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Cu^{2+} removal in a biosorption column by immobilized bacterial biomass

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ABSTRACT

Removal of excess heavy metal ions from waste waters is essential due to their extreme toxicity towards aquatic life and humans. Microorganisms are being increasingly studied for the removal of heavy metal ions from aqueous solution. In this study, bacterial biomass, which was isolated from soil beforehand, was identified as *Bacillus subtilis*. Then the bacterial biomass was immobilized in agar and polyacrylamide gel. The immobilized particles packed in a column were used to remove Cu^{2+}. The effects of the immobilization method, bed-length, flow rate and initial metal concentration on performance of the Cu^{2+} removal by the fixed bed columns were systematically investigated. The highest Cu^{2+} uptake efficiency 58.0% (Y_i) was obtained by *Bacillus subtilis* immobilized on agar, 10cm bed length, 180ml/h flow rate and 100mg/l initial Cu^{2+} concentration. This research showed that *Bacillus subtilis* had a potential to be used in removal of Cu^{2+} from waste waters. Freundlich constants were determined from the Freundlich adsorption isotherms.

Keywords: Immobilized cells; Biosorption; Waste water; Copper removal; *Bacillus subtilis*

1. INTRODUCTION

The presence of copper ions in water may cause toxic and harmful effects to the living organisms present and as well as to consumers. Copper present in industrial wastes is primarily in the form of the bivalent Cu^{2+} as a hydrolysis product, $CuCO_{3(aq)}$ and/or organic complexes. Several industries, for example, dyeing, paper, petroleum, copper/brass-plating, and copper-ammonium rayon, release undesired amounts of Cu^{2+}. In the copper-cleaning, copper plating, and metal-processing industries, Cu^{2+} concentrations approach 100-120mg/l; this value is very high in relation to water quality standards, and Cu^{2+} concentrations of waste waters should be reduced to a value of 1.0-1.5mg/l (Aksu *et al.*, 1992; Sag *et al.*, 1995).

Microorganisms may be used to remediate waste waters contaminated with heavy metals. Microorganisms accumulate metals by a number of different processes such as uptake by transport, biosorption to cell walls and entrapment in extracellular capsule, precipitation and oxidation-reduction reactions (Macaskie and Dean, 1989; Gadd 1990).

Although freely suspended biomass may have better contact with the adsorbates during the adsorption, the biomass suspension is normally not the practical form for the direct use in biosorption processes. The biomass is often immobilized to enhance its stability, mechanical strength, reusability and the ease of handling. Thus cell immobilization techniques have been incorporated into the development of biosorption processes. Biosorption with immobilized cells is frequently designed as a fixed-bed reactor, inside which desired types of immobilized biomass are packed (Chang *et al.*, 1998).

In this study, the bacterial biomass were immobilized in agar and polyacrylamide gel. The immobilized particles packed in a column were used to remove Cu^{2+}. The effects of the

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immobilization method, bed-length, flow rate and initial metal concentration on performance of the Cu^{2+} removal by the fixed-bed columns were systematically investigated.

2. MATERIAL AND METHODS

2.1. Bacterial strain and identification

Bacillus sp. OGUB 001 was used as biosorbent which had been isolated in our previous work (Nourbaksh *et al.*, 2002). The bacterial strain was identified according to Bergey's Manual of Determinative Bacteriology and The Prokaryotes (Kandler and Weis, 1986; Slepecky and Hemphill, 1992).

2.2. Preparation of Cu^{2+} solutions

The stock solution of Cu^{2+} (1.0g/l) was prepared by dissolving a weighed quantity of $CuSO_4.5H_2O$ (Merck) in distilled water. The selected inlet metal ion concentration range corresponds to 50-150mg/l on the basis of weight. The pH of each was adjusted to the required value for the biosorption Cu^{2+}, by adding 1mol/l of H_2SO_4.

2.3. Preparation of biomass

The bacterial isolate was cultivated aerobically at 30°C in Nutrient Broth (NB) (Merck) by constantly agitating at 150rpm in glass flasks. The cells were harvested by centrifugation (5000rpm, 10min) from culture at early-stationary phase. After rinsing in distilled water the cells were again centrifugated.

