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Simultaneous sulfate reduction and copper removal by a PVA-immobilized sulfate reducing bacterial culture

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ABSTRACT

The effect of a sulfate reducing bacteria immobilized in polyvinyl alcohol (PVA) on simultaneous sulfate reduction and copper removal was investigated. Batch experiments were designed using central composite design (CCD) with two parameters, i.e. the copper concentration (10–100 mg/L), and the quantity of immobilized SRB in culture solution (19–235 mg of VSS/L). Response surface methodology (RSM) was used to model the experimental data, and to identify optimal conditions for the maximum sulfate reduction and copper removal. Under optimum condition, i.e. ~138.5 mg VSS/L of sulfate reducing bacteria immobilized in PVA, and ~51.5 mg/L of copper, the maximum sulfate reduction rate was 1.57 d^{-1} as based on the first-order kinetic equation. The data demonstrate that immobilizing sulfate reducing bacteria in PVA can enhance copper removal and the resistance of the bacteria towards copper toxicity.

1. Introduction

Sulfate is one of the most abundant anions found in the environment. It is generated and discharged from many industrial processes including molasses fermentation, tannery operations, food production, coal burning power plants, and pulp and paper processing (Austin, 1984; Liamleam and Annachhatre, 2007; Shin et al., 1997). Other technological activities have resulted in the generation of large quantities of aqueous effluents that contain high levels of heavy metals (Kadukov and Vircikova, 2005). The ability of sulfate reducing bacteria (SRB) to produce hydrogen sulfide and the high affinity of sulfide to react with divalent metallic cations provide an excellent option for achieving simultaneous removal of heavy metals and reduction of sulfate from wastewater (Bai et al., 2008; Jimenez-Rodriguez et al., 2009; Neculita et al., 2007; Radhika et al., 2006; Remoudaki et al., 2003; Southam et al., 1995; Teclu et al., 2009; Velasco et al., 2008). Anaerobic reduction of sulfate is the key step in the biological treatment of heavy metals, i.e. biogenic metal removal (Alvarez et al., 2007; Baskaran and Nemati, 2006), and the recent advances in molecular microbial ecology have provided a further impetus to promote biogenic metal removal (Ike et al., 2007; Remoudaki et al., 2003; Southam et al., 1995; Wang et al., 2001; Zhao et al., 2005).

The effects of various process parameters including pH, temperature, carbon source, sulfate concentration, and the inhibitory effects of heavy metals and sulfide on metal removal have been investigated (Alvarez et al., 2007). Moreover, SRB have been tested for removing heavy metals (Quan et al., 2003; Tabak et al., 2003; Velasco et al., 2008). The role of these bacteria in the biogenic metal removal including bioprecipitation and bioaccumulation has been investigated extensively (Alvarez et al., 2007; Jin et al., 2007; Kleikemper et al., 2002; Lyew and Sheppard, 1997). Many studies have shown the inhibition of SRB by high metal concentrations especially when the SRB cells are freely suspended in the medium (Sani et al., 2001; Utgikar et al., 2001, 2002). The application of the bioprecipitation process has been constrained due to problems such as poor cell retention within continuous bioreactors (Baskaran and Nemati, 2006). If the bacteria are growing in suspension, a continuous operating system requires long hydraulic retention times to prevent washout of the cells (Neculita et al., 2007). Therefore, immobilized cells can be used to shorten the hydraulic retention time while avoiding cell washout so that a high sulfate reduction efficiency can be maintained.

The application of immobilized microorganism has been widely investigated to increase the biological activity of the microorganisms and to maintain the higher bacterial cell retention in the reactor. Several natural polymeric materials including agar, *k*-carrageenan, alginate and chitosan, and synthetic polymeric materials such as polyacrylamide, polyethylene glycol, Polyvinyl alcohol (PVA) and cellulose triacetate have been tested

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for cell immobilization (Lozinsky and Plieva, 1998). Although a variety of supporting materials has been recommended for immobilizing SRB, PVA has received considerable attention due to its non-toxicity to microorganisms and low cost (Chen and Lin, 1994; Lozinsky and Plieva, 1998). However, very few systematic studies have been carried out to investigate the application of immobilized SRB for biogenic metal removal.

The objective of this study was to investigate the utilization of PVA as a gel matrix for the immobilization of SRB. Moreover, a set of biogenic copper removal experiments were carried out to estimate the optimum quantity of immobilized SRB in culture solution for achieving a maximum copper removal. The central composite design (CCD) and response surface methodology (RSM) were applied to achieve this goal.

2. Methods

2.1. Chemicals

PVA (with 99.4–99.8% saponification) used in this study was supplied by Chang Chun Petrochemical Co. Ltd., Taiwan. All other chemicals used in this study were of analytical grade; they are provided by local suppliers.

Table 1
Experimental conditions of biogenic copper removal experiments designed by using the CCD.

