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Biosorption of Cu(II) by immobilized biomass of *Bacillus cereus* M^1_{16} from aqueous solution

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Biosorption of Cu(II) ion from aqueous solution was studied using *Bacillus cereus* M^1_{16} immobilized in calcium alginate and agar agar gel in batch mode. Uptake of metal was very fast initially and equilibrium was attained within 240 min. Sorption data conformed well to both Freundlich and Langmuir isotherm model. Highest Cu(II) uptake (87.32%) by selected biomass (3.09 g/l, dry wt) immobilized in 3% calcium alginate occurred at 30°C, 120 rpm when initial copper concentration was 50 mg/l.

Keywords: Biosorption, Copper, Immobilized biomass

Introduction

Wastes containing metals are directly or indirectly discharged by various industries into environment causing pollution[1-3]. Copper is present in industrial wastes primarily in the form of bivalent Cu(II) ion as a hydrolysis product, $CuCo_3$ (aqueous) and/or organic complexes. In copper cleaning, copper plating and metal processing, Cu(II) ion concentrations approach 100 -120 mg/l, which is very high in relation to water quality standards and permissible[4] Cu(II) concentration (1.0-1.5 mg/l) of wastewaters. Conventional methods for removing metal ions from aqueous streams have certain limitations[5] like incomplete metal removal, high reagent and energy requirements, and generation of toxic sludge.

Biological methods, which have better performance and low cost for remediation, are hindered by small particle size with low density, poor mechanical strength and rigidity[6-7]. Immobilization of biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity and porous characteristics to biological material[8]. Immobilization of biomass, which allows higher biomass concentration and column operation, may be well suited for non- destructive recovery[3].

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In this study , adsorption ability of immobilized *Bacillus cereus* M^1_{16} was investigated for removal of Cu(II) from aqueous solution.

Materials & Methods

Organism

A mutated strain[9] *Bacillus cereus* M^1_{16} immobilized in different carriers was used for biosorption of Cu(II) ions from aqueous solution. It was maintained by monthly subculturing using nutrient agar and stored at 4°C.

Biomass Production and Biosorption Method

B. cereus M116 was grown in a 250 ml Erlenmeyer flask containing 50 ml medium (beef extract, 1.0; yeast extract, 2.0; peptone, 5.0; NaCl, 5.0 g/l and pH 6.0 at 30±1°C and 120 rpm for 24 h). Cell suspension (2%) was used as inoculum. After 24 h, biomass was harvested by centrifugation at 5500 rpm for 15 min at room temperature and washed twice with normal saline. Then required amount of biomass was used for immobilization. Experiments were carried out with wet biomass of selected strain and results were calculated on dry biomass basis[10]. Amount of metal adsorbed was taken to be the difference between initial and final metal ion concentration[11].

Preparation of Dry cells

Washed biomass from a measured amount of whole-cell broth was placed in a previously weighed aluminium

cup and dried at 70°C over night. It was weighed again and weight of dry cell mass was calculated from the difference.

Immobilization Using Calcium Alginate Gel

Cell suspension (3 ml) containing definite amount of cell was added to solution (6 ml) of sodium alginate (4.5%) and mixed thoroughly. Final concentration of sodium alginate was 3.0%. Slurry was dispersed drop by drop into 2% calcium chloride solution by a hypodermic syringe and kept for 2 h at 4°C. Then beads were washed thoroughly with distilled water and air-dried. For storage, beads were dipped in normal saline and kept at 4°C.

Immobilization within Agar Matrix

Agar (270 mg) was melted in 6 ml of distilled water and then cooled to 45-60°C. Cell suspension (3 ml) in saline having definite amount of cells maintained at same temperature was added to molten agar (6 ml) and shaken thoroughly so that agar concentration becomes 3.0%. It was then added to ice-cold toluene: chloroform (3:1) mixture drop by drop using a hypodermic syringe. Spherical beads were then rinsed thoroughly with 0.01 % triton X – 100 to eliminate residual phase[12]. For storage, beads were dipped in normal saline and kept at 4°C.

