulfate reducing bacteria (SRB) at the base of each riser. Each compartment was equipped with sampling ports that allowed drawing biological sludge, and liquid samples. To maintain anaerobic conditions, the sampling ports of the reactor and the fittings were sealed after inoculation. The reactor was maintained at 35°C using a heater fan in a cabin. The produced methane gas was measured using gas-liquid displacement method and a safety bottle was used to avoid the vacuum of NaOH to the reactor. HRT was kept constant at 2 days throughout the study. To do this, the synthetic wastewater (pH 4.5–7.0 and 2000 mg/L sulfate) containing 2563 mg/L MgSO$_4$·7H$_2$O; 1479 mg/L Na$_2$SO$_4$; 56 mg/L KH$_2$PO$_4$; 110 mg/L NH$_4$Cl; 11 mg/L Ascorbic acid, 50 mg/L yeast extract and 1340 mg COD/L lactate was fed to the reactor at a rate of 10 L/day. The composition of AMD may show great variation (Johnson, 2003) and we aimed to simulate a moderately acidic and Zn containing AMD. Lactate, which is one of the best organic source for SRB (Liamleam and Annachhatre, 2007; Kaksonen et al., 2003) was used as a carbon and electron source stoichiometrically to reduce sulfate to hydrogen sulfide and oxidize lactate completely to CO$_2$ and H$_2$O. Hence, throughout the study COD/SO$_4^{2-}$ ratio was kept at 0.67. The feed solution was prepared daily. COD removal, Zn precipitation and sulfate reduction was not observed in the feed container.

#### 2.2. Experimental procedure

The reactor performance was investigated at different feed organic, sulfate and Zn loadings for 304 days (Table 1). Firstly, the reactor was fed with an alkaline solution containing 1000 mg/L SO$_4^{2-}$ without Zn (Period I, days 0–46) to enrich SRB. Then, the reactor performance was investigated at increased Zn, sulfate and organic loadings (Table 1) with decreasing pH.

The reactor feed, each compartment, and the effluent were sampled 3–4 times in a week for the measurement of pH, alkalinity, total volatile fatty acids (VFA), chemical oxygen demand (COD), sulfate, dissolved sulfide, and soluble Zn.

#### 2.3. Analytical techniques

Before the measurement of sulfate, dissolved sulfide, soluble Zn and COD, samples were centrifuged using Hettich Rotofix 32 centrifuge at 4000 rpm for 10 min. Before centrifugation for sulfide measurement, the pH was increased to around 10 with 1 M NaOH and glass sample tube was sealed using a Teflon crimp cap not to cause any loss of sulfide. Total sulfide was analyzed spectrometrically using a Shimadzu UV-1601 Spectrophotometer following the method described by Cord-Ruwisch (1985). A turbidimetric method was used to measure sulfate concentrations. COD and alkalinity were also measured according to Standard Methods (APHA, 1999).

Before COD measurements, sample pH was decreased to below 2 with concentrated H$_2$SO$_4$ and the sample was purged with N$_2$ gas around 5 min to remove H$_2$S from the sample. Total VFA concentration was measured following the procedure described by Alvarez et al. (2007). For soluble Zn measurements sample was first filtered through 0.45 μm polyethersulfone membrane syringe filters and then acidified with concentrated HCl to pH below 2. For total Zn concentration measurements, samples were first acidified with concentrated HCl to solubilise Zn particles. Then, samples were filtered through 0.45 μm to remove biomass and other particles. Zn concentration was measured with an atomic absorption spectrophotometer (Varian AA 140). Panalytical Axios-Advanced wavelength dispersive X-ray fluorescence spectrometer (XRF) was employed to analyze the elemental composition of sludge samples.
drawn from the compartment 1 at the end of reactor operation (day 304).

All measurements were done at least in duplicate. Mean values and standard deviations were presented.

