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Biosorption of copper from wastewater by *Bacillus subtilis* in a packed bed bioreactor

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Abstract

The potential for copper (Cu^{2+}) reduction in a fixed-film bioreactor system was investigated using a copper reducing bacterial species *Bacillus subtilis*. This work on the process of biosorption of copper from wastewater in a continuous-flow, fixed packed-bed bioreactor was conducted in a search for solution to the environmental problem caused by heavy metals. The Cu^{2+} bearing solution with initial concentration in the range of 50 - 150 mg/L was continuously pumped downward into the column. The Cu^{2+} loading rates were ranged from 180 to 900 ml/h. Analysis of the results demonstrated that the biosorbent had an extraordinary capacity for biosorption of copper (II) studied at about 4.5 pH of the effluent, with a removal percentage of 71.2% and 63.2% for the two immobilization methods used. Agar immobilized *Bacillus subtilis* biomass was found to be more efficient than polyacrylamide immobilized biomass in the packed bed bioreactor.

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Introduction

In India, water pollution comes from three main sources: Domestic sewage, industrial effluents and run-off from agriculture. Earth's surface comprising of 70% water is the most valuable natural resource existing on our planet (WHO). Without this invaluable compound, the life on the Earth would not exist. Although this fact is widely recognized, pollution of water resources is a common problem being faced today. Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water (Vijayaraghavan and Yun, 2008). Modern industry is, to a large degree, responsible for contamination of the environment. Lakes, rivers and oceans are being overwhelmed with many toxic contaminants. Among toxic substances reaching hazardous levels are heavy metals (Vieira and Volesky, 2000). Heavy metals are the group of contaminants of concern, which comes under the inorganic division. Typical concentrations vary from several thousand mg/L from plating bath waste to less than 1 ppm from copper cleaning operations (Vieira and Volesky, 2000). Copper is present in the wastewater of several industries, such as metal cleaning and plating baths, refineries, paper and pulp, fertilizer, and wood preservatives. The excessive intake of copper by man leads to severe mucosal irritation, widespread capillary damage, hepatic and renal damage, central nervous problems followed by depression, gastrointestinal irritation, and possible necrotic changes in the liver and kidney (Kalavathy *et al.,* 2005). All heavy metals have a specific pH underneath where their solubility is drastically increased. For copper, this pH is 5.5 (Martinez and Motto, 2000), which is close to the ideal farmland pH of 6.0–6.5 (Eriksson *et al.,* 1997). Copper can be found in many kinds of food, in drinking water and in air. Copper uptake by *Bacillus subtilis* and *P.fluorescens* was passive and uptake depended on nutrients available in the medium. Copper adsorption by *Rhizopus arrhizus, Cladosporium resinae* and *Penicillium italicum* was reported by Rome and Gadd (1987). Copper specific uptake by polymers extracted from activated sludge suggests that retention of copper in this type of system was primarily binding by bacterial extracellular polymers (Wang and Chen 2006).

Literature indicates that *Bacillus subtilis* can remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents. The advantages of *Bacillus subtilis* as biosorbents in metal biosorption, the forms of *Bacillus subtilis* in biosorption research, biosorptive capacity of *Bacillus subtilis*, the selectivity and competitive biosorption by *Bacillus subtilis* were depicted in detail by Wang and Chen (2006). The magnitude order of metal uptake capacity by *Bacillus subtilis* can be estimated for copper, in the order of 1–2, less than 20 mg Cu/g dry weight of cells. As *Bacillus subtilis* is the efficient biosorbent for the removal of copper (II) from wastewater, it was used in the immobilized form in a packed bed bioreactor and studied the biosorption rates (Kalavathy *et al.,* 2005).

Materials and methods

Preparation of stock solution and biomass

Bacillus subtilis was used as biosorbent which had been isolated from wastewater of NIT Warangal wastewater treatment plant in our previous work (Narasimhulu *et al.*,2010). The stock solution of Cu^{2+} (1.0 g/L) was prepared by dissolving a weighed quantity of $CuSO_4.5H_2O$ in distilled water. The selected inlet metal ion concentration range corresponds to 50 – 150 mg/l on the basis of weight. The pH of each was adjusted to the required value for the biosorption Cu^{2+}, by adding 1 mol/L of H_2SO_4.

The bacterial isolate was cultivated aerobically at 30 ^{0}C in Nutrient Broth (NB) by constantly agitating at 150 rpm in glass flasks. The cells were harvested by centrifugation (5000 rpm, 10 min) from culture at early-stationary phase. After rinsing in distilled water the cells were again centrifuged.