Biosorption Isotherm

Langmuir model[13] [$q_e = q_m b.C_e/(1+b.C_e)$] may be rearranged as

$$C_e/q_e = 1/b.q_m + C_e/q_m \quad ...(1)$$

where C_e is equilibrium concentration (mg/l) and q_e is adsorbed amount of metal ion per g of biomass at equilibrium (mg/g). q_m is maximum amount of metal ion per unit weight of biomass to form a complete mono layer on the surface bound at high Ce (mg/l). b is a constant related to affinity of binding sites (l/mg). A plot of C_e / q_e vs C_e should indicate a straight line of slope $1/q_m$ and an intercept of $1/bq_m$. Freundlich model equation[14] ($q_e = k.Ce^{1/n}$) is conveniently used in linear form as

$$\ln q_e = \ln k + (1/n) \ln C_e \quad ...(2).$$

where k and n are Freundlich constants characteristics of the system. k is relative indicator of adsorption capacity (l/g) and n indicates intensity of adsorption.

Kinetic Modeling

First- order rate expression[15-17] based on solid capacity is generally expressed as

$$- \log_{10}(q_e-q_t)/q_e = k_1 t/2.3 \quad ...(3)$$

where k_1 is rate constant of First order biosorption (min^{-1}).

Pseudo second order equation is also based on sorption capacity of solid phase[17-19] as

$$1/(q_e - q_t) = 1/q + k_2 t \quad ...(4)$$
$$t / q_t = 1/h + (1 / q_e)t \quad ...(5)$$

where $h = k_2.q_e^2$ can be regarded as initial sorption rate as $t \rightarrow 0$. If pseudo second order kinetics is applicable, plot of t/q_t versus t gives a linear relationship, which allows computation of q_e, k_2 values.

Desorption of Metal from Biosorbed Cell

Efficiency of various eluents (0.1M) like HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH was examined to recover copper from biosorbed bacterial cells of the selected strain at 30°C and 120 rpm. To investigate desorption efficiency of different eluents, metal laden immobilized cells were filtered and after soaking in filter paper to remove any liquid adhered, these beads were transferred to 50 ml eluent taken in 250 ml Erlenmeyer flask. Each flask containing eluent solution was incubated for 4 h at 30°C and 120 rpm.It was then centrifuged and supernatant was collected. Desorption capacity is defined as (concentration of desorbed copper / concentration of adsorbed copper) x 100%.

Estimation of Copper

Concentration of Cu(II) in solution was estimated using Atomic Absorption Spectrophotometer (Chemito Technologies, Model - AA302, 324.5 nm).

Results and Discussion

Screening of Suitable Matrix for Immobilization

From batch studies on Cu(II) biosorption with *B. cereus* M^1_{16} immobilized on different carriers, a high adsorption (90.35% using calcium alginate, 55% using agar) was obtained at pH 6.0. At higher pH (> 6.0), copper precipitation takes place. At low pH, concentration of proton is high, so metal binding sites become positively charged and metal cations and protons compete for binding sites, resulting in lower uptake of metal. With increase in pH, bifunctional groups on cell wall with

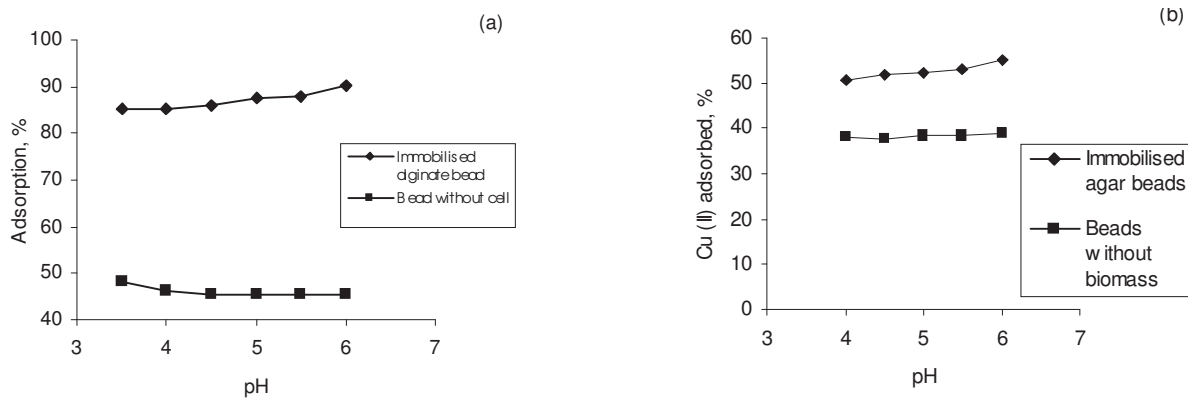

Fig. 1—Effects of initial pH on Cu(II) biosorption : a) Immobilized biomass and calcium alginate bead without biomass; and b) Immobilized biomass and agar agar beads without biomass