Immobilization with agar (A): For preparation of agar gel particles, the method described below was followed (Kierstan and Coughlan, 1985). Agar (100mg) dissolved in 4.5ml of 0.9% (w/v) NaCl by heating at 100°C and then cooling to 50°C. Cell slurry (10-30mg dry weight per 100ml) was suspended in 0.9% (w/v) NaCl solution. 0.5ml of the cell slurry was added to 4.5ml of the agar solution at 50°C and mixed. The solution was poured into petri dishes. The gel was cut into particles (18-30mesh) using sieve. Then the particles of gel were washed with 0.9% NaCl solution to separate it from intact cells.

Immobilization with polyacrylamide (PAA): Detailed procedures were described elsewhere (Skryabin and Koscheenko, 1987). In general, 6ml of the prepared cell suspension (17-100mg dry cell per ml) was rapidly mixed with a solution containing 1.9g of acrylamide monomer, 0.1g of N,N'-methylene-bisacrilamide, 3ml ammonium persulfate (0.5%, w/v), and 0.2ml TEMED (50%, w/v) on a shallow plate. All the chemical reagents described here were obtained from Sigma. After completion of polymerization (about 1h), the resulting gel-like slice was cut into (18-30mesh) cubes, and then were rinsed with 0.9% NaCl solution.

2.4. Column studies

The immobilized cells were stacked into glass columns (1.5cm in diameter) of the bed length in the range of 3 to 10cm. The Cu^{2+}-bearing solution (50, 100 and 150mg/l) was continuously pumped upward into the column to avoid channel effects. The Cu^{2+} loading rates were ranged from 180 to 900ml/h. Samples were collected from the effluent to measure for residual Cu^{2+} concentrations.

2.5. Adsorption equilibrium experiments

Immobilized cells were suspended in solution amended with Cu^{2+} in the concentration range of 25-200mg/l and 4.5 pH. In general, each adsorption batch contained 4 grams of immobilized cells per 100ml of Cu^{2+} solution. The adsorption solutions were gently agitated at 27°C. As the adsorption reached equilibrium, samples were taken from each batch, and the metal concentration in the supernatants was measured.

2.6. Measurement of Cu^{2+}

Total metal concentration in the solution was measured with a Perkin Elmer 3110 Atomic Absorption Spectrometer. Before measurement, the heavy metal solutions were appropriately diluted with deionized water to ensure that the heavy metal concentration in the sample was linearly dependent on the absorbance detected.

3. FREUNDLICH ISOTHERM AND MATHEMATICAL EQUATIONS

The equilibrium occurring during physical adsorption at a definite concentration range could be represented by the Freundlich adsorption isotherm equation:

$$q_{eq} = K_F C_{eq}^{1/n} \tag{1}$$

where K_F and n are Freundlich constants, and are indicators of adsorption capacity and adsorption intensity, respectively. The variables q_{eq} and C_{eq} also show the amount of metal adsorbed onto cells and the metal (residual) concentration at equilibrium. This equation can be linearized in logarithmic form and Freundlich constants can be determined from the gradient and intercept, which are equal to $1/n$ and K_F at C=1.0 respectively (Chang *et al.*, 1998; Sag *et al.*, 1995; Aksu *et al.*, 1999).

The breakthrough curves for the biosorption of Cu^{2+} were measured as a function of bed length, initial metal concentration and flow rate. The results are given in terms of the maximum (equilibrium) capacity of the column, $C_{i,max}$ (mg) the amount of metal loading on the bacterium surface, $q_{i,eq}$ (mg/g), and the adsorption yield (adsorbed metal percentage), $\%Y_i$. The maximum (equilibrium) capacity of the column for a given feed concentration is equal to the area under the plot of the adsorbed metal ion concentration $C_{i,ads}$ (mg/l) vs. time (min) or the area behind the breakthrough curve. The amount of metal that remains in the effluent $C_{i,eq}$, is the area under the breakthrough curve (Sag *et al.*, 2000a; Sag and Aktay, 2001).

$$C_{i,eq} = \frac{C_i t - \int_0^a C_{i,ads} dt}{t} \quad or \quad C_{i,eq} = \frac{W_i - q_{i,eq} X}{Qt} \tag{2}$$

$$C_{i,\max} = Q \int_0^a C_{i,ads} dt \tag{3}$$

The amount of metal loading on the bacterium surface is calculated from the weight of metal adsorbed per unit dry weight of bacterium in the column, that is, the ratio of the maximum capacity of the column to the amount of biosorbent in the column, X (mg).

$$q_{i,eq} = \frac{C_{i,\max}}{X} \tag{4}$$

The adsorption yield is the ratio of the maximum capacity of the column to the amount of metal loading into the column, W_i (mg).

$$W_i = C_i Q t \tag{5}$$

$$Y_i = \frac{C_{i,\max}}{W_i}.100 \tag{6}$$

4. RESULTS AND DISCUSSION

In this study, the bacterial strain was identified according to Bergey's Manual of Determinative Bacteriology and The Prokaryotes (Kandler and Weiss, 1986; Slepecky and Hemphill, 1992). Biochemical tests performed for identification and other properties are in

Table 1. *Bacillus sp.* OGUB 001 was determined as *Bacillus subtilis* according to these tests.