Immobilization with agar (A)

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

Immobilization with polyacrylamide (PAA)

6 ml of the prepared cell suspension (17-100 mg dry cell per ml) was rapidly mixed with a solution containing 1.9 g of acrylamide monomer, 0.1 g of N,N'-methylene-bisacrilamide, 3 ml ammonium persulfate (0.5%, w/v), and 0.2 ml Tetramethylethylenediamine (TEMED) (50%, w/v) on a shallow plate (Skryabin and Koscheenko, 1987). After completion of polymerization for about one hour, the resulting gel-like slice was cut into 18-30 mesh cubes, and then were rinsed with 0.9 % NaCl solution.

Column studies

The immobilized cells were stacked into glass column of the bed length in the range of 10 to 25 cm. The Cu^{2+} bearing solution with initial concentration of 50, 100 and 150 mg/l was continuously pumped downward into the column. The Cu^{2+} loading rates were ranged from 180 to 900 ml/h. Samples were collected from the effluent to measure for residual Cu^{2+} concentration.

Adsorption equilibrium experiments

Immobilized cells were suspended in solution amended with Cu^{2+} in the concentration range of 25-200 mg/l and pH of 4.5. Each adsorption batch contained 4 grams of immobilized cells per 100 ml 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. 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.

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:

$$q_{eq} = K_F \, C_{eq}^{\,1/n} \qquad \text{--- (1)}$$

where K_F and n are Freundlich constants, and are indicators of adsorption capacity and adsorption intensity, respectively.

The linearized form of this model is as follows:

$$lnq_{eq} = lnk_f + \left(\frac{1}{n}\right) lnC_e \qquad \text{----- (2)}$$

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 was linearized in logarithmic form and Freundlich constants were determined from the gradient and intercept, equal to $1/n$ and K_F at C=1.0 respectively (Chang *et al.*, 1997; Sag *et al.*, 2000; 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 were 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.*, 2000).

$$C_{i,eq} = \frac{C_i\, t - \int_0^a C_{i,ads}\ dt}{t} \quad \text{------ (3)}$$

Or

$$C_{i,eq} = (W_i - q_{i,eq}\, X)/Q_t$$

$$C_{i,max} = Q \int_0^a C_{i,ads}\ dt \quad \text{------ (4)}$$

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).

$$q_{i,eq} = \frac{C_{i,max}}{X} \quad \text{------ (5)}$$

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).

$$W_i = C_i Q_t \quad \text{------- (6)}$$

$$Y_i = \frac{C_{i,max}}{W_i}.100 \quad \text{------ (7)}$$

Other isotherm models such as Reddlich and Peterson, Sips, and the Toth models were tested for validation of experimental data of more efficient immobilization method.

Reddlich and Peterson isotherm model

$$Q_e = \frac{K_R\, C_e}{1 + a_R\, C_e^{\beta}} \quad \text{------- (8)}$$

Where K_R (l/g), a_R (l/mg) and β (varied between 0 and 1) are empirical parameters without physical meaning.

Sips isotherm model

$$Q_e = \frac{K_s\, C_e^{\frac{1}{bs}}}{1 + a_s C_e^{\frac{1}{bs}}} \quad \text{------- (9)}$$

Where Ks, a_s and b_s are the sips isotherm parameters.

The Toth isotherm model

$$Q_e = (K_t C_e)/[(a_t + C_e)^{\frac{1}{t}}] \quad \text{------ (10)}$$

Where K_t (mg/g), a_t and t represent the Toth isotherm constants.

Results and discussion

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. 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.

Effect of column operating conditions on adsorption behaviour

Fixed-bed columns with the bed length of 10, 15, 20 and 25 cm were operated at a constant flow rate of 180 ml/h and inlet-metal-ion concentration of approximately 100 mg/l. Increasing the bed-length from 10 to 25 cm led to prolongation of equilibrium time. Table 1 indicates that, the adsorption yields (Yi) of both A immobilized *Bacillus subtilis* and PAA immobilized *Bacillus subtilis* were high for the bed length of 20 and 25 cm, whereas the 10 cm had lower Y_i value especially PAA immobilized *Bacillus 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 *Bacillus subtilis* and PAA immobilized *Bacillus subtilis* in bed length of 20 cm. The maximum value of $C_{i,max}$ was obtained at a bed-length of 25 cm. Maximum adsorption yields (Y_i) were 71.2% and 63.2%, respectively, for the A immobilized *Bacillus subtilis* and PAA immobilized *Bacillus subtilis* (Table 1). The change in the inlet ionic concentration of the feed has affected the operating characteristics of the fixed-bed column. When the flow rate (180 ml/h) and bed length (25 cm) were kept constant, inlet-metal-ion concentrations were changed from 50 to 150 mg/l.