negative charge increase, due to deprotonation of metal binding sites, promoting metal uptake. Ionic forms of metal in solution and electric charge of biomass depend on solution pH. Biosorption of Cu(II) ion decreased with increase in pH up to 5.0 and 4.5 for calcium alginate and agar beads having no biomass respectively and then remained constant with increase in pH (Fig. 1).

Effect of Biomass Concentration

Cu(II) ion uptake by immobilized *Bacillus cereus* M^1_{16} was studied using different biomass (1.62-5.29 g/l for calcium alginate entrapped and 1.82-7.64 g/l for agar entrapped) using 50 ml solution (pH 6.0) containing 50 mg/l Cu(II) ion in a 250 ml Erlenmeyer flask at 30°C and 120 rpm for 4 h. With increase in biomass concentration in immobilized beads, biosorption increased up to 3.09 g/l for calcium alginate and 3.64 g/l for agar agar matrices and then decreased with increase in biomass concentration (Fig. 2). Availability of Cu(II) adsorption sites increases with increasing cell mass concentration, but due to agglomeration of biomass, total adsorption sites are not available and Cu(II) adsorption is decreased.

Effect of Initial Cu(II) Ion Concentration

Biosorption carried out with different initial Cu(II) ion concentrations (50, 100, 200, 500, 1000 mg/l) was optimum at biomass concentration (dry wt) of 3.09 g/l for calcium alginate entrapped cells and 3.64g/l for agar entrapped cells with other conditions remaining the same. With increase in initial concentration of Cu(II) ion, specific uptake increased (Fig. 3a), may be due to an increase in electrostatic interactions involving sites

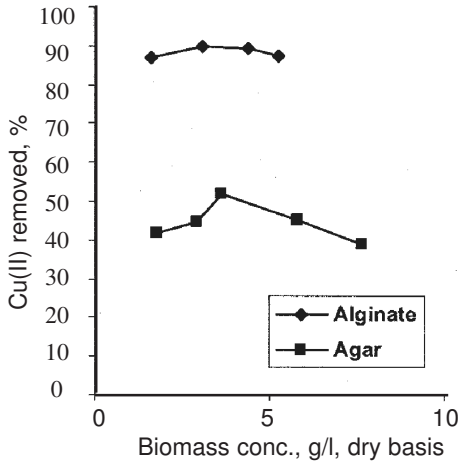

Fig. 2—Effect of biomass concentration on Cu(II) ion biosorption by *Bacillus cereus* M^1_{16} biomass immobilized in calcium alginate and agar agar gel

of progressively lower affinity for metal ions, but metal removal decreased (Fig. 3b).

Time Course of Biosorption

Immobilized beads containing 3.09 g/l and 3.64 g/l biomass in calcium alginate and agar agar matrices respectively were taken in each of 50 ml normal saline containing 50 mg/l Cu (II) ion (pH 5.0) in each 250 ml Erlenmeyer flask and incubated at 30°C and 120 rpm for 280 min. Biosorption of Cu(II) ion was rapid and occurred during first 40 min of sorption (82.72% removal using calcium alginate entrapped , 46.1% removal using agar entrapped biomass) but thereafter equilibrium was reached at 240 min (Fig. 4). Maximum removal were possible for calcium alginate entrapped (85.22%) and agar entrapped biomass (54.28%).