Table 1
Biochemical tests for identification of *Bacillus sp.* OGUB 001 and other determined properties

	Properties
Cell shape	Rod
Width (μm)	1
Length (μm)	3
Gram reaction	+
Spores round	-
Sporangium swollen	-
Catalase	+
Anaerobic growth	-
Voges-Proskauer tset	+
Acid from D-Glucose	+
Acid from D-Xylose	+
Acid from D-Mannitol	+
Hydrolysis of casein	+
Hydrolysis of gelatin	+
Hydrolysis of starch	+
Utilization of citrate	+
Formation of indole	-
Growth at pH 6.8, NB	+
Growth at pH 5.7, NB	+
Growth in 2% NaCl	+
Growth in >5% NaCl	+
Growth at 5 °C	-
Growth at 30 °C	+
Growth at 40 °C	+
Growth at >40 °C	-

+: 90% or more strain positive, -: 90% or more strain negative

The walls of gram positive bacteria are efficient metal chelators. The carboxyl group of the glutamic acid of peptidoglycan is the major site of metal deposition. Carbohydrate groups are important metal binding sites. Some *Bacillus* species produce carbohydrate capsules (Gadd, 1988; Kuyucak and Volesky, 1988; Slepecky and Hemphill, 1992; Schulze-Lam, *et al.*, 1993; Puranik and Paknikar, 1999).

For metal uptake by cell walls of *Bacillus subtilis*, a two step mechanism consisting of an initial binding reaction with reactive sites in the cell wall, followed by a further deposition nucleated by the bound metal has been proposed. Carboxyl groups are identified as providing the major site of metal deposition, while phosphodiester groups are also involved in metal binding, although these do not mediate the additional deposition step (Tobin *et al.*, 1990; Beveridge *et al.*, 1997).

The optimum initial pH and temperature of the adsorption medium for Cu^{2+} adsorption to the bacterium were found to be pH 4.5 and 27°C in our previous work (Nourbaksh *et al.*, 2002). These conditions were fixed in this study. The adsorption of copper ions to the bacterial biomass immobilized with agar and polyacrylamide was investigated as a function of bed length, initial metal concentration and flow rate in the fixed-bed column.

4.1. Effect of column operating conditions on adsorption behaviour

Fixed-bed columns with the bed length of 3, 6 and 10cm were operated at a constant flow rate of 180ml/h and inlet-metal-ion concentration of approximately 100mg/l. Increasing the bed-length from 3cm to 10cm led to prolongation of equilibrium time. Table 2 indicates that, the adsorption yields (Y_i) of both A immobilized *B. subtilis* and PAA immobilized *B. subtilis* were high for the bed length of 6 and 10cm, whereas the 3cm had lower Y_i value especially PAA immobilized *B. subtilis*, which may be due to a relatively small amount of adsorbents in a shorter bed. It was observed that Y_i value obtained were quite high for both A immobilized *B. subtilis*

and PAA immobilized *B. subtilis* in bed length of 6cm. As expected, maximum value of $C_{i,max}$ was obtained at a bed-length of 10cm. Maximum adsorption yields (Y_i) were 58.0% and 52.8%, respectively, for the A immobilized *B. subtilis* and PAA immobilized *B. subtilis* (Table 2).

As generally expected, change in the inlet ionic concentration of the feed affected the operating characteristics of the fixed-bed column. When the flow rate (180ml/h) and bed length (10cm) were kept constant, inlet-metal-ion concentrations were changed from 50 to 150mg/l.

The biosorption capacity of the biomass increased firstly with increasing of the initial Cu^{2+} concentration and then reached a saturation value. These saturation values were around 100mg/l for A and PAA immobilized *B. subtilis* (Table 3). Maximum adsorption yields to A immobilized *B. subtilis* and PAA immobilized *B. subtilis* were determined as 58.0 and 52.8 percent, respectively, at their optimum initial Cu^{2+} concentrations (Table 3). The maximum (equilibrium) capacity of the column, $C_{i,max}$ (mg), the amount of metal loading on the bacterium surface, $q_{i,eq}$ (mg/g), and the adsorption yield (adsorbed metal percentage), $\%Y_i$, are given in Table 3.