Table 1. The effect of bed length on the biosorption of Cu^{2+} on immobilized *Bacillus subtilis* (pH 4.5, flow rate180 ml/h,$W_{biosorbent}$ 18 g of 10 cm bed length,36 g of 20 cm bed length,60 g of 25 cm bed length, initial metal concentration 100 mg/l).

Immobilized *Bacillus subtilis (A)*					*Immobilized Bacillus 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 (%)
10	40.0	112.3	6.6	35.3	10	20.5	114.5	3.4	17.9
15	51.1	112.3	4.2	45.5	15	34.8	114.5	2.9	30.4
20	65.1	112.3	3.3	58.0	20	59.3	112.3	3.0	52.8
25	69.3	112.3	2.9	71.2	25	64.6	112.3	3.2	63.2

Table 2. The effect of initial metal concentration on the biosorption of Cu^{2+} on immobilized *Bacillus subtilis* (pH :4.5, flow rate:180 ml/h,$W_{biosorbent}$: 60 g of 25 cm bed length).

Immobilized *Bacillus subtilis (A)*					*Immobilized Bacillus 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	149.0	149.0	17.3	165.3	0.8	10.5
165.0	16.1	174.4	0.7	156.0	162.4	16.0	171.2	0.65	16.5

Table 3. The effect of flow rate on the biosorption of Cu^{2+} on immobilized *Bacillus subtilis* (pH :4.5, initial metal concentration 100 mg/l,$W_{biosorbent}$: 60 g of 25 cm bed length).

Immobilized *Bacillus subtilis (A)*					*Immobilized Bacillus 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

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 100 mg/l for A and PAA immobilized *Bacillus subtilis* (Table 2). Maximum adsorption yields to A immobilized *Bacillus subtilis* and PAA immobilized *Bacillus subtilis* were determined as 71.2% and 63.2%, respectively, at their optimum initial Cu^{2+} concentrations (Table 2). 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 2. Adsorption rates of Cu^{2+} to biomass increased with increasing metal ion concentrations up to 100 mg/l for both immobilized *Bacillus subtilis*. Even though this value is very high in relation to water quality standard especially A immobilized *Bacillus subtilis* could be used for removal Cu^{2+} from waste water. When the inlet-metal-ion concentration, approximately 100 mg/l, and bed length, 25 cm,

were kept constant, flow rate were changed from 180 to 900 ml/h. As indicated in Table 3, 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 180 ml/h.

Table 4. Copper ion adsorption characteristics for cell-free A and PAA (pH :4.5, flow rate:180 ml/h, initial metal concentration 100 mg/l,Wbiosorbent : 60 g of 25 cm bed length).

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

Table 5. 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

Table 6. Correlation coefficient values of the models for Agar immobilized *Bacilluz subtilis*.

Model	Reddlich and Peterson model	Sips model	Toth model
R^2	0.9798	0.9789	0.4563

In our previous study, the adsorption equilibrium between adsorbed Cu^{2+} on this bacterium and un-adsorbed Cu^{2+} in solution was observed to occur within 30-40 min in batch stirred experiments (Nourbakhsh *et al.*, 2002). However, in this study, adsorption equilibrium was reached within 5-10min. The un-adsorbed Cu^{2+} concentration in the effluent was high at the beginning of the column operation. According to Sag *et al.*, (2000), 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 *Bacillus 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 *Bacillus 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 *Bacillus subtilis* and PAA immobilized *Bacillus subtilis* were illusturated in Figure 1. Figure 1 demonstrate a similar trend of concentration profiles for the two type of biomass but A immobilized *Bacillus subtilis* was higher than PAA immobilized *Bacillus subtilis*.

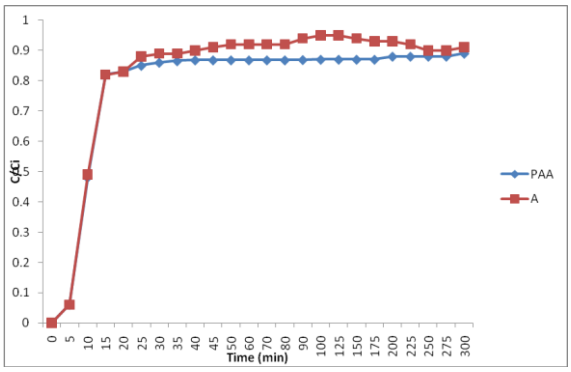

Fig. 1. Comparison of the breakthrough curves of Cu^{2+} for A immobilized *B. subtilis* and PAA immobilized *B. subtilis* (determined optimum conditions; flow rate: 180ml/h, initial metal concentration: approximately 100mg/l, $W_{biosorbent}$: 20g of 25 cm bed-length).

