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Biological hydrogen sulfide production in an ethanol–lactate fed fluidized-bed bioreactor

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ABSTRACT

Sulfate-reducing fluidized-bed bioreactor (FBR) fed with ethanol–lactate mixture was operated at 35 °C for 540 days to assess mine wastewater treatment, biological hydrogen sulfide production capacity and acetate oxidation kinetics. During the mine wastewater treatment period with synthetic wastewater, the sulfate reduction rate was 62 mmol SO_4^{2-} L^{-1} d^{-1} and Fe and Zn precipitation rates were 11 mmol Fe L^{-1} d^{-1} and 1 mmol Zn L^{-1} d^{-1}. After this, the hydrogen sulfide production was optimized, resulting in sulfate reduction rate of 100 mmol SO_4^{2-} L^{-1} d^{-1} and H_2S production rate of 73.2 mmol H_2S L^{-1} d^{-1}. The limiting step in the H_2S production was the rate of acetate oxidation, being 50 mmol acetate L^{-1} d^{-1}. Therefore, FBR batch assays were designed to determine the acetate oxidation kinetics, and following kinetic parameters were obtained: K_m of 63 μmol L^{-1} and V_{max} of 0.76 μmol acetate g VSS^{-1} min^{-1}. The present study demonstrates high-rate hydrogen sulfide production and high-rate mine wastewater treatment with ethanol and lactate fed fluidized-bed bioreactor.

1. Introduction

Sulfate-reducing bacteria (SRB) produce hydrogen sulfide in the presence of a suitable electron donor. In 2006, the operating biological sulfate reduction plants treated 15 tons of sulfur per day (Buisman et al., 2007). Biological sulfate reducing reactors used for metal precipitation can have either one or more stages, i.e. the sulfate reduction and metal precipitation can occur simultaneously, or in separate process units (Hao, 2000). Single-stage processes are low-cost to operate, but more prone to inhibition by the treated wastewater (Hao, 2000). Therefore, careful balancing of the wastewater loading is required to match the sulfate reduction capacity of the reactor.

The electron donor constitutes the highest operational cost for a sulfate reducing bioreactor. Currently operating sulfate reducing bioreactors are fed with, for example, ethanol, cellulosic or fermentation industry wastewater and hydrogen (Buisman et al., 2007). Sulfidogenic ethanol (1), lactic acid (2) and acetate oxidation (3) proceed according to the following reactions (Thauer et al., 1977):

$$CH_3CH_2OH + \tfrac{1}{2}SO_4^{2-} \rightarrow CH_3COO^- + \tfrac{1}{2}HS^- + \tfrac{1}{2}H^+ + H_2O \quad (1)$$

$$CH_3CHOHCOO^- + \tfrac{1}{2}SO_4^{2-} \rightarrow CH_3COO^- + HCO_3^- + \tfrac{1}{2}HS^- + \tfrac{1}{2}H^+ \quad (2)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (3)$$

The Gibbs free energy ($\Delta G'$) for the Eqs. (1)–(3) are −66.4 kJ mol^{-1}, −80.0 kJ mol^{-1} and −47.6 kJ mol^{-1}, respectively (Thauer et al., 1977). These reactions show that complete electron donor oxidation yields more hydrogen sulfide and alkalinity. Therefore, acetate oxidation is desirable, and results in less residual effluent Chemical Oxygen Demand (COD). Alkalinity production is needed to maintain neutral reactor pH. If the reactor hydraulic retention time (HRT) is not sufficient for complete acetate oxidation, acetate accumulates and decreases the pH (Kaksonen et al., 2004a). Sulfidogenic acetate oxidation yields less energy than sulfidogenic ethanol or lactic acid oxidation to acetate (Thauer et al., 1977), thus acetate oxidizing SRB grow at slow rates. Complete acetate oxidation may not be obtained even when excess sulfate is provided, and enrichment of acetate oxidizing SRB may be difficult (Lens et al., 2003). On the other hand, Kaksonen et al. (2003a) showed that sulfidogenic ethanol oxidation is more prone to H_2S toxicity than sulfidogenic acetate oxidation. The undissociated form of acetic acid is inhibitory, as it passes through the cell membrane and results in acidification of cytoplasm when dissociating within the cell (Thauer et al., 1977). The concentration of undissociated acetic acid can be controlled by maintaining the reactor pH in the neutral area, as the pK_a of acetic acid is 4.8.