3. Results and discussion

3.1. Sulfate reduction and alkalinity production

The performance of the reactor throughout the study is summarized in Fig. 1 and Table 2. The operational conditions were not changed until steady state conditions were reached. The reactor was assumed to be operating at steady state when the measured effluent sulfate, alkalinity, sulfide and COD concentrations did not show variations higher than 10% at least for the last five measurements. The steady state performance of the reactor was provided in Table 2. Between days 0 and 46 (Period I), reactor was fed with 1000 mg/L sulfate at pH 6.5–7 without Zn supplementation to enrich SRB. COD, Zn and sulfate concentrations decreased from compartments 1 to 4. For the first 10 days, sulfate removal efficiencies for compartments 1–4 were 20%, 23%, 25% and 30%, respectively, and increased steadily to around 50%, 65%, 75% and 80%, respectively, at the end of period 1. Increasing Zn concentration during periods 2 and 3 to 65 mg/L and 130 mg/L, respectively, did not adversely affect the system performance and percent sulfate reductions increased to around 40%, 70%, 85% and 90% for compartments 1–4, respectively, at the end of period 1. Increasing Zn concentration during periods 2 and 3 to 65 mg/L and 130 mg/L, respectively, did not adversely affect the system performance and percent sulfate reductions increased to around 40%, 70%, 85% and 90% for compartments 1–4, respectively, at the end of period 1. With increasing feed sulfate concentration to 2000 mg/L (Period IV), sulfate removal efficiencies decreased and it was only 62% for the compartment 4. Although sulfate-reduction efficiency decreased, sulfate reduction rate increased from 450 to 620 mg/L d with increasing feed sulfate concentration from 1000 to 2000 mg/L.

Fig. 1. Sulfate (A), COD (B), and Zn (C) removal performances. Left column shows feed and effluent concentrations, right column shows percent removals.
Moreover, the sulfate-reduction efficiency and the rate increased to 73% and 730 mg/L, respectively, at the end of Period V. This increase should be due to the adaptation of SRB to the new operational conditions (e.g. increased sulfide concentration). After Period V, the feed pH was decreased steadily (Table 1) keeping the other operational conditions constant. Decreasing feed pH from around 7.0 to 4.5 did not adversely affect the system performance and sulfate-reduction efficiency increased from 20% to 85% within 70 days of operation. 

Fig. 2A shows feed and effluent pH variations for each compartment throughout the reactor operation. The pH increased from compartments 1 to 4 due to alkalinity production (Fig. 2B) during sulfate reduction (Eq. (1)). The pH at the effluent of compartment 4 was close to 7.0 although the feed pH was decreased to around 4.5 at Period VIII. This shows that ABR is a potential alternative for the treatment of acidic metal- and sulfate-containing wastewater.

3.2. COD removal and VFA production

Similar to sulfate, COD concentration decreased from compartments 1 to 4. During the start-up of the reactor (Period I), the COD removal efficiencies were 46%, 62%, 65% and 79%, respectively, for the compartments 1–4, respectively. Removal efficiencies increased to around 50%, 80%, 90% and 95% for the compartments 1–4, respectively, at the end of Period III. This increase showed that the COD removal performance was not adversely affected by Zn addition and SRB were enriched during the operation. With increasing feed COD concentration from 670 to 1340 mg/L (Period IV), COD removal efficiency slightly decreased from 95% to 87% at compartment 4. Similar to sulfate reduction, although COD removal efficiency decreased, the COD removal rate increased from 320 to 585 mg/L with increasing feed COD concentration. Decreasing feed pH steadily from 7 to 4.5 (Periods VI–VIII) did not adversely affect the COD removal efficiency (Table 2 and Fig. 1) and it averaged 86%.