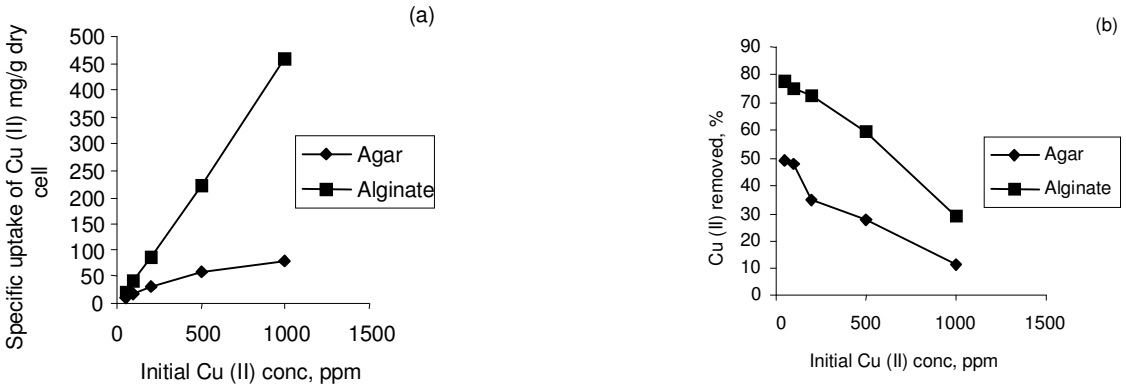

Fig. 3—Effect of initial metal ion concentration on: a) specific uptake of Cu(II) by immobilized biomass; and b) Cu(II) biosorption on immobilized biomass

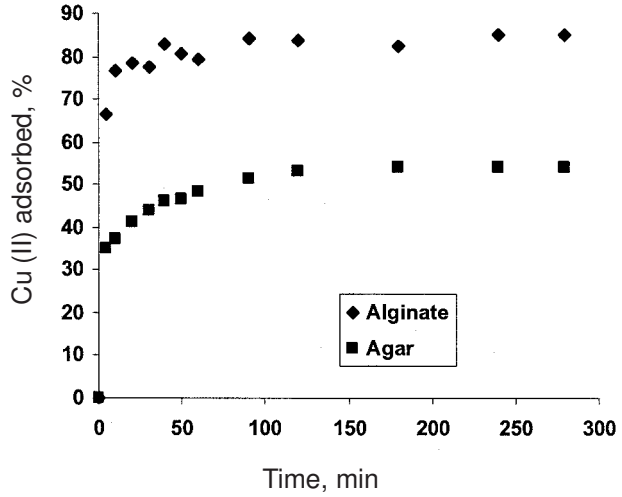

Fig. 4—Effect of incubation time on Cu(II) biosorption by immobilized biomass of *Bacillus cereus* M^{1}_{16}

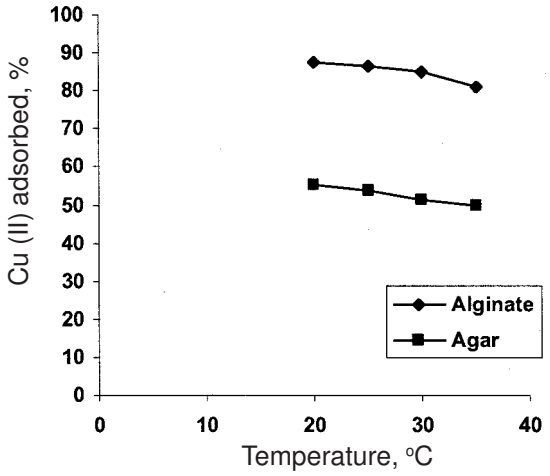

Fig. 5—Effect of temperature on Cu(II) biosorption by *Bacillus cereus* M^{1}_{16} entrapped in calcium alginate and agar agar gel

Effect of Temperature

Agar and alginate immobilized biomass adsorption decreased with increase in temperature (20-35°C) and maximum copper removal was observed at 20°C (Fig. 5), may be a case of physical adsorption, which is normally an exothermic process.

Desorption Efficiency of Different Eluents

Among eluents (0.1 M each; HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH) tested to recover copper from biosorbed immobilized bacterial cells of selected strain at 30°C and 120 rpm, mineral acid may act as better desorbing agents than organic acids (Fig. 6). HCl showed maximum desorption (92%) from biosorbed cells immobilized in calcium alginate gel whereas for agar immobilized biomass, H_2SO_4 was found most efficient eluent (89.12%)

Adsorption Equilibrium Isotherm

Freundlich (Fig. 7, Table 1) and Langmuir (Fig. 8, Table 2) models exhibited good fit to Cu(II) sorption at solution pH of 6.0. Pb(II) sorption by *P. ostreatus* immobilized in calcium alginate gel gave k and 1/n values as 0.4350 l/g and 0.9642 respectively[20].

Kinetics of Adsorption

Kinetics were studied with a constant adsorbent amount of 3.09 g/l and 3.64 g/l (dry wt) entrapped