WATER RESEARCH 40 (2006) 2561–2571

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Microbial sulfate reduction under sequentially acidic conditions in an upflow anaerobic packed bed bioreactor

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ARTICLE INFO

Article history:
Received 16 March 2004
Received in revised form
1 May 2006
Accepted 1 May 2006

Keywords:
Microbial sulfate reduction
SRB
Conductivity
AMD
Sulfide
Bioremediation

ABSTRACT

The aim of this study was to operate an upflow anaerobic packed bed reactor (UAPB) containing sulfate reducing bacteria (SRB) under acidic conditions similar to those found in acid mine drainage (AMD). The UAPB was filled with sand and operated under continuous flow at progressively lower pH and was shown to be capable of supporting sulfate reduction at pH values of 6.0, 5.0, 4.5, 4.0 and 3.5 in a synthetic medium containing 53.5 mmol l^{-1} lactate. Sulfate reduction rates of 553–1052 mmol m^{-3} d^{-1} were obtained when the influent solution pH was progressively lowered from pH 6.0 to 4.0, under an optimal flow rate of 2.61 ml min^{-1}. When the influent pH was further lowered to pH 3.5, sulfate reduction was substantially reduced with only about 1% sulfate removed at a rate of 3.35 mmol m^{-3} d^{-1} after 20 days of operation. However, viable SRB were recovered from the column, indicating that the SRB population was capable of surviving and metabolizing at low levels even at pH 3.5 conditions for at least 20 days. The changes in conductivity in the SRB column did not always occur with changes in pH and redox potential, suggesting that conductivity measurements may be more sensitive to SRB activity and could be used as an additional tool for monitoring SRB activity. The bioreactor containing SRB was able to reduce sulfate and generate alkalinity even when challenged with influent as low as pH 3.5, indicating that such treatment systems have potential for bioremediating highly acidic, sulfate contaminated waste waters.

1. Introduction

High levels of metals, sulfate and other salt constituents and low pH are common characteristics of wastewater produced in mining, metal processing and petrochemical industries (Tuppurainen et al., 2002). For example, the potential for contamination of the environment by acid mine drainage (AMD) has been well documented (Herlihy et al., 1987). The impact on terrestrial and aquatic ecosystems by AMD is potentially enormous not only because of its high acidity and elevated dissolved metal content, but also because of the large quantities of this water that can be produced. Harries (1997) estimated that about 54 sites in Australia are managing significant amounts of potentially acid generating wastes. The additional cost of managing such wastes at operating mine sites in Australia, as a whole, was estimated to be about $60 million/year. The treatment of metal and sulfate contaminated waters by sulfate reducing bacteria (SRB) is based on the ability of these organisms to use sulfate ions as the terminal electron acceptor for the metabolism of organic (Jalali and Baldwin, 2000; Tsukamoto and Miller, 1999; Tuppurainen et al., 2002; Utgikar et al., 2002) and inorganic

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doi:10.1016/j.watres.2006.05.001

(Van Houten et al., 1994) substrates to produce sulfide ions, which readily reacts with most dissolved metals to form insoluble metal sulfide precipitates, although precipitation with hydroxides and carbonates and sorption into biomass are also possible (Drury, 1999; Groudev et al., 1999). The oxidation of lactate coupled to sulfate reduction and precipitation of metal cations (M^{2+}) can be summarized by the following reaction equations:

$$2CH_3CHOHCOOH + SO_4^{2-} \rightarrow 2CH_3COOH + H_2S + 2HCO_3^-, \quad (1)$$

$$CH_3COOH + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-, \quad (2)$$

$$2CH_3CHOHCOOH + 3SO_4^{2-} \rightarrow 3H_2S + 6HCO_3^-, \quad (3)$$

$$M^{2+} + H_2S \rightarrow MS_{(s)} + 2H^+. \quad (4)$$

The potential advantages of metal sulfide precipitation include the production of a denser sludge, lower sludge volume and lower solubility products as compared to hydroxide precipitation produced in chemical treatment processes (Jalali and Baldwin, 2000; Peters et al., 1985; Whang et al., 1982). Moreover, valuable metals from biologically precipitated metal sulfide can be recovered and recycled (Boonstra et al., 1999).