Similar to COD and sulfate, VFA concentrations decreased from compartments 1 to 4 (Fig. 2C). This claims that the lactate was converted to VFA in the initial compartment and VFA concentration decreased as the wastewater moved through the reactor. Similar to the trends of sulfate and COD, the effluent VFA concentration increased slightly with increasing feed lactate concentrations from 670 to 1340 mg COD/L. Results showed that VFA may accumulate in the reactor at higher loadings. The accumulation of acetate under sulfidogenic conditions has been reported both under mesophilic (Omil et al., 1997; Nagpal et al., 2000a,b; Kaksonen et al., 2003) and thermophilic conditions (Vallero et al., 2004; Sahinkaya et al., 2007) and thermophilic conditions (Vallero et al., 2004; Sahinkaya et al., 2007). Moreover, acetate oxidation as the limiting step in sulfate reducing bioprocesses.

3.3. Metal removal and sulfide production

The soluble Zn concentrations at the effluent of each compartment did not change significantly as the effluent Zn concentration for compartment 4 slightly increased from 0.4 to around 1.2 mg/L (Fig. 1 and Table 2) with increasing feed Zn concentration from 65 to 200 mg/L. Hence, the Zn removal efficiency was always higher than 99%. Similar results were also reported by Kaksonen et al. (2003) for FBR and Sahinkaya (2008) for completely stirred tank reactor (CSTR). Another significant point is that around 99% of the Zn was precipitated in the first compartment. Hence, precipitated Zn can directly be recovered from compartment 1 without the accumulation of precipitated metals within the reactor. Moreover, the recovery of precipitated metal is possible because the biomass can also be completely or partly lost during metal removal from the reactor (Sahinkaya, 2008). Moreover, Herreta et al. (1997)
reported, that the metal sulfide precipitates accumulated in biofilm reactors may adversely affect the activity of SRB and the mechanism of inhibition is that metal sulfides act as barriers preventing the access of the reactants (sulfate, organic matter) to the necessary enzymes (Utgikar et al., 2002).

Similar to studies in the literature (Celis-Garcia et al., 2007), the increased sulfide concentration (Fig. 2D) did not adversely affect the reactor performance. The sulfide concentration increased from compartments 1 to 4 and the sulfide concentration at compartment 4 averaged around 150 and 350 mg/L when feed sulfate concentration was 1000 and 2000 mg/L, respectively. Although sulfide concentration in the reactor was very high, we still detected measurable Zn concentration (up to 1.4 mg/L at compartment 4) (Table 2 and Fig. 2). Similar result was also observed by Sahinkaya (2008). The reason for this observation may be the formation of small ZnS particles at high sulfide concentrations that can pass through the 0.45 \( \mu \text{m} \) membrane filter (Veeken et al., 2003). Hence, the measured Zn concentration may consist of both soluble Zn and ZnS particles.

In addition to soluble Zn, total Zn concentration at the effluent of the reactor was measured when the reactor received 200 mg/L Zn. Although soluble Zn concentration at the effluent of the reactor (or compartment 4) averaged 1.2 mg/L, total Zn concentrations were between 3 and 6 mg/L. This result showed that only small amount of the ZnS particles escaped from the reactor and around 98% of the ZnS particles were retained in the reactor. The result was in agreement with the previous findings as Sahinkaya (2008) reported that after 2 h settling around 95% of the Zn-sulfide particles can be settled. In another study Kaksonen et al. (2003) reported that in sulfidogenic FBR and UASB reactor around 73–86% of the Zn and Fe accumulated into the reactors and water level adjustors. The remaining 14–23% of the metals washed out from the reactors as precipitates. The reason for higher efficiency of metal-sulfide settling (98%) in our study was much lower up-flow velocity as no recycle was applied in the ABR.