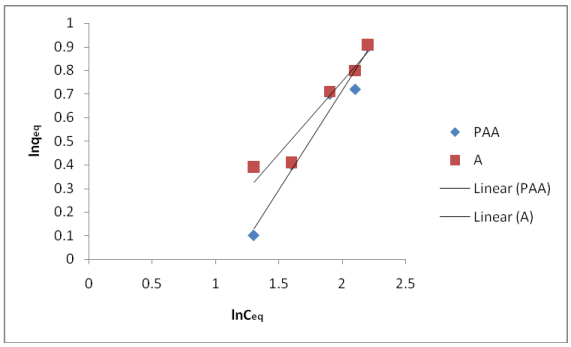

Fig. 2. The Freundlich adsorption isotherms of each immobilized *B. subtilis* (PAA, polyacrylamide; A, agar) for copper ion adsorption (pH 4.5, 270C).

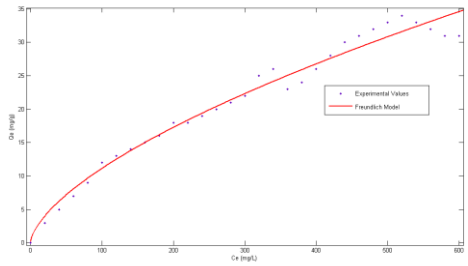

Fig. 3. The Freundlich adsorption isotherm model for copper biosorption.

Maximum adsorption yields to A immobilized *Bacillus subtilis* and PAA immobilized *Bacillus subtilis* were determined as 71.2% and 63.2%, respectively, at their optimum initial Cu^{2+} concentrations.

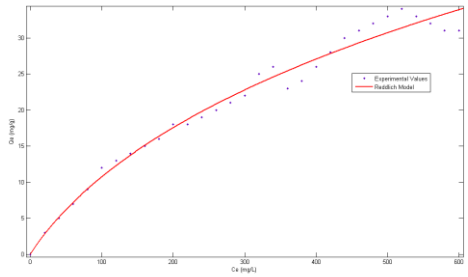

Fig. 4. Reddlich and Peterson isotherm model for copper biosorption by *Bacillus subtilis*.

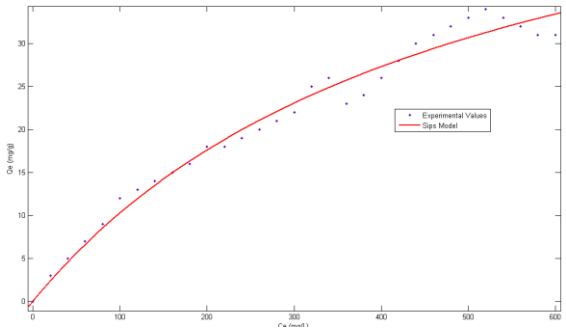

Fig. 5. Sips isotherm model for copper biosorption by *Bacillus subtilis*.

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 4).

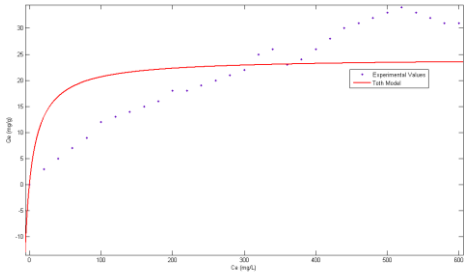

Fig. 6. The Toth isotherm model for copper biosorption by *Bacillus subtilis*.

Adsorption modelling

For the adsorption of Cu^{2+} to both immobilized microbial biomass, the equilibrium occurred within 20-40min. At the optimum initial pH value (4.5) and 27^{0}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 5. The magnitude of K_F and n illustrate the separation of heavy metal ions from wastewater and the high adsorption capacity of bacterial biomass.

Among the three models, Redlich and Peterson model and Sips model were fitted with the experimental data and the Toth model was fitted (Fig. 4, 5, 6).

Conclusion

Based on the results obtained, it was observed that the *Bacillus subtilis* was efficient biosorbent for the removal of copper ions from wastewater. *Bacillus subtilis* biomass was immobilized by agar and polyacrylamide and used as packing material in the packed bed bioreactor. Agar immobilized biomass was found to be more efficient than polyacrylamide immobilized biomass in the packed bed bioreactor.

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