



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

Hsiu-Feng Hsu^{a,b}, Yu-Sheng Jhuo^a, Mathava Kumar^a, Ying-Shih Ma^c, Jih-Gaw Lin^{a,*}

^aInstitute of Environmental Engineering, National Chiao Tung University, 1001, University Road, Hsinchu, Taiwan

^bChin Min Institute of Technology, 110 Hsueh-Fu Road, Toufen, Miaoli, Taiwan

^cDepartment of Environmental Engineering and Health, Yuanpei University, 306 Yuanpei Street, Hsinchu, Taiwan

ARTICLE INFO

Article history:

Received 22 June 2009

Received in revised form 19 January 2010

Accepted 21 January 2010

Available online 12 February 2010

Keywords:

Bioprecipitation

Sulfate reducing bacteria

Heavy metal

Polyvinyl alcohol (PVA)

Central composite design

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.

© 2010 Elsevier Ltd. All rights reserved.

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

* Corresponding author. Tel.: +886 3 5722681; fax: +886 3 5725958.
E-mail address: jglin@mail.nctu.edu.tw (J.-G. Lin).

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.

Table 2

Sequence of runs for the CCD.

Run order	Quantity of PVAbeads added (g)	Copper		Lag time (d)	Time to max. sulfate reduction efficiency (d)	Maximum sulfide production (mg/L)	Dependent variables				
		removal by blank PVA after 24 h					Copper removal by bioprecipitation			Sulfate reduction rate constant K (d^{-1})	
		mg/L	R_{blank} (%)				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.

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 $4000 \times g$ 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 ($\sim 35^\circ C$) 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 $30^\circ C$ 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

quantities of sulfate reducing bacterial culture immobilized in PVA, and copper concentrations as shown in Table 1. Different quantities of PVA-immobilized beads (Table 2) were added in each run to maintain the predetermined levels of protein concentration.

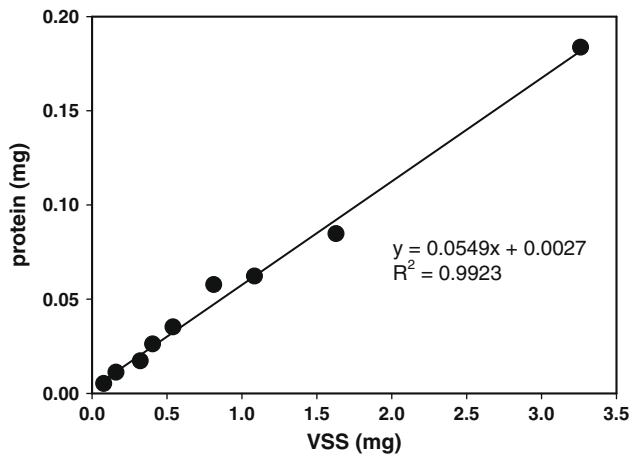


Fig. 1. Relationship between protein and VSS of sulfate reducing bacterial culture.

Subsequently, the pH of the medium was adjusted to 7.5 ± 0.1 , and nitrogen gas was purged into the flask to maintain the anaerobic condition. The flask was incubated for 7 d at 30 °C on a shaker set at 100 rpm. For each run, eight flasks were operated in duplicate under same conditions. At each sampling time, two flasks were used for measuring sulfate, sulfide and copper concentrations. In addition, the protein concentration in the sample was measured and correlated to the biomass concentration.

Subsequently, control experiments were conducted using blank PVA beads. The quantities of blank PVA beads in control groups and the PVA-immobilized SRB beads in experimental groups were the same in each run. The control experiments were used to estimate the copper removal through physical absorption and chemical precipitation. Statistical analyses were done using the analysis of variance (ANOVA) available in MINITAB® 14.1 statistical software.

3. Analytical technique

3.1. Spectrophotometry

Copper concentration was determined using a flame/graphite atomic absorption spectrophotometer (Hitachi, Z-8100) in an