3.4. Fate of electron donor and acceptor in the reactor

Methane production ceased after 10 days of reactor operation and electron flow to sulfate reduction increased very sharply subsequently (Fig. 3). This showed that SRB out-competed MA within a short period. There are two possible reasons for this observation. First, lactate is a well known substrate for SRB especially at low COD/SO4\(^{2-}\) ratios. Second is the increased sulfide concentration as sulfide is much more toxic to MA compared to SRB (Hulshoff Pol et al., 1998). Around 86 ± 11% of the electrons produced from lactate oxidation were used by sulfate reduction. Fermentative reactions and biomass growth were the possible sinks for the rest of carbon source as methane production was ceased after day 10. This result is in agreement with previous studies. Sahinkaya (2008) observed this value as 83% in a sulfidogenic CSTR treating acidic and Zn-containing wastewater. Similarly Kaksonen et al. reported.
(2004) reported the average electron donor utilized for sulfate reduction as 76 ± 10% in a mesophilic FBR fed with ethanol. In another study Sahinkaya et al. (2007) observed that 62 ± 15% and 72 ± 13% of the added electron donor was utilized by SRB in FBRs operated at low (8 °C) and high temperatures (65 °C), respectively.

As for the fate of electron acceptor (sulfate) Fig. 4 shows the reduced sulfate, produced total soluble sulfide and sulfide recovery throughout the reactor operation. In the calculation of produced total sulfide (soluble sulfide + precipitated sulfide as ZnS), it was assumed that 1 mmol sulfide was used to precipitate 1 mmol Zn according to the following reaction:

\[ \text{H}_2\text{S} + \text{Zn}^{2+} \rightarrow \text{ZnS(s)} + 2\text{H}^+ \]  

The recovery of sulfide was calculated using the following equation:

\[ \text{% Recovery} = \left( \frac{\text{Total sulfide as mmol}}{\text{Sulfate reduced as mmol}} \right) \times 100 \]  

Fig. 4 shows that the reduced amount of sulfate was always higher than the sum of total sulfide produced (soluble sulfide + precipitated sulfide) and the sulfide recovery averaged 70 ± 15%. Hence, there was around 30% uncoupling between reduced sulfate and total sulfide produced. There are three possible reasons for this observation:

The first one is the chemical oxidation of sulfide to elemental sulfur within the reactor. Observing yellow precipitates on the wall of the reactor supports this hypothesis. Oxygen, chemically oxidizing sulfide to elemental sulfur, may come with the influent wastewater as the reactor was sealed to avoid any oxygen leakage to the reactor. The second reason is the volatilization of sulfide to the gas phase. This is especially true in the first compartment due to relatively low pH values (6.67 ± 0.3) compared to following compartments. Lastly, partial loss of sulfide may also occur during the sampling and measurement.

3.5. Composition of Zn precipitate

The sludge samples, collected from the compartment 1 at the end of reactor operation (day 304), were analyzed to determine the composition of the Zn precipitate. XRF results showed that sludge samples contain high amount of Zn and S as the % atomic masses were 56.8 and 43.2, respectively. In another study Radhika et al. (2006) reported that the atomic % masses of Zn and S for high purity ZnS were 52.6 and 47.4, respectively. Hence, the chemical composition of the produced Zn-sulfide precipitate was in close agreement with the composition of high purity ZnS reported by Radhika et al. (2006).

4. Conclusions

In the study, lactate supplemented sulfidogenic ABR fed with acidic sulfate– and Zn-containing wastewater enriched sulfate reducing bacteria. Methane production ceased within ten days of operation and average electron flow to sulfate reduction was close to 85%. The reactor did not suffer from increasing feed Zn up to 200 mg/L and decreasing feed pH down to 4.5. Sulfate removal efficiency reached around 90% and 80% at feed sulfate concentration of 1000 and 2000 mg/L. Similarly COD removal efficiency was always between 80% and 95%. The alkalinity produced during sulfate reduction increased wastewater pH remarkably to neutral values. Throughout the study, over 99% Zn removal (precipitation rate was around 100 mg Zn/L d) was attained even at very high feed Zn concentrations (up to 200 mg/L). Accumulation of precipitate Zn in the first compartment allows recovery of Zn directly from the first compartment, which makes recovery of Zn possible without interrupting reactor performance. Hence, ABR is a potential alternative in the treatment of AMD and recovery of valuable metals. The good performance of ABR is due to its compartmentalized structure as the first compartment acts as a buffer zone to toxic metals in the feed; hence, the later compartments are loaded with a relatively harmless influent.

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