Performance and ethanol oxidation kinetics of a sulfate-reducing fluidized-bed reactor treating acidic metal-containing wastewater

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Abstract

The treatment of simulated acidic wastewater (pH 2.5–5) containing sulfate $(1.0–2.2 \text{ g l}^{-1})$, zinc $(15–340 \text{ mg l}^{-1})$ and iron (57 mg l^{-1}) was studied in a sulfate-reducing fluidized-bed reactor (FBR) at 35 °C. The original lactate feed for enrichment and maintenance of the FBR culture was replaced stepwise with ethanol over 50 days. The robustness of the process was studied by increasing stepwise the Zn, sulfate and ethanol feed concentrations and decreasing the feed pH. The following precipitation rates were obtained: $360 \text{ mg l}^{-1} \text{ d}^{-1}$ for Zn and $86 \text{ mg l}^{-1} \text{ d}^{-1}$ for Fe, with over 99.8% Zn and Fe removal, with a hydraulic retention time of 16 h. Under these conditions, 77–95% of the electrons were accepted by sulfate reduction. The alkalinity produced from ethanol oxidation increased the wastewater pH from 2.5 to 7.5–8.5. Michaelis–Menten constants (K_m) determined in batch FBR experiments, were $4.3–7.1 \text{ mg l}^{-1}$ and $2.7–3.5 \text{ mg l}^{-1}$ for ethanol and acetate oxidation, respectively. The maximum oxidation velocities (V_{max}) were $0.19-0.22 \text{ mg gVS}^{-1} \text{ min}^{-1}$ and $0.033-0.035 \text{ mg gVS}^{-1} \text{ min}^{-1}$, for ethanol and acetate, respectively. In summary, the FBR process produced a good quality effluent as indicated by its low organic content and Zn and Fe concentrations below 0.1 mg l^{-1} .

Abbreviations: COD – chemical oxygen demand; DOC – dissolved organic carbon; FBR – fluidized-bed reactor; HPLC – high performance liquid chromatograph; HRT – hydraulic retention time; K_m – Michaelis–Menten constant; RFBR – recirculating fluidized-bed reactor; S – initial substrate concentration; SRB – sulfate-reducing bacteria; T – temperature; TSS – total suspended solids; v – oxidation velocity; $V_{\rm max}$ – maximum oxidation velocity; VS – volatile solids; VVS – volatile suspended solids

Introduction

The exploitation of sulfide minerals results in the oxidation of iron and sulfur, and thus in the production of acidic metal-containing wastewaters (Foucher et al. 2001). The techniques traditionally used for the treatment of these wastewaters have been based on chemical neutralization and precipitation. The disadvantages of chemical treatment include high cost of the chemical reagents and production of a bulky sludge, which must be disposed of (García et al. 2001). In view of this, efforts have been made to develop biological alternatives for wastewater treatment.

Recently, the interest in the application of sulfate reduction as the dominant step of wastewater treatment has been growing (for a review, see Hulshoff Pol et al. 2001). The process is based on biological hydrogen sulfide production (Equation (1)) by sulfate-reducing bacteria (SRB), metal sulfide precipitation (Equation (2)) and neutralization of the water with the alkalinity produced by the microbial metabolism (Equation (3)) (Dvorak et al. 1992; Christensen et al. 1996):

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

where CH_2O = electron donor

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

where M = metal, such as Zn^{2+}

$$HCO_3^- + H^+ \to CO_2(g) + H_2O.$$
 (3)

The sulfidogenic process has several advantages over conventional chemical processes, such as lime addition. Most metal sulfides form a denser sludge and are less soluble (i.e., more stable) than the hydroxides produced by chemical treatment. In addition, metal sulfides can be recovered and recycled (Jalali & Baldwin 2000).