Single-stage H_2S production may inhibit the SRB growth, activity and reactor operation (Okabe et al., 1995). The inhibitory effects of H_2S are reversible, and SRB can recover from shock concentrations (Okabe et al., 1995). Table 1 lists the reported toxic concentrations of total dissolved sulfide and H_2S for sulfidogenic biomass. On-site production of biological hydrogen sulfide may

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Table 1
Toxic concentrations of total dissolved sulfide (DS) and H_2S reported for sulfidogenic biomass.

Biomass type	Electron donor	pH	DS (mmol L^{-1})	H_2S (mmol L^{-1})	Reference
AMD treatment sludge[a]	Ethanol	7.2	27.4	8.9	O'Flaherty et al. (1998)
AMD treatment sludge[a]	Acetate	7.2	17.2	5.6	O'Flaherty et al. (1998)
Sulfate adapted sludge[a]	Ethanol	7.2	24.6	8.0	O'Flaherty et al. (1998)
Anaerobic hybrid reactor sludge[a]	Ethanol	7.5	42.1	7.6	O'Flaherty et al. (1999)
Anaerobic hybrid reactor sludge[a]	Acetate	7.5	20.9	3.8	O'Flaherty et al. (1999)
Anaerobic digester sludge[b]	Lactate	6.6	–	16.1	Reis et al. (1992)
AMD treating FBR[c]	Ethanol	6.9–7.3	7.7	2.6	Kaksonen et al. (2004a)
AMD treating FBR[c]	Acetate	6.9–7.3	11.1	3.9	Kaksonen et al. (2004a)

AMD = acid mine drainage.
[a] IC50 value.
[b] Complete inhibition of growth.
[c] 50% Inhibition on the electron donor oxidation rate.

be feasible, as the variation in scale of production can be controlled via electron donor supply (Huisman et al., 2006).

Fluidized-bed bioreactor (FBR) configuration was chosen for this study due to the efficient mixing and dilution rate, which protect the biomass from the toxic effects of the influent. The performance of an ethanol–lactate fed biological sulfate-reducing fluidized-bed bioreactor process was examined for biological hydrogen sulfide production, metal precipitation and acetate oxidation kinetics. The acetate oxidation kinetics of the FBR biomass was studied using FBR in batch mode. Moreover, the precipitation of the Fe and Zn in the FBR was estimated using chemical modeling program.

2. Methods

2.1. Experimental design

The FBR was operated as a wastewater treatment reactor with synthetic wastewater for the first 1–188 days (period A). Thereafter, biological hydrogen sulfide production capacity was studied on days 189–331 (period B) and finally, acetate kinetic batch assays were performed during days 332–540 (period C) (Fig. 1). The aim of periods A and B was to stabilize the reactor operation and to minimize the HRT (Fig. 1), then to maximize the hydrogen sulfide production with minimal metal load in period B, and simultaneously gradually replace the lactate with ethanol in the influent. The aim of the period C was to determine the acetate oxidation kinetics of the FBR biomass with batch assays.

2.2. Reactor set-up

The total liquid volume of the FBR was 0.65 L. The volume of the carrier material bed was increased by 20% with the high recycling rate, resulting in active fluidized-bed volume of 0.33 L. The FBR set-up was as shown in Fig. 2A and B. The carrier material was silicate mineral (Ø 0.5–1 mm, bulk density 770 g L^{-1}, Filtralite, Norway). The FBR temperature was maintained at 35 °C with a temperature control system. The FBR inoculum originated from an ethanol-fed sulfate reducing FBR operated at 35 °C from the experiments by Kaksonen et al. (2004a,b,c).