Adsorption rates of Cu^{2+} to biomass increased with increasing metal ion concentrations up to 100mg/l for both immobilized *B. subtilis*. Even though this value is very high in relation to water quality standard especially A immobilized *B. subtilis* could be used for removal Cu^{2+} from waste water.

When the inlet-metal-ion concentration, approximately 100mg/l, and bed length, 10cm, were kept constant, flow rate were changed from 180 to 900ml/h. As indicated in Table 4, increase in the flow rate resulted in a decrease in the yield (Y_i). Maximum adsorption equilibrium yield for both immobilized biomass were obtained at a flow rate of 180ml/h.

Table 2
The effect of bed length on the biosorption of Cu^{2+} on immobilized *B. subtilis*

Immobilized B. subtilis(A)					Immobilized B. subtilis (PAA)				
Bed-length (cm)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg/g)	Y_i (%)	Bed-length (cm)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg/g)	Y_i (%)
3	40.0	112.3	6.6	35.3	3	20.5	114.5	3.4	17.9
6	51.1	112.3	4.2	45.5	6	34.8	114.5	2.9	30.4
10	65.1	112.3	3.3	58.0	10	59.3	112.3	3.0	52.8

(pH 4.5, flow rate: 180ml/h, $W_{biosorbent}$: 6g of 3cm bed-length; 12g of 6cm bed-length; 20g of 10cm bed-length, initial metal concentration: approximately 100mg/l)

Table 3
The effect of initial metal concentration on the biosorption of Cu^{2+} on immobilized *B. subtilis*

Immobilized B. subtilis(A)					Immobilized B. subtilis(PAA)				
Initial metal concentration (mg/l)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg/g)	Y_i (%)	Initial metal concentration (mg/l)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg/g)	Y_i (%)
49.6	23.6	47.2	1.1	50.0	50.3	24.3	55.8	1.2	43.5
101.2	65.1	112.3	3.1	58.0	101.2	59.3	112.3	2.8	52.8
152.0	18.8	168.7	0.8	11.1	149.0	17.3	165.3	0.8	10.5

(pH 4.5, flow rate: 180ml/h, bed-length: 10cm, $W_{biosorbent}$: 20g)

Table 4
The effect of flow rate on the biosorption of Cu^{2+} on immobilized *B. subtilis*

Immobilized B. subtilis(A)					Immobilized B. subtilis(PAA)				
Flow rate (ml/h)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg/g)	Y_i (%)	Flow rate (ml/h)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg/g)	Y_i (%)
180	65.1	112.3	3.1	58.0	180	59.3	112.3	2.8	52.8
300	123.1	332.3	5.8	37.0	300	137.6	330.2	6.5	41.6
540	46.6	158.5	2.2	29.4	540	47.4	170.2	2.2	27.8
900	83.4	531.6	3.9	15.7	900	91.0	495.6	4.3	18.3

(pH 4.5, bed-length: 10cm, $W_{biosorbent}$: 20g, initial metal concentration: approximately 100mg/l)

In our previous study, the adsorption equilibrium between adsorbed Cu^{2+} on this bacterium and unadsorbed Cu^{2+} in solution was observed to occur within 30-40min in batch stirred

experiments (Nourbakhsh *et al.*, 2002). However, in this study, adsorption equilibrium was reached within 5-10min.

The unadsorbed Cu^{2+} concentration in the effluent was high at the beginning of the column operation. According to Sag *et al.*, (1995), the initial drop in adsorption may be attributed to diffusion limitations. This effect was observed especially at low flow rates. After a while, effluent concentration of Cu^{2+} decreased in a short time. Equilibrium was then reached everywhere in the column system, and breakthrough occurred. The effluent concentration did not show any change with time, and this step was considered to be equilibrium state.

The Cu^{2+} adsorption behaviour of both A and PAA immobilized *B. subtilis* were similar to that of matrices (A and PAA). It is worth noting that in the first effluent, adsorbed Cu^{2+} concentrations by immobilized A and PAA *B. subtilis* were much the same at the all flow rates because of diffusion limitations. Thereafter adsorbed metal amounts increased at lower flow rates.