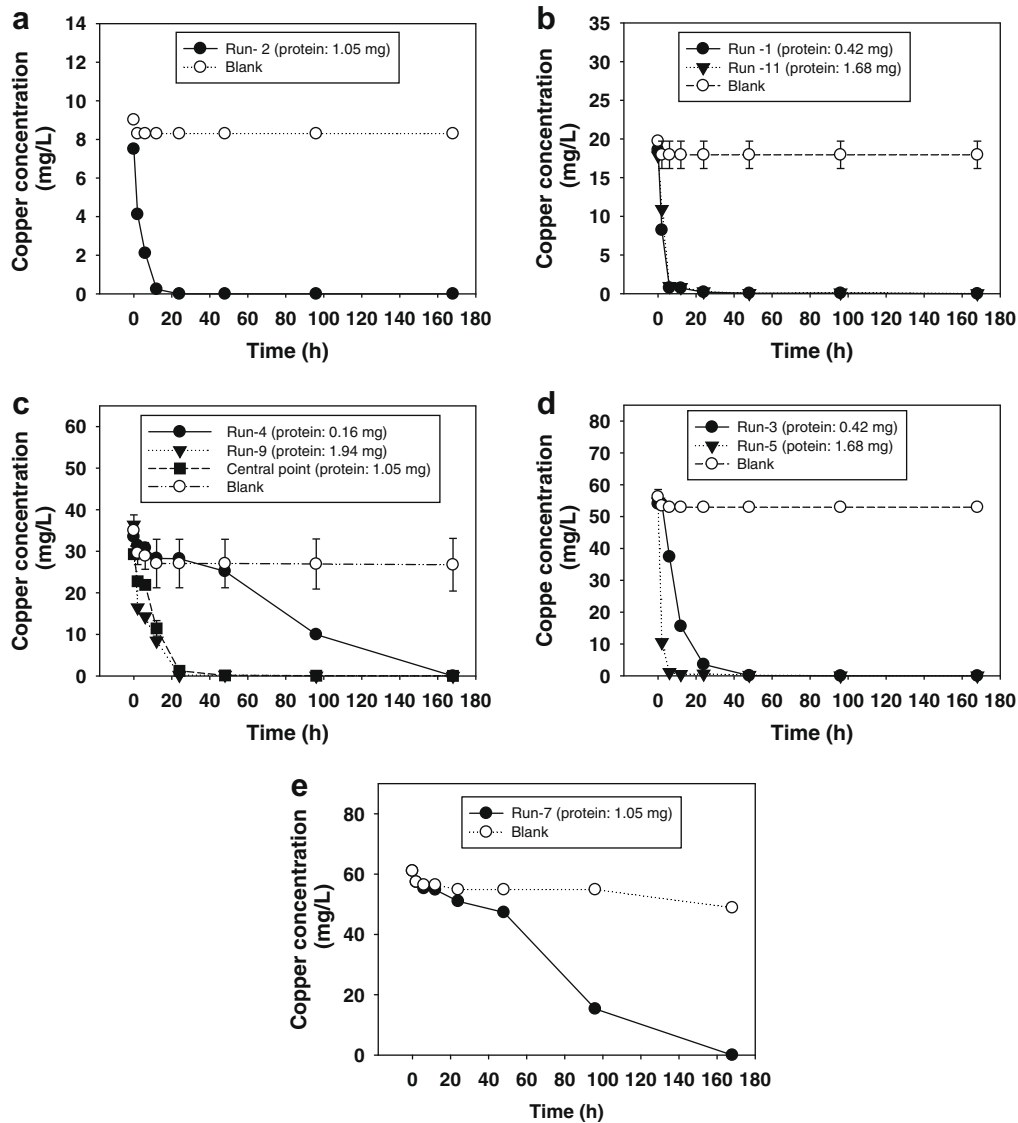


Fig. 2. Copper removal profiles under various initial copper concentrations (mg/L) of the CCD experiments (a) 10, (b) 23.2, (c) 55, (d) 86.8 and (e) 100.

air-acetylene flame at 324.8 nm wavelength. The sample was prepared by centrifuging at $4000\times g$ for 10 min, and filtering through 0.2 μm membrane filter (ADVANTEC, Japan). The sulfate concentration in the filtrate was analyzed by using the turbidimetric method with a UV spectrophotometer (Hitachi, U3210) as outlined in Standard Methods (APHA, 2005). For measuring dissolved sulfide concentration, the filtered sample was analyzed using the methylene blue method (APHA, 2005). The detection limits are 0.72 mg/L for sulfate, 0.85 mg/L for sulfide and 0.083 mg/L for copper. The recovery percentages of sulfate, sulfide and copper were $98.8 \pm 2.8\%$, $93.3 \pm 6.7\%$ and $105 \pm 5.0\%$, respectively. The VSS concentration in the sample was determined according to the procedures in the Standard Methods (APHA, 2005).

3.2. Biomass concentration in the PVA-immobilized SRB beads

The quantity of biomass immobilized in each PVA bead was determined using a biomass estimation method based on the cell protein content. The cell protein was measured according to Bradfords protein assay (Bradford, 1976) with bovine serum albumin (BSA) protein as the standard. The seed sludge containing SRB was diluted to different concentrations of volatile suspended solids (VSS). Subsequently, the corresponding protein content in the dif-

ferent diluted SRB samples was measured. Also, the total biomass of diluted SRB samples was measured as VSS by determining the dry-cell weight at 550°C (APHA, 2005).