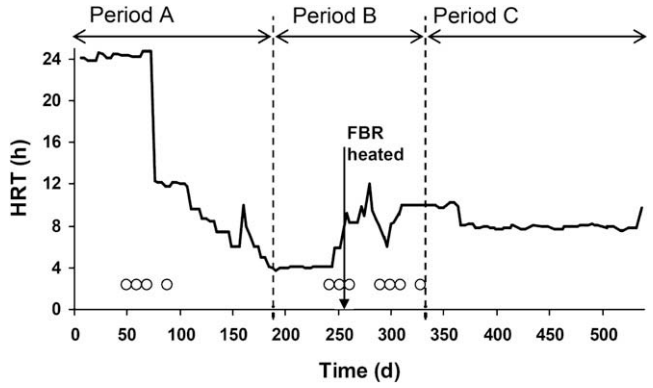

Fig. 1. The hydraulic retention time (HRT) and inoculation periods (○) of the fluidized-bed bioreactor (FBR). The operation periods of the FBR were following; A: mine wastewater treatment (days 1–188), B: biological hydrogen sulfide production (days 189–331) and C: Acetate kinetic batch assays (days 332–540).The FBR heated unintentionally to 55 °C on day 253.

2.3. Continuous flow operation

The FBR liquid was sampled daily for dissolved hydrogen sulfide (DS) and pH, and twice a week for alkalinity, sulfate, ethanol, acetic acid, dissolved organic carbon (DOC) and dissolved metals. The lactate concentration was calculated from DOC, ethanol and acetic acid results. The influent was sampled weekly for pH, acidity, sulfate, ethanol, acetic acid and DOC. The FBR carrier material and liquid were sampled monthly for analysis of carrier material bound biomass (volatile solids, VS), biomass concentration in the FBR liquid (volatile suspended solids, VSS) and inorganic and organic solids in the FBR liquid (total suspended solids, TSS). The FBR inoculum consisted of a diverse microbial community described by Kaksonen et al. (2004b,c).

The compositions of the FBR influents were as shown in Table 2. The FBR operation did not stabilize in the beginning and, therefore, on day 62 carrier material from the previous lactate-fed FBR (Kaksonen et al., 2003b) was added to the FBR. Also the FBR loading was decreased and the influent was supplemented with lactate, Fe, Zn, vitamin and trace-element solutions, yeast extract, sodium thioglycolate and ascorbic acid. To support the acetate oxidation, the FBR was regularly inoculated during days 52–77, 247–265 and 289–330 (see Fig. 1) with enrichment cultures made from the original FBR inoculum. These enrichment cultures were grown in batch bottles at 35 °C in Postgate medium with acetate as described by Kaksonen et al. (2004b).

2.4. Acetate oxidation kinetic batch assays

Prior to kinetic assays the FBR operation was stable for 50 days, fed with influent C (Table 1) and maintained constant biomass concentration. The acetate oxidation kinetic assays were performed for the FBR operated in batch mode. Between individual assays, the FBR was operated on continuous mode at HRT of 8 h to flush out excess acetate. The inhibition constants (K_i) of DS and H_2S on acetate oxidation for the FBR culture determined by Kaksonen et al. (2004a) were 11.1 and 3.9 mmol L^{-1} for DS and H_2S, respectively. To study the acetate oxidation at optimal conditions, the undissociated acetic acid concentration was controlled by maintaining the FBR pH around 7.5, and the DS and H_2S concentrations were maintained below 2 and 1 mmol L^{-1}, respectively. The final acetate concentration in the batch assays was 0.05–52 mmol L^{-1}, and the proportion of the undissociated acetic acid in this concentration range was below