Under the determined optimum conditions, the breakthrough curves of Cu^{2+} for A immobilized *B. subtilis* and PAA immobilized *B. subtilis* were illusturated in Figure 1. Figure 1 demonstrate a similar trend of concentration profiles for the two type of biomass but A immobilized *B. subtilis* was higher than PAA immobilized *B. subtilis*.

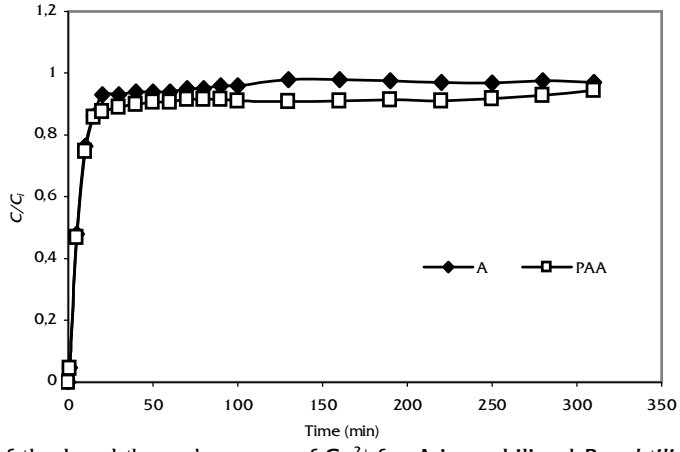

Figure 1. Comparison of the breakthrough curves of Cu^{2+} for A immobilized *B. subtilis* and PAA immobilized *B. subtilis* (determined optimum conditions; flow rate: 180ml/h, initial metal concentration: approximately 100mg/l, $W_{biosorbent}$: 20g of 10cm bed-length)

Under the determined optimum conditions, adsorption experiments for cell-free A and PAA matrices, the blank control, were also carried out. It was found that the cell free A and PAA matrix showed poor Cu^{2+} adsorption ability (Table 5).

Table 5
Copper ion adsorption chararcteristics for cell-free A and PAA

	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg g^{-1})	Y_i (%)
A	3.6	100.8	0.1	3.6
PAA	4.0	106.4	0.1	3.7

(pH 4.5, flow rate: 180ml/h, bed-length: 10cm, $W_{sorbent}$: 20g, initial metal concentration: approximately 100mg/l)

4.2. Adsorption modelling

For the adsorption of Cu^{2+} to both immobilized microbial biomass, the equilibrium occured within 20-40min. At the optimum initial pH value (4.5) and 27°C, the Freundlich adsorption isotherms of both immobilized microorganism for Cu^{2+} adsorption are given in Figure 2. For

isotherm experiments, the initial Cu^{2+} concentrations were varied while the immobilized biomass weight in each sample was kept constant. Values of K_F and $1/n$ obtained from the isotherms are compared in Table 6. The magnitude of K_F and n illustrate the separation of heavy metal ions from wastewater and the high adsorption capacity of bacterial biomass.

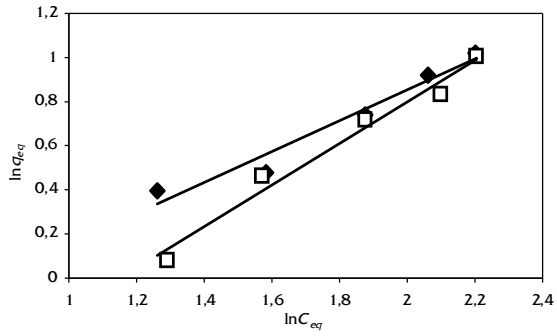

Figure 2. The Freundlich adsorption isotherms of each immobilized *B. subtilis* (□, polyacrylamide; ◆, agar) for copper ion adsorption (pH 4.5, 27°C).

Table 6
Kinetic parameters of adsorption isotherms estimated by Freundlich Model

Type of adsorbent	K_F	1/n
Polyacrylamide immobilized cells	12.280	0.101
Agar immobilized cells	3.535	0.199

5. CONCLUSIONS

Fixed bed column experiment results showed that the maximum Cu^{2+} uptake was efficiency with 10cm bed length, at 180ml/h flow rate and at 100mg/l initial Cu^{2+} concentration. Under the determined this optimum conditions the adsorption yield of the A immobilized *B. subtilis* was 58.0% but that of PAA immobilized *B. subtilis* was 52.8%.

According to these results, it could be considered that both two type of biomass can be used in removing Cu^{2+}. However, A immobilized *B. subtilis* could be preferred in use, because of the fact that both agar, as a matrix, is cheaper than polyacrylamide gel and also method of immobilization is much more easier.

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