Approximately 1 g of PVA-immobilized SRB beads was placed on a clear glass plate; each bead was cut into 20–30 pieces with a sharp knife. The gel pieces were collected in a test tube, added with 1% sodium dodecyl sulfate (SDS) solution and sonicated for 2 h to extract the cell protein using an ultrasonic cleaner (Branson, 40 kHz, 130 W). After centrifugation at $16,060\times g$ for 20 min, the cell protein was measured according to Bradfords protein assay.

4. Results and discussion

Using the Brunauer, Emmett and Teller (BET) method (Brunauer et al., 1938), the average surface area and the pore size of the PVA-immobilized SRB beads were found to be $75.6 \pm 0.5\text{ m}^2/\text{g}$ and 178.6 \AA , respectively. The average surface area and the pore size of the blank PVA beads (without SRB) were $47.8 \pm 0.5\text{ m}^2/\text{g}$ and 211.4 \AA , respectively. The scanning electron microscopic images of the PVA-immobilized SRB beads revealed that the average diameter of large pores was measured as $40 \pm 12\text{ }\mu\text{m}$. Under the complete mixing condition at 100 rpm for 7 d, the PVA-immobilized SRB beads had not shown any crack on the surface confirming that

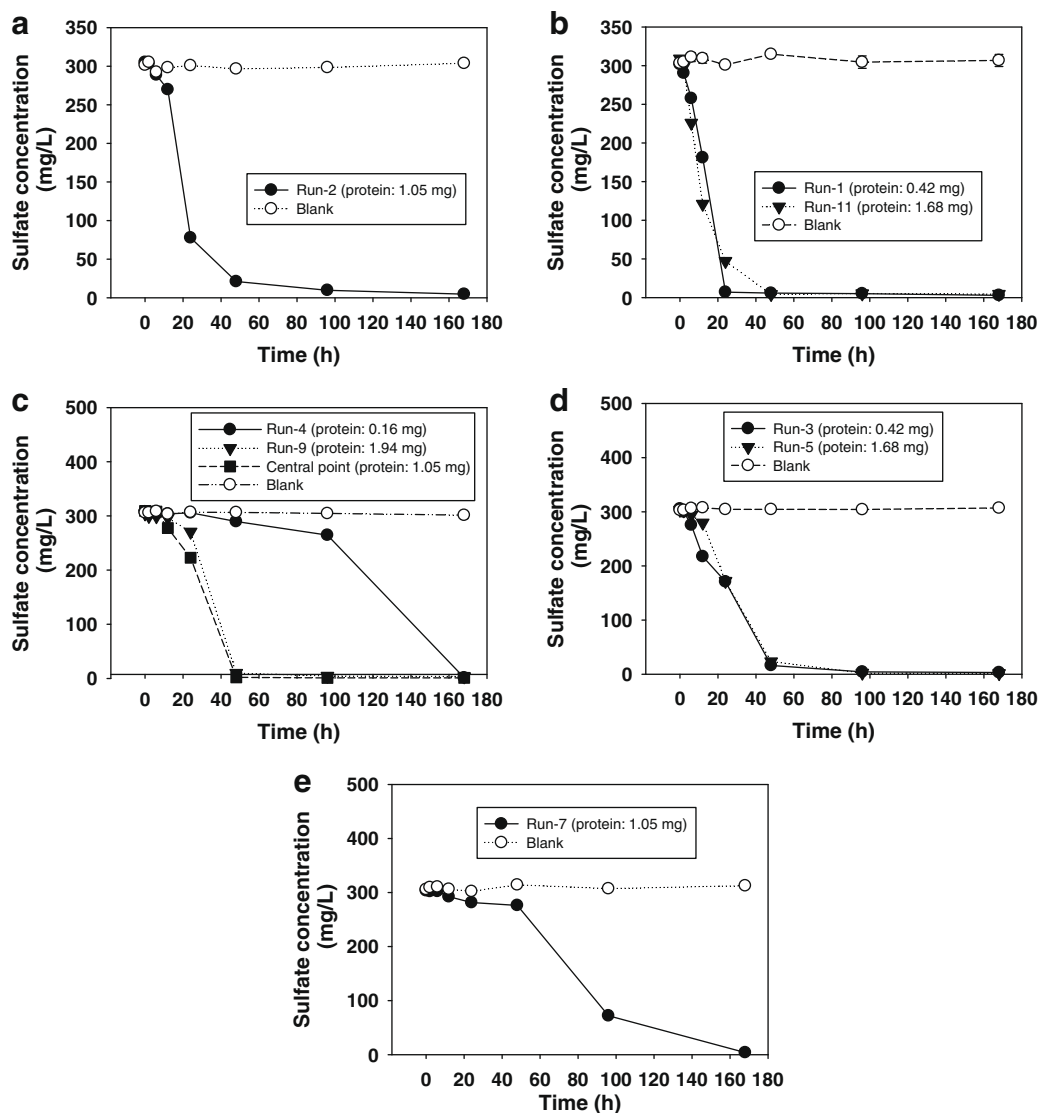


Fig. 3. Sulfate reduction profiles under various initial copper concentrations (mg/L) of the CCD experiments (a) 10, (b) 23.2, (c) 55, (d) 86.8 and (e) 100.

the beads were durable for conducting biogenic copper removal studies.

The protein concentration was correlated to the corresponding VSS concentration in the samples as shown in Fig. 1. Thereafter, the cell weight per immobilized bead was estimated from the standard curve of dry-cell weight versus protein concentration. The

average protein concentration in 1 g of PVA-immobilized SRB beads was 0.213 ± 0.033 mg (wet weight basis).

4.1. Copper concentration in CCD experiments