The use of biological sulfate reduction to treat contaminated groundwater containing sulfate and dissolved heavy metals has been widely investigated (Barnes et al., 1992; Boonstra et al., 1999; Dvorak et al., 1992; Tuttle et al., 1969). Biological sulfate reduction has been studied in various reactor designs, such as anaerobic contact process (Haas and Polprasert, 1993), anaerobic filter (Dvorak et al., 1992; Elliott et al., 1998; Farmer et al., 1995), stirred tank reactor (Moosa, 2000), upflow anaerobic sludge blanket reactor (Boonstra et al., 1999; de Vegt et al., 1998; Hammack and Dijkman, 1999), hybrid reactor (Nedwell and Reynolds, 1996; Steed et al., 2000) and fluidized-bed reactors (Ma and Hua, 1997; Somlev and Tishkov, 1992). There have been few successful applications of SRB mediated AMD treatment systems, even though the possibility of using SRB to remediate AMD have long been appreciated. The main reason being that the pH optimum for growth of SRB is between pH 5–9 (Postgate, 1984), whereas AMD generally has a pH between 2 and 4 (Béchard et al., 1994), and commonly less than pH 3 (Hammack et al., 1993).

In a previous study, Drury (1999) used an anaerobic solid-substrate reactor containing cow manure and sawdust and supplemented with whey additions for the treatment of pH ~3.2 acid mine drainage. He achieved sulfate reduction rates of 120 mmol m^{-3} d^{-1}, with effluent pH staying relatively constant at 6.5. Elliott et al. (1998) found that significant sulfate reduction occurred at pH 3.25 in an anaerobic bioreactor enriched with SRB, isolated from sediment samples taken from Dawsley Creek, South Australia. Contrastingly, Lyew et al. (1994) reported that 90% of dissolved metals and 11% of sulfate was removed in a downflow column reactor operated at pH 4.8, but SRB activity ceased when the influent pH was decreased to 3.5. Kolmert and Johnson (2001) employed acidophilic SRB in an upflow reactor packed with porous glass beads to treat pH 4.0 media. They found average sulfate conversion rates of 26.0–31.2 mmol m^{-3} d^{-1} in bioreactors utilizing various permutations of glycerol, lactic acid and ethanol as carbon sources. In the present study, our aim was to promote SRB activity under acidic conditions similar to those encountered in AMD. This was done by evaluating the sulfate reduction rates in an upflow anaerobic packed bed (UAPB) operated under sequentially more acidic conditions using a general, mixed-population of neutrophilic SRB supplied with lactate.

2. Materials and methods

2.1. UAPB bioreactor

The laboratory scale UAPB bioreactor system consisted of six main components: influent medium reservoir tank, nitrogen gas source, peristaltic pump, fixed bed column, effluent tank and a volatile gas trap. The substrates were pumped from the influent medium reservoir tank to the bottom inlet of the reactor by means of a calibrated variable speed peristaltic pump. The UAPB bioreactor was constructed from a light gray polyvinyl chloride (PVC) pipe with an overall height of 800 mm, an internal diameter of 90 mm and a net empty working volume, V_w, of 4.78 ± 0.01 l. It was equipped with a total of ten ports used for sampling either liquid or solid material along the height of the reactor. Seven 12.5 mm diameter sampling ports were located on one side of the column and located at 0 (inlet), 225, 335, 445, 555, 665 and 800 (outlet) mm from the base of the column. The other three were 38 mm diameter sampling ports located directly on the opposite side of the column with respect to the second, fourth and sixth 12.5 mm sampling ports and the inlet. The reactor, all connecting tubing, valves and vessels were thoroughly cleaned by soaking in 1% Decon™ followed by soaking in 2% HNO_3 for 48 h and rinsing with Hi–Pure water (Permutit).

The flow was dispersed with the aid of a frustum shaped cowling located at the base of the reactor (near the inlet), which also served to contain the porous media. The reactor was filled with >2 mm fraction of a commercially available coarse pool filter sand (Commercial Minerals Limited, Melbourne, Australia). This support material was further pretreated by washing with distilled water, soaking in 5% HNO_3 for 72 h to remove organic material, and washing with distilled water again before rinsing with Hi–Pure water and drying in a 60 °C oven before use. Before conducting an experiment, a volume of medium, equivalent to approximately 1.0 pore volume, was pumped through the UAPB reactor to further stabilize and condition the sand bed. A slow and continuous purge of high purity nitrogen (Air Liquide) was bubbled through the medium reservoir tank. The experiments were conducted at room temperature (23 ± 1 °C).

2.2. Column parameters

The bioreactor parameters used for this study are presented in Table 1. For practical purposes, the pool sand itself was assumed to be a non-porous media. The particle density, ρ_p, of the sand was estimated by quantifying the volume of water displaced by a given mass of particles. This was determined