Run order	Coded value		Natural value	
	Quantity of immobilized SRB in culture solution, X_1	Copper concentration, X_2	Quantity of immobilized SRB in culture solution as VSS per liter, X_1 (mg of protein)	Copper concentration, X_2 (mg/L)
1	−1	−1	51 (0.42)	23.2
2	0	−1.414	127 (1.05)	10.0
3	−1	1	51 (0.42)	86.8
4	−1.414	0	19 (0.16)	55.0
5	1	1	204 (1.68)	86.8
6	0	0	127 (1.05)	55.0
7	0	1.414	127 (1.05)	100.0
8	0	0	127 (1.05)	55.0
9	1.414	0	235 (1.94)	55.0
10	0	0	127 (1.05)	55.0
11	1	−1	204 (1.68)	23.2

Note: in Run-1, X_1 (0.42 mg of protein) = (51/18.2) * working volume (0.15 L). The value "18.2" is based on Fig. 1, i.e. 18.2 mg of VSS contains 1 mg of protein.

2.2. Bacterial source and population of SRB

A mesophilic sulfate reducing bacterial culture, enriched and maintained using modified Postgate's C medium (MM) for nearly 5 years, was used as the seed for this study (Hsu et al., 2009). The MM solution contains 3.5 g/L sodium lactate (70%), 1.8 g/L Na_2SO_4, 0.25 g/L KH_2PO_4, 1.0 g/L NH_4Cl, 0.06 g/L $CaCl_2{\cdot}6H_2O$, 0.1 g/L yeast extract, 0.04 g/L $FeCl_3{\cdot}7H_2O$ and 2.52 g/L $NaHCO_3$ with the final pH adjusted to 7.5 ± 0.1 (Postgate, 1984). The presence and relative abundance of SRB in the seeding sludge were determined by using the fluorescence in situ hybridization (FISH) method. The SRB population in the seed sludge (the sum of cells hybridized with probes SRB385 and SRB385Db) was 81% (Hsu et al., 2009). Before the experiment, the SRB in the seed sludge was centrifuged at 4000×*g* for 10 min, washed twice with sterilized–deionized water and resuspended in sterilized–deionized water with the final volume adjusted to 10 mL.

2.3. Preparation of PVA-immobilized SRB beads

The phosphorylated PVA method as outlined by Chen and Lin (1994) was followed for preparing PVA-immobilized SRB beads. Initially, PVA (20% w/v) was heated until dissolved, cooled (~35 °C) and then mixed with an equal volume of concentrated sulfate reducing bacterial culture (~20 g of VSS/L). The PVA-cell mixture was added drop by drop into a saturated boric acid and gently stirred for 30 min to form spherical beads. The gel beads formed were then submerged in a sodium phosphate solution (0.5 M, pH 5) for 1 h for hardening, and subsequently washed with tap water. The average diameter of the beads was between 2 and 3 mm. After immobilization, the beads were placed in a flask containing 500 mL of MM under anaerobic condition, i.e. the head space was replaced with nitrogen gas, and incubated at 30 °C for 8 h.

2.4. Experimental design and optimization of parameters

The CCD was used to design a set of biogenic copper removal experiments. A 2^2-factorial central composite experimental design was employed, using four axial points ($\alpha = 1.414$) and three replications at the central points with a total of 11 experiments (Table 1). Predetermined ranges of independent variables, i.e. the quantity of immobilized SRB in culture solution (e.g. 19, 51, 127, 204, and 235 mg of VSS/L) and copper concentration (e.g. 10, 23.2, 55, 86.8, and 100 mg/L), were used for the CCD; the data were analyzed by using MINITAB® 14.1 statistical software (Minitab Inc.).

All experiments were conducted in 250 mL flasks containing 150 mL MM (sulfate concentration 300 mg/L) with preselected

Table 2
Sequence of runs for the CCD.

Run order	Quantity of PVAbeads added	Copper removal by blank PVA after 24 h		Lag time	Time to max. sulfate reduction efficiency	Maximum sulfide production	Dependent variables				
							Copper removal by bioprecipitation			Sulfate reduction rate constant K (d^{-1})	
	(g)	mg/L	R_{blank}, (%)	(d)	(d)	(mg/L)	mg/L	Observed R_{bio}, (%)	Predicted R_{bio}, (%)	Observed	Predicted
1	2.33	3.99	17.2	0.08	1.0	50	19.21	82.8	77.5	0.858	0.785
2	4.75	1.70	17.0	0.25	4.0	57	8.30	83.0	87.0	0.709	0.898
3	1.71	33.77	38.9	0.25	2.0	42	53.03	61.1	57.3	0.790	0.603
4	0.65	24.42	44.4	2.00	7.0	50	30.58	55.6	59.3	0.554	0.673
5	8.15	33.94	39.1	0.25	2.0	54	52.86	60.9	47.4	0.883	0.811
6	5.35	24.75	45.0	0.50	2.0	53	30.25	55.0	54.9	1.685	1.555
7	4.27	51.10	51.1	1.00	7.0	35	48.90	48.9	58.4	0.521	0.640
8	5.35	24.81	45.1	0.50	2.0	54	30.20	54.9	54.9	1.444	1.555
9	8.78	35.53	64.6	0.50	2.0	53	19.47	35.4	45.3	0.790	0.981
10	5.35	24.75	45.0	0.50	2.0	49	30.25	55.0	54.9	1.538	1.555
11	8.44	6.52	28.1	0.08	1.0	56	16.68	71.9	67.6	1.279	0.994

Note: R_{bio}, (%) is calculated based on 7 d reaction time.