Fig. 2 shows the profiles of copper removal for samples containing various initial copper concentrations in the CCD experiments including copper removal using blank PVA beads (without SRB). After 24 h of reaction, copper concentration in the control experiments reached pseudo equilibrium. The percentage removal of copper in the control experiments (R_{blank}) was in the range of 17.0–64.4% as shown in Table 2. In all biotic run with PVA-immobilized SRB beads (except Run-4 and Run-7), the copper concentration was reduced to below 1 mg/L within 2 d. However, the copper removal percentage declined for runs with samples containing the maximum copper concentration, i.e. 100 mg/L (Run-7), and the lowest protein concentration, i.e. 0.16 mg (Run-4). In both these runs, the overall copper removal by PVA-immobilized SRB beads was around 99.2% at the end of 7 d. This indicates that the quantity of immobilized SRB in culture solution and the initial copper concentration have significant influence on copper removal.

The results reported by Utgikar et al. (2002) show that when the copper concentration exceeds 50 mg/L, the copper ion can produce toxicity to SRB. The apparent decline in the copper removal rate at 100 mg/L copper concentration could be due to copper toxicity that

Table 3

Estimated parameters and AVONA analysis for copper removal by bioprecipitation (%) and sulfate reduction rate constant (d^{-1}) with the quantity of immobilized SRB in culture solution and copper concentration in CCD.

Variable	Copper removal by bioprecipitation R_{bio} (%)		Sulfate reduction rate constant K (d^{-1})	
	Regression coefficient	p -Value	Regression coefficient	p -Value
Constant	103.456	0.000	-0.649	0.118
X_1	-0.007	0.969	0.017	0.005
X_2	-1.282	0.021	0.040	0.006
X_1^2	0.000	0.732	-0.00006	0.006
X_2^2	0.009	0.053	-0.00039	0.004
Model		0.040		0.013
Lack-of-fit		0.000		0.216
R^2 (R^2_{adj})	77.1% (61.8%)		84.6% (74.3%)	