Acidic metal-containing wastewaters usually contain relatively low concentrations of organic substances. Therefore, addition of a suitable carbon source and electron donor for sulfate reduction is often necessary to promote biogenic H2S production (Kolmert & Johnson 2001). SRB utilize several low molecular weight substrates, such as lactate, formate, acetate, ethanol, and hydrogen. Some SRB oxidize organic substrates completely to CO2, while others oxidize them incompletely to acetate (Widdel 1988). Failures of anaerobic wastewater treatment processes as a result of shock loadings or decreasing pH are often characterized by an increasing concentration of acetic acid in the effluent (Kus & Wiesmann 1995). Lactate is a good growth substrate for most SRB (Postgate 1979), but the use of lactate in wastewater treatment processes would result in high operational costs (Barnes et al. 1991; Nagpal et al. 2000a). The use of lactate may speed-up the process start-up due to the relatively high growth yields of sulfate-reducing populations growing on lactate. A low-cost substrate, such as ethanol, is needed for large-scale operations, once a reasonable biomass yield has been achieved (Nagpal et al. 2000a). Another factor that needs consideration is the organic content in the effluent from the reactor that results from the unused electron donor. For example waste products, such as sewage sludge, produce a secondary chemical oxygen demand (COD) load, which restricts its disposal to the environment.

The slow growth rate of SRB has resulted in the development of several immobilized biomass reactors for treatment of large volumes of wastewater. Anaerobic fixed-bed or packed-bed bioreactors are effective in wastewater remediation (Foucher et al. 2001; Kolmert & Johnson 2001). However, they are subject to clogging and channelling which decreases their long term efficiencies (Kolmert & Johnson 2001). In upflow anaerobic sludge blanket reactors (UASB),

methanogens support the formation of granules (Visser 1995; Omil et al. 1996). On the other hand, the methanogenic activity reduces the sulfide yield. A large-scale process utilizing an ethanol-fed UASB reactor is being used in the Netherlands for the treatment of metal-contaminated groundwater (Barnes et al. 1991; de Vegt & Buisman 1995). In fluidizedbed reactors (FBR), biomass is retained on an inert carrier material, which is fluidized with recycled water or a gas stream (gas lift reactors). The advantage of a fluidized-bed reactor (compared to packed-bed or UASB reactors) is enhanced mass-transfer of both substrates and toxic products, such as H₂S (Nagpal et al. 2000b). For acidic, metal containing wastewaters, the high recycle ratio dilutes the metal concentrations and acidity in the reactor influent, thus allowing the biological sulfate reduction and metal precipitation to occur in a single reactor. In addition, due to the intensive mixing, the FBRs are less subject to clogging compared to fixed-bed reactors.

In a previous study, Ma & Hua (1997) demonstrated the suitability of lactate-fed FBR for the precipitation of Cd from synthetic wastewater (pH not reported). Furthermore, lactate-supplemented sulfatereducing FBRs were used to precipitate Zn and Fe from acidic (pH 2.5) wastewater (Kaksonen et al., in press). The alkalinity produced in lactate oxidation increased the initial pH of 2.5 resulting in effluent pH of 7.5–8.5 (Kaksonen et al., in press). In the completely mixed FBR, the reactor pH was close to that of the effluent. Nagpal et al. (2000b) attained sulfate reduction rates up to 6.33 g sulfate 1^{-1} d⁻¹ in an ethanol-fed system consisting of a FBR and a separate recirculation vessel (recirculating fluidized-bed reactor RFBR) at a hydraulic retention time (HRT) of 5.1 h. In their experiments, the efficiency of sulfate reduction increased considerably as the HRT increased until the bacteria became strongly substrate-limited at 55 h HRT.

In the present work, the effects of the change of electron donor from lactate to ethanol on the FBR performance were studied. The loading limits of the ethanol-fed process were determined in continuous-flow experiments. In addition, in batch FBR kinetic experiments, maximum velocities $(V_{\rm max})$ and Michaelis–Menten constants (K_m) were determined for the oxidation of the ethanol and its major degradation intermediate, acetate (Equations (4) and (5)).

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O,$$
 (4)

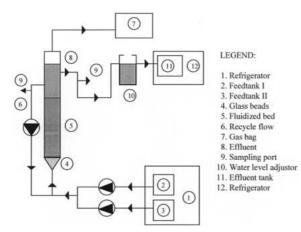


Figure 1. Schematic diagram of the process configuration of the fluidized-bed reactor (FBR).

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-.$$
 (5)

Materials and methods

Bioreactors

A laboratory-scale FBR with a 20% fluidization rate was used to enrich and maintain SRB at 35 °C (Figure 1). Silicate mineral (particle size 0.5–1 mm) was used as the carrier material. The reactor was originally inoculated with sediment from a mining area and methanogenic granular sludge, respectively (Kaksonen et al. in press). A modified Postgate B medium (Postgate 1979) (Table 1) containing sulfate (1.0–2.2 g l $^{-1}$), zinc (15–340 mg l $^{-1}$) and iron (57 mg l $^{-1}$) was fed to the reactor at a HRT of 16 h.

Replacement of the electron donor and loading experiments

For enrichment and maintenance of the FBR culture, lactate was fed to the reactor for 210 days. Maximally, 60–75% of the electrons were used for sulfate reduction. The lactate feed was replaced stepwise with ethanol over 50 days. The sulfate, ethanol and Zn influent concentrations were increased stepwise and feed pH was decreased gradually until process failures occurred. The experimental design is shown in Figure 2. Rates of sulfate reduction, dissolved organic carbon (DOC) removal and metal precipitation were determined as the difference of feed and effluent sulfate,

Table 1. Composition ($mg \, l^{-1}$ except for pH) of the reactor feeds. The changes in the feed during the loading experiment were as presented in Figure 2.

	Loading experiments	Batch kinetic experiments ^a
Sodium lactate	$780 \rightarrow 0$	
Ethanol	$0 \rightarrow 710$	160
KH_2PO_4	56	28
NH ₄ Cl	110	55
Ascorbic acid	11	5.5
Thioglycolic acid	11	5.5
$Na_2SO_4 \cdot 10H_2O$	$1430 \rightarrow 5690$	
Na ₂ SO ₄		1820
$MgSO_4 \cdot 7H_2O$	1130	580
FeSO ₄ ·7H ₂ O	280	140
$ZnCl_2$	$30 \rightarrow 700$	15
pН	$5.0 \rightarrow 2.5$	4

^a Feed to the reactor between individual batch experiments was continuous.

DOC and soluble metal concentrations, respectively. Samples for the determination of total dissolved sulfide were taken directly from the sampling port placed in the effluent line (see Figure 1).

Batch kinetic experiments

Batch kinetic experiments were conducted in the FBR to obtain the kinetic parameters $(V_{\text{max}} \text{ and } K_m)$ for the oxidation of electron donor. Before and between individual batch experiments, the reactor was continually fed (HRT 24 h) with wastewater (Table 1) to wash out the excess hydrogen sulfide formed during the batch experiments, and to maintain a constant biomass content in the reactor. The pH of the feed was 4 and the resulting pH in the reactor and effluent was 6.5–7.5. Sulfate was added in excess (three times as much as stoichiometrically required) for complete oxidation of the electron donor. For each batch experiment, the feed flow was discontinued and the FBR was operated in recycle mode. A portion (50 ml) of the reactor liquid was replaced through the sampling valve placed in the recycling line (Figure 1) with fresh solution (pH 7) containing Na₂SO₄, nutrients and ethanol or acetate. This solution was purged with nitrogen before adding it to the FBR. During the batch experiments, samples of the reactor liquid were collected through the sampling valve placed in the recycling line at regular intervals and analyzed for ethanol and acetate. In addition, sulfate and dissolved sulfide were

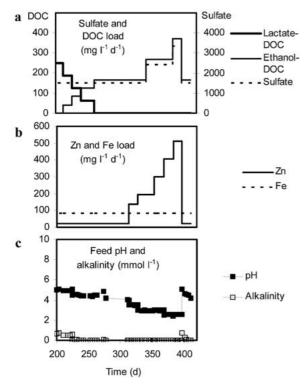


Figure 2. Experimental design of continuous-flow fluidized-bed reactor experiments showing changes in the feed composition.

determined in the first and last sample of each batch experiment.

Analytical methods

For sulfate, DOC, ethanol, acetate, soluble metal and sulfide analyses, the samples were filtered through $0.45 \mu m$ polyethersulfone membrane syringe filters. For the loading experiments, sulfate was determined by ion chromatography (Dionex DX-120, USA) and total dissolved sulfide was analyzed spectrometrically (Shimadzu UV-1601, Japan) by the methylene blue method (Trüper & Schlegel 1964). For batch kinetic experiments, sulfate was determined using a high performance liquid chromatograph (HPLC) system consisting of a Millipore Waters IC-PakTM Anion column $(4.6 \text{ mm} \times 5 \text{ cm})$, GBC LC 1610 Autosampler, GBC LC 1150 HPLC pump and a Millipore® Waters Model 430 Conductivity Detector, and total dissolved sulfide was analyzed spectrometrically (Helios Epsilon, 9423 UVE 100E, USA) by the colorimetric method described by Cord-Ruwisch (1985). DOC was measured with a TOC-5000 Analyzer (Shimadzu, Japan), and metals with an atomic absorption spectrophotometer (Philips PU9200X, Great Britain) according to the Finnish standards SFS 3044 (SFS 1980a) and SFS 3047 (SFS 1980b). Liquid pH was determined in unfiltered samples using a pH electrode (WTW SenTix41, Germany). Total alkalinity was analysed by titrating unfiltered samples with 0.02 M HCl to pH 4.5 according to the standard SFS-EN ISO 9963-1 (SFS 1996). Total suspended solids (TSS) and volatile suspended solids (VSS) were analysed in effluent samples by filtering a measured volume of sample through a glass fiber filter (Whatman GF/A), drying the filter for 1 hour at 105 °C, to determine TSS, with subsequent ignition at 550 °C for 1 hour, for the determination of VSS. Ethanol and acetate were determined using a gas chromatograph (Hewlett Packard 5890A, USA) equipped with a 25 m \times 0.32 mm (ID) capillary column coated with a 0.25 μ m film of polyethylene glycol treated with TPA (BP21, SGE Australia) and a flame ionisation detector. The amount of biomass in the reactor was estimated as volatile solids (VS) according to the Finnish standard SFS 3008 (SFS 1990). The ethanol and acetate oxidation rates were standardized to the total amount of biomass in the FBR. Michaelis–Menten equation (Equation (6)) and five different plotting approaches: Lineweaver-Burk plot (Equation (7)), Hanes plot (Equation (8)), Eadie–Hofstee plot (Equation (9)), Direct linear plot (Equation (10)) and Modified linear plot (Equation (11)) were used to obtain the kinetic parameters (V_{max} and K_m) (Cornish-Bowden 1995).

$$\nu = \frac{V_{\text{max}} \cdot S}{K_m + S} \tag{6}$$

$$\frac{1}{\nu} = \frac{1}{V_{\text{max}}} + \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{S} \tag{7}$$

$$\frac{S}{v} = \frac{K_m}{V_{\text{max}}} + \frac{1}{V_{\text{max}}} \cdot S \tag{8}$$

$$\nu = V_{\text{max}} - K_m \cdot \frac{\nu}{S} \tag{9}$$

$$V_{\text{max}} = \nu + \frac{\nu}{S} \cdot K_m \tag{10}$$

$$\frac{1}{V_{\text{max}}} = \frac{1}{\nu} - \frac{1}{S} \cdot \frac{K_m}{V_{\text{max}}}.$$
 (11)

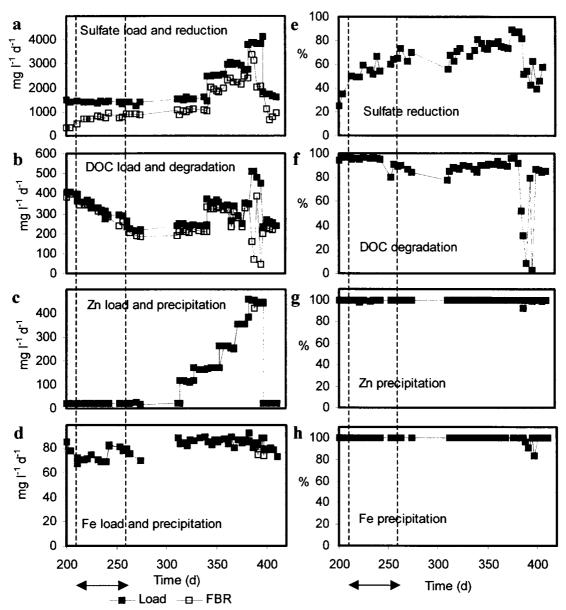


Figure 3. Quantitative sulfate load and reduction rate (a), DOC load and degradation rate (b), Zn (c) and Fe (d) load and precipitation rate, and percent sulfate reduction (e), DOC degradation (f), Zn (g) and Fe (h) precipitation for the fluidized-bed reactor (FBR) during the electron donor change (period indicated by the arrow and dashed lines) and subsequent loading experiments.

Results and discussion

Replacement of the electron donor and loading experiments

During the electron donor change (between days 210 and 260), 51–72% of the DOC was consumed by sulfate reduction (Figure 3a, b, e and f) with 80–97% DOC removal. In a previous study by Barnes et al. (1991) methanol was used as an intermediate

substrate for a sulfidogenic UASB during an electron donor change from lactate to ethanol. Methanol accelerated the growth of methanogens and hence complete degradation of the acetate occurred. In the present study, no significant acetate accumulation was observed during the electron donor change.

The capacity of the FBR-process was studied by increasing stepwise the Zn, sulfate and ethanol feed concentrations and decreasing the feed pH (Figure 2). The following precipitation rates were obtained: 360 mg l⁻¹ d⁻¹ for Zn (Figure 3c) and 86 mg l⁻¹ d⁻¹ for Fe (Figure 3d), with over 99.8% Zn and Fe removal (Figure 3g and h), at a HRT of 16 h. Ma & Hua (1997) attained greater Cd precipitation rates with a lactate-fed FBR; the pH of the feed was not reported. Metal precipitation and pH neutralization are interdependent, since the precipitation reaction (Equation (2)) produces acidity. In the present study, the pH (2.5) of the influent water was neutralized by the alkalinity produced (Figure 4b and c) and the effluent pH remained at 7.5–8.5 during stable reactor performance.

Zinc has been reported to completely inhibit sulfate-reduction in the concentration range of 25 to 60 mg l $^{-1}$ (Morton et al. 1991; Ueki et al. 1991). In the present study, the sulfate-reducing FBR was able to treat wastewater with 240 mg Zn l $^{-1}$ resulting in an effluent with less than 0.1 mg Zn l $^{-1}$. In the completely mixed FBR, bacteria became exposed to Zn concentrations close to those of the effluent, and thus, toxicity problems were avoided.

The effluent DOC during stable FBR performance with the highest loadings was 7–20 mg l⁻¹. Concentrations of TSS (Figure 4d) and VSS (Figure 4e) in the effluent increased as loading rates were increased. The efficiency of the ethanol-fed FBR to precipitate metals and neutralize acidity was similar to that reported with lactate-fed FBRs (Kaksonen et al., in press). However, at the highest loadings, a greater percentage of the added electron donor was utilized for sulfate reduction in the ethanol-fed FBR (77–95%) as compared to the lactate-fed FBR (60–75%). The rest of the electron flow was most likely coupled with fermentative reactions. Biogas was not produced and hence, methanogenesis was insignificant.

This study focused on treatment of simulated zinc and iron containing wastewater. The sulfate-reducing FBR process can potentially be used to precipitate other toxic metals, which have high affinity to S^{2-} ions, such as Cu, Pb and Cd. The low solubilities required for highly toxic metals such as mercury are often achievable only by speciation as sulfides, since most metal sulfides (As and Cr are exceptions) have solubilities several orders of magnitude lower than the hydroxides throughout the pH range (Conner 1990).

Batch kinetic experiments

Ethanol and acetate oxidation kinetics were studied in batch FBR experiments. Oxidation of ethanol

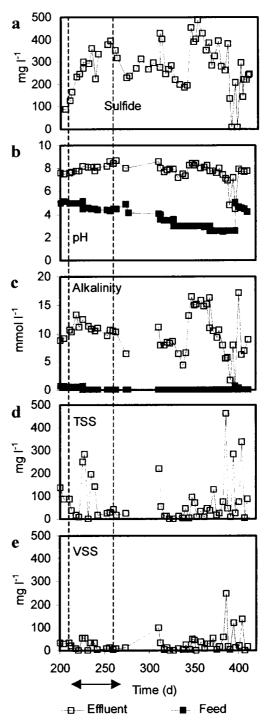


Figure 4. Effluent sulfide (a), feed and effluent pH (b), feed and effluent alkalinity (c), effluent total suspended solids (TSS) (d) and volatile suspended solids (VSS) (e) for the fluidized-bed reactor (FBR) during the electron donor change (period indicated by the arrow and dashed lines) and subsequent loading experiments.

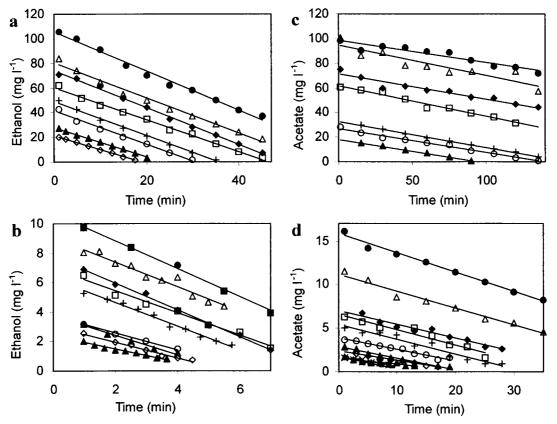


Figure 5. Oxidation of ethanol (a and b) and acetate (c and d) in the batch kinetic experiments with different initial substrate concentrations.

and acetate at different initial concentrations was as presented in Figure 5. Acetate oxidation was the ratelimiting step in ethanol oxidation (Equations (4) and (5)), as demonstrated by the accumulation of acetate in the FBR during the batch experiments (Figure 6). The total quantity of biomass in the FBR was 4.0 g and 3.8 g during the ethanol and acetate oxidation experiments, respectively. Approximately 85-86% of the biomass was carrier bound, 10–11% on the metal precipitates accumulated above the carrier material and 3–4% occurred in the bulk water. The effects of the initial substrate concentrations on oxidation rates are shown in Figure 7a and the Lineweaver-Burk plot, Hanes plot, Eadie-Hofstee plot, Direct linear plot and Modified linear plot in Figures 7b to 7f, respectively. Michaelis-Menten constants (K_m) were 4.3- $7.1 \text{ mg } 1^{-1} \text{ and } 2.7-3.5 \text{ mg } 1^{-1} \text{ for ethanol and acetate,}$ respectively (Table 2). The maximum oxidation velocities (V_{max}) were 0.19–0.22 mg gVS⁻¹ min⁻¹ and $0.033-0.035 \text{ mg gVS}^{-1} \text{ min}^{-1}$, for ethanol and acetate, respectively (Table 2). The results show that acetate was oxidized to CO₂ considerably more slowly

than ethanol was oxidized to acetate. Sulfate and sulfide analysis confirmed that excess sulfate was present in the FBR during the experiments and initial sulfide concentrations remained below 100 mg l^{-1} .

The V_{max} for ethanol oxidation was greater than the observed oxidation velocities during the continuous loading experiments, whereas V_{max} for acetate was lower (Figure 3b), if the biomass content of the FBR is assumed to be the same as during the batch experiments. The K_m and V_{max} values for acetate oxidation were lower than previously reported for mesophilic sulfate-reducing cultures (Table 3). The observed low $V_{\rm max}$ indicates that only part of the total biomass in the FBR utilized acetate as an electron donor. Many of the known sulfate-reducers incompletely oxidize their substrates producing acetate as an end product (Castro et al. 2000). As shown in the loading experiments, up to 77-95% of the added electron flow was used for sulfate reduction, the rest was coupled most likely to fermentative reactions.

To the best of our knowledge, ethanol oxidation kinetics in sulfate-reducing systems have been pre-

Table 2. Michaelis–Menten constants (K_m) and maximum oxidation velocities (V_{\max}) for ethanol and acetate oxidation obtained with different linearization approaches.

Linearization	$K_m \text{ (mg l}^{-1}\text{)}$		V _{max} (mg	$V_{\rm max}~({\rm mg~gVS^{-1}~min^{-1}})$		
approach	Ethanol	Acetate	Ethanol	Acetate		
Lineweaver-Burk	5.0	2.9	0.20	0.034		
Hanes plot	7.1	3.5	0.22	0.035		
Eadie-Hofstee plot	4.9	2.7	0.20	0.034		
Direct linear plot	5.1	2.9	0.20	0.034		
Modified linear plot	4.3	2.5	0.19	0.033		

Table 3. Kinetic parameters for acetate oxidation by mesophilic sulfate-reducing pure and enrichment cultures.

Strain/culture	T (°C)	K_m (mg l ⁻¹)	$V_{\rm max}$ (mg gVS ⁻¹ min ⁻¹)	Reference
Desulfobacter postgatei	30	13.6	0.4-1.2 ^a	Schönheit et al. (1982)
Desulfobacter postgatei	30	3.8-4.5	3.0-3.1	Ingvorsen et al. (1984)
Desulfobacter postgatei	30		7.1-9.4	Widdel (1988
Desulforhabdus amnigenus	37	35	1.7 ^a	Oude Elferink (1998)
Desulfobacca acetoxidans	37	35	2.5 ^a	Oude Elferink (1998)
Enrichment culture	31	5.9		Middleton & Lawrence (1977)
Mixed culture of SRB and methanogens	30	9.5		Yoda (1987)
Enrichment culture	35	2.7–3.5	0.033-0.035	This study

^a Biomass determined as protein instead of VS.

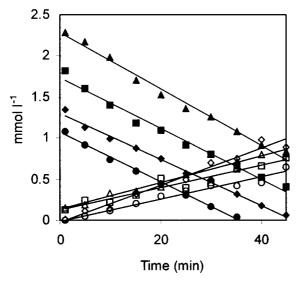


Figure 6. Ethanol concentration decrease (filled symbols) and acetate accumulation (unfilled symbols) in the sulfate-reducing FBR during four batch experiments.

viously reported only by Nagpal et al. (2000a). In their study, the half saturation constant for ethanol

 $(0.2~{\rm g~l^{-1}})$ was obtained using a batch-enriched sulfate reducing culture and batch bottle assays (Nagpal et al. 2000a) and was over one order of magnitude greater than that reported in our study (5 mg ${\rm l^{-1}}$). This shows that the FBR system selectively enriches for sulfate reducers with high affinities for the electron donor.

Based on the low K_m values, the ethanol and acetate oxidation rates remain high even at low substrate concentrations. This implies low residual organic content in the effluent, and hence a potentially low environmental impact associated with the discharge of the effluent water. This was also shown as the low concentration of DOC in the effluent water of the FBR during the continuous flow operation. In comparison, Nagpal et al. (2000b) did not report significant acetate consumption in the ethanol-fed sulfate-limited RFBR containing Desulfobacter postgatei (complete oxidizer) and Desulfovibrio desulfuricans (incomplete oxidizer). Nagpal et al. (2000b) discussed that this could be due to the inhibition of acetate-consuming bacteria either by sulfide or acetate concentrations, or the better ability of Desulfovibrio to compete for the

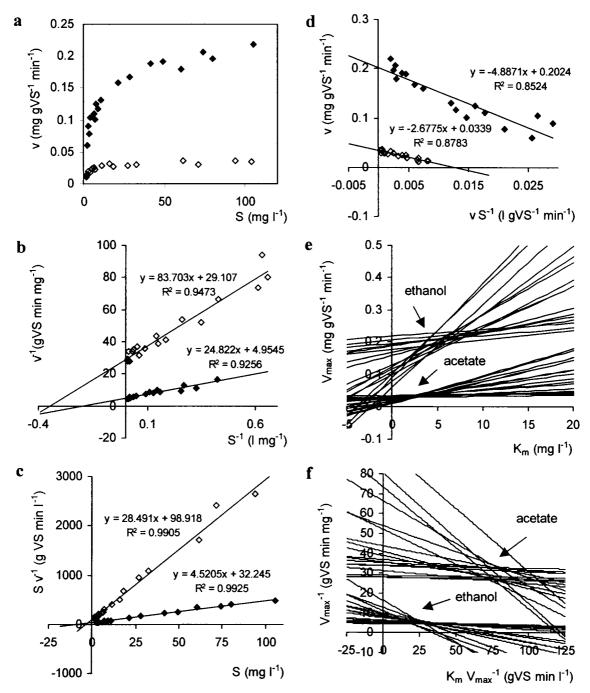


Figure 7. (a) Effect of initial concentrations (S) on ethanol (filled symbols) and acetate (unfilled symbols) oxidation rates (v) in the batch kinetic experiments. Linearization of the results with (b) Lineweaver–Burk plot, (c) Hanes plot, (d) Eadie–Hofstee plot, (e) direct linear plot and (f) modified linear plot. The arrows point to the groups of ethanol and acetate plots, respectively.

limiting amounts of sulfate (Laanbroek et al. 1984; Nagpal et al. 2000b).

The acetate oxidation step (Equation (5)) in ethanol oxidation produces the bicarbonate alkalinity, which neutralizes the acidic wastewater. In addition, the acetate oxidation produces twice as much hydrogen sulfide per mole than does ethanol oxidation to acetate (Equation (4)). In summary, acetate oxidation is the rate limiting step, the kinetics of which define the design criteria for the ethanol-supplemented sulfate-reducing bioprocess for metal precipitation.

Conclusions

The sulfate-reducing FBR culture, that was originally enriched with sodium lactate, utilized ethanol with a higher efficiency (77–95% of the electrons used for sulfate reduction) when compared to the efficiency of lactate utilization (60–75%). The FBR-treatment precipitates metals as sulfides and neutralizes the wastewater from an influent pH of 2.5, at a HRT of 16 h. Acetate oxidation is the rate-limiting step in sulfidogenic ethanol oxidation. The sulfidogenic FBR system enriches selectively bacteria with low K_m values for ethanol and acetate oxidation. In conclusion, the design criteria for ethanol-supplemented sulfate-reducing bioprocess for metal precipitation are defined by acetate oxidation kinetics.

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