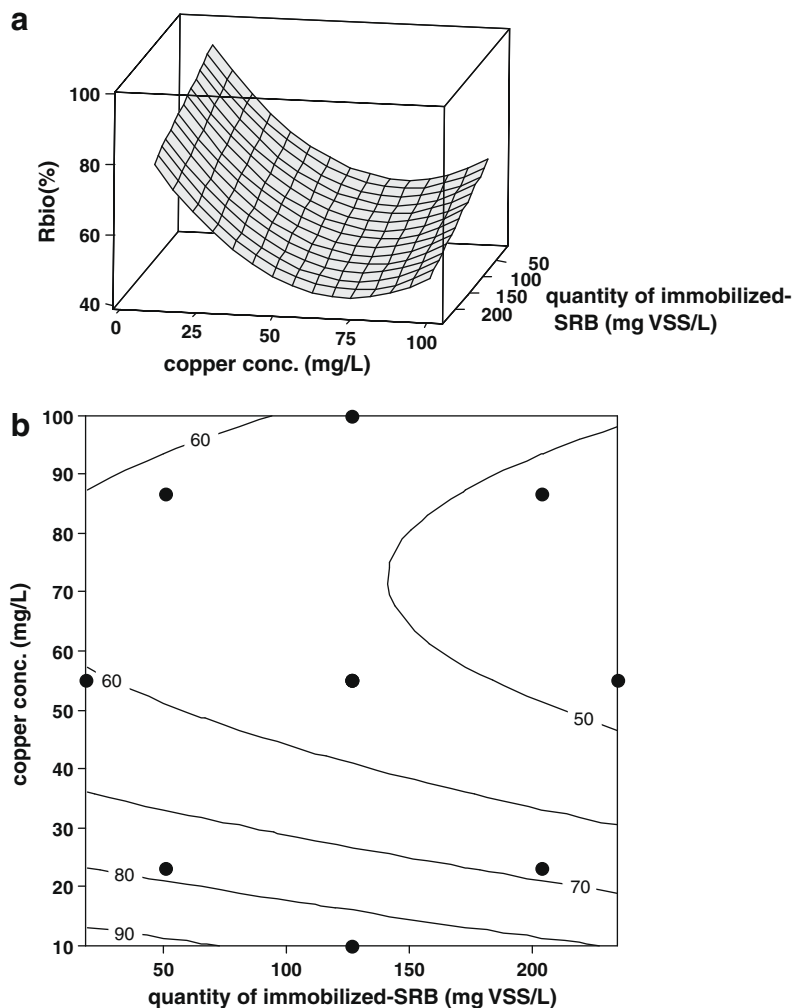


Fig. 4. (a) Response surface and (b) contour plot for copper removal by bioprecipitation, R_{bio} (%) versus the quantity of immobilized SRB in culture solution (%) and copper concentration.

adversely affected the microbial growth and activity. However, the sulfide generated by the activity of SRB could have reduced the copper toxicity by precipitating copper as copper sulfide. The copper concentrations that inhibit 50% (EC_{50}) and 100% (EC_{100}) of the mixed SRB were 10.5 and 12.0 mg/L, respectively, with a reaction time of 7 d (Utgikar et al., 2003). Jin et al. (2007) also pointed out that the SRB activity in pond sediment consortia was completely inhibited at 30 mg of Cu/L. However, if immobilized in PVA, SRB was not inhibited by 10–100 mg of Cu/L as observed in this study except that higher copper concentrations caused longer lag periods for SRB. This indicates that the immobilization of SRB in PVA can effectively protect the SRB from copper toxicity by avoiding direct contact with the copper ions.

The mechanism of copper removal by SRB is rather complex including physical absorption, chemical precipitation and bioprecipitation (Neculita et al., 2007). Copper removal by physical absorption and chemical precipitation, i.e. R_{blank} , and the biogenic copper removal, i.e. R_{bio} , could be calculated by Eqs. (1) and (2), respectively.

$$R_{\text{blank}}(\%) = \frac{(Cu_i - Cu_{\text{blank}})}{Cu_i} \times 100\% \quad (1)$$

$$R_{\text{bio}}(\%) = \frac{(Cu_{\text{blank}} - Cu_{\text{PVA,t}})}{Cu_i} \times 100\% \quad (2)$$

where Cu_{blank} (mg/L) is the copper concentration remaining in the control experiment after 24 h; $Cu_{\text{PVA,t}}$ (mg/L) is the copper concentration remaining in the experiment with PVA-immobilized SRB beads after 7 d reaction time; and Cu_i is the initial copper concentration (mg/L). The values of R_{blank} and R_{bio} in each run of the CCD experiments are shown in Table 2. A minimum R_{bio} value of 35.4% was observed in Run-9 when the initial copper concentration was at 55 mg/L while the maximum R_{bio} value of 83% was observed in Run-2 when the initial copper concentration was at 10 mg/L. At the highest copper concentration (100 mg/L) in Run-7 and the lowest biomass in the culture solution (19 mg of VSS/L) in Run-4, the lag time of the culture was increased considerably, i.e. 1 and 2 d, respectively. However, Run-4 and Run-7 show R_{bio} values of 55.6% and 48.9%, respectively, at the end of 7 d. This could be due to the immobilization of SRB in PVA and avoiding the direct contact of SRB to copper toxicity.

4.2. Sulfate concentration in CCD experiments

The overall copper removal efficiency of the biogenic metal removal process depends on the sulfate reduction by SRB, and the formation and utilization of sulfide produced in the system (Lyew and Sheppard, 1997). Moreover, the amount of sulfate reduction is a major indicator of the SRB activity (Johnson and Hallberg, 2005). Fig. 3

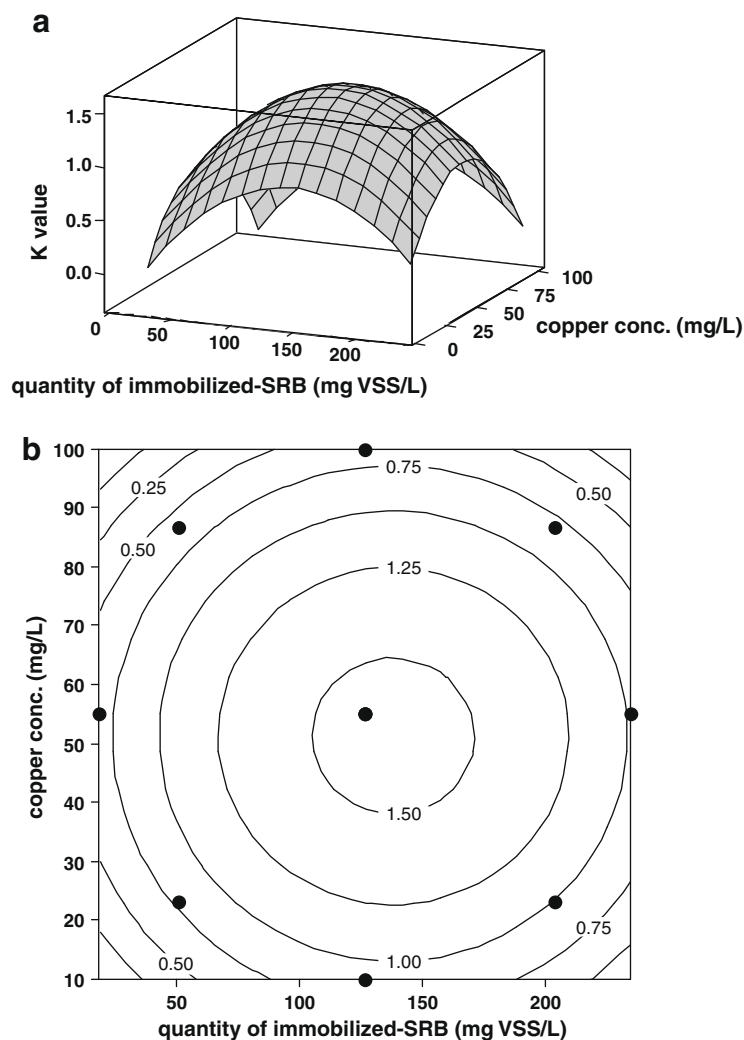


Fig. 5. (a) Response surface and (b) contour plot for sulfate reduction rate constant, K (d^{-1}), versus the quantity of immobilized SRB in culture solution and copper concentration.

shows the profiles of sulfate concentration under a range of initial copper concentrations for the CCD experiments. The initial sulfate concentration in the CCD experiments was maintained at 306 ± 3 mg/L. No change in the sulfate concentration was observed in the control tests, whereas almost 99% of the sulfate reduction was observed in runs conducted with PVA-immobilized SRB beads. However, an apparent decline in the sulfate reduction rates was observed in Run-7 that had the maximum copper concentration, and also in Run-4 that had the lowest quantity of immobilized SRB in culture solution; the decline was probably due to copper toxicity to affect SRB (Fig. 3). The lag time in the activity of SRB in Run-4 and Run-7 were 2 and 1 d, respectively (Table 2). The profiles of copper and sulfate concentrations (Figs. 2 and 3) indicate that the copper concentration and the quantity of immobilized SRB in culture solution have significant influence on the biogenic copper removal.

In the CCD experiments (Run-1 to Run-11), the pH was maintained between 7.0 and 8.5. Moreover, the initial ORP in all runs (around +100 mV) rapidly decreased to around -400 mV within 24 h that was caused by the activity of sulfate reducing bacterial culture; the ORP become constant thereafter. The comparison of PVA-immobilized SRB runs (Run-1 to Run-11) with control runs indicate that the majority of copper removal was through the sulfide produced by SRB.

4.3. Statistical analysis and kinetics of the CCD experiments

The relationship between the factors (X_1 and X_2) and responses (R_{bio} and K) were investigated. The first-order equation as shown in Eq. (3) was used to determine the K value based on the sulfate reduction.

$$(\ln C - \ln C_0) = -Kt \quad (3)$$

where C is the sulfate concentration (mg/L); C_0 is the initial concentration of sulfate (300 mg/L); K is the reaction rate constant (d^{-1}); and t is the reaction time (d). The K value computed based on the variation of sulfate concentration with time was used for carrying out regression analysis. The analysis of variance (ANOVA) was used to determine the most appropriate representation of the biogenic copper removal. The outcomes of ANOVA shown in Table 3 reveal that R_{bio} and K can be represented by second-order polynomial equations shown in Eqs. (4) and (5), respectively.

$$Y_{R_{\text{bio}}} (\%) = 103.456 - 0.007X_1 - 1.282X_2 + 0.009X_2^2 \quad (4)$$

$$Y_K (\text{d}^{-1}) = -0.649 + 0.017X_1 + 0.040X_2 - 0.00006X_1^2 - 0.00039X_2^2 \quad (5)$$

$Y_{R_{\text{bio}}}$ is R_{bio} (%), Y_K is the K value of the sulfate reduction rate constant (d^{-1}); X_1 is the quantity of immobilized SRB in culture solution (mg of VSS/L); and X_2 is the copper concentration (mg/L). Table 3 indicates that both models are significant at the 95% confidence level in terms of p -value (p -value < 0.005) and regression coefficients (R^2 and adjusted R^2). However, the ANOVA results reveal that the sulfate reduction rate constant (K) could be more suitable than the biogenic copper removal (R_{bio}) for the representation of the process efficiency. Moreover, this could be noticed by comparing the response surfaces (Figs. 4a and 5a) and contour plots (Figs. 4b and 5b) of the two responses, i.e. R_{bio} and K , with respect to X_1 and X_2 . Based on the contour plots of K value (Fig. 5b), the estimated maximum sulfate reduction rate constant under the conditions used in the present study was 1.57 d^{-1} , which could be achieved by maintaining 138.5 mg of VSS/L and 51.5 mg of Cu/L.

5. Conclusions

The quantity of immobilized-SRB in culture solution and the initial copper concentration were in good correlation with sulfate

reduction rate constant. The second-order polynomial models formulated based on the CCD experiments could be used to predict the sulfate reduction rate constant under the experimental ranges used in this study. The results demonstrated that biogenic metal removal from wastewater could be improved by alleviating copper toxicity with the use of PVA-immobilized SRB beads.

Acknowledgement

The financial support of this research (NSC-95-2211-E-264-017) was provided by the National Science Council, Taiwan, Republic of China.

References

- Alvarez, M.T., Crespo, C., Mattiasson, B., 2007. Precipitation of Zn(II), Cu(II) and Pb(II) at bench-scale using biogenic hydrogen sulfide from the utilization of volatile fatty acids. *Chemosphere* 66, 1677–1683.
- APHA, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
- Austin, G.T., 1984. *Shreve's Chemical Process Industries*. McGraw Hill.
- Bai, H.-J., Zhang, Z.-M., Yang, G.-E., Li, B.-Z., 2008. Bioremediation of cadmium by growing *Rhodobacter sphaeroides*: Kinetic characteristic and mechanism studies. *Bioresour. Technol.* 99, 7716–7722.
- Baskaran, V., Nemati, M., 2006. Anaerobic reduction of sulfate in immobilized cell bioreactors, using a microbial culture originated from an oil reservoir. *Biochem. Eng. J.* 31, 148–159.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* 72, 248–254.
- Brunauer, S., Emmett, P.H., Teller, E., 1938. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* 60, 309–319.
- Chen, K.C., Lin, Y.F., 1994. Immobilization of microorganisms with phosphorylated polyvinyl-alcohol (PVA) gel. *Enzyme Microb. Technol.* 16, 79–83.
- Hsu, H.-F., Kumar, M., Ma, Y.-S., Lin, J.-G., 2009. Extent of precipitation and sorption during copper removal from synthetic wastewater in the presence of sulfate-reducing bacteria. *Environ. Eng. Sci.* 26 (6), 1087–1096.
- Ike, A., Sriprang, R., Ono, H., Murooka, Y., Yamashita, M., 2007. Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. *Chemosphere* 66, 1670–1676.
- Jimenez-Rodriguez, A.M., Duran-Barrantes, M.M., Borja, R., Sanchez, E., Colmenarejo, M.F., Raposo, F., 2009. Heavy metals removal from acid mine drainage water using biogenic hydrogen sulphide and effluent from anaerobic treatment: effect of pH. *J. Hazard. Mater.* 165, 759–765.
- Jin, S., Drever, J.I., Colberg, P.J.S., 2007. Effects of copper on sulfate reduction in bacterial consortia enriched from metal-contaminated and uncontaminated sediments. *Environ. Toxicol. Chem.* 26, 225–230.
- Johnson, D.B., Hallberg, K.B., 2005. Acid mine drainage remediation options: a review. *Sci. Total Environ.* 338, 3–14.
- Kadukov, J., Vircikova, E., 2005. Comparison of differences between copper bioaccumulation and biosorption. *Environ. Int.* 31, 227–232.
- Kleikemper, J., Pelz, O., Schroth, M.H., Zeyer, J., 2002. Sulfate-reducing bacterial community response to carbon source amendments in contaminated aquifer microcosms. *FEMS Microbiol. Ecol.* 42, 109–118.
- Liamleam, W., Annachhatre, A.P., 2007. Electron donors for biological sulfate reduction. *Biotechnol. Adv.* 25, 452–463.
- Lozinsky, V.I., Plieva, F.M., 1998. Poly(vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments. *Enzyme Microb. Technol.* 23, 227–242.
- Lyew, D., Sheppard, J.D., 1997. Effects of physical parameters of a gravel bed on the activity of sulphate-reducing bacteria in the presence of acid mine drainage. *J. Chem. Technol. Biotechnol.* 70, 223–230.
- Neculita, C.M., Zagury, G.J., Bussiere, B., 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. *J. Environ. Qual.* 36, 1–16.
- Postgate, J.R., 1984. *The Sulphate-Reducing Bacteria*. Cambridge University Press, New York.
- Quan, Z.X., La, H.J., Cho, Y.G., Hwang, M.H., Kim, L.S., Lee, S.T., 2003. Treatment of metal-contaminated water and vertical distribution of metal precipitates in an upflow anaerobic bioreactor. *Environ. Technol.* 24, 369–376.
- Radhika, V., Subramanian, S., Natarajan, K.A., 2006. Bioremediation of zinc using desulfotomaculum nitrificans: bioprecipitation and characterization studies. *Water Res.* 40, 3628–3636.
- Remoudaki, E., Hatzikioseyan, A., Kousi, P., Tsezos, M., 2003. The mechanism of metals precipitation by biologically generated alkalinity in biofilm reactors. *Water Res.* 37, 3843–3854.
- Sani, R.K., Geesey, G., Peyton, B.M., 2001. Assessment of lead toxicity to desulfotomaculum nitrificans G20: influence of components of lactate C medium. *Adv. Environ. Res.* 5, 269–276.

- Shin, H.S., Sae-Eun, O., Chae-Young, L., 1997. Influence of sulphur compounds and heavy metals on the mechanisation of tannery wastewater. *Water Sci. Technol.* 35, 239–245.
- Southam, G., Ferris, F.G., Beveridge, T.J., 1995. Mineralized bacterial biofilms in sulfide tailings and in acid mine drainage system. In: Lappin-Scott, H.M., Costerton, J.W. (Eds.), *Microbial Biofilms*. Cambridge University Press, Cambridge, pp. 148–170.
- Tabak, H.H., Scharp, R., Burckle, J., Kawahara, F.K., Govind, R., 2003. Advances in biotreatment of acid mine drainage and biorecovery of metals: 1. Metal precipitation for recovery and recycle. *Biodegradation* 14, 423–436.
- Teclu, D., Tivchev, G., Laing, M., Wallis, M., 2009. Determination of the elemental composition of molasses and its suitability as carbon source for growth of sulphate-reducing bacteria. *J. Hazard. Mater.* 161, 1157–1165.
- Utgikar, V.P., Chen, B.Y., Chaudhary, N., Tabak, H.H., Haines, J.R., Govind, R., 2001. Acute toxicity of heavy metals to acetate-utilizing mixed cultures of sulfate-reducing bacteria: EC100 and EC50. *Environ. Toxicol. Chem.* 20, 2662–2669.
- Utgikar, V.P., Harmon, S.M., Chaudhary, N., Tabak, H.H., Govind, R., Haines, J.R., 2002. Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. *Environ. Toxicol.* 17, 40–48.
- Utgikar, V.P., Tabak, H.H., Haines, J.R., Govind, R., 2003. Quantification of toxic and inhibitory impact of copper and zinc on mixed cultures of sulfate-reducing bacteria. *Biotechnol. Bioeng.* 82, 306–312.
- Velasco, A., Ramirez, M., Volke-Sepulveda, T., Gonzalez-Sanchez, A., Revah, S., 2008. Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation. *J. Hazard. Mater.* 151, 407–413.
- Wang, C.L., Ozuna, S.C., Lum, A.M., Clark, D.S., Keasling, J.D., 2001. Aerobic sulfide production and cadmium precipitation by *Escherichia coli* expressing the *Treponema denticola* cysteine desulfhydrase gene. *Appl. Environ. Microbiol.* 56, 425–430.
- Zhao, X.W., Zhou, M.H., Li, Q.B., Lu, Y.H., He, N., Sun, D.H., Deng, X., 2005. Simultaneous mercury bioaccumulation and cell propagation by genetically engineered *Escherichia coli*. *Process Biochem.* 40, 1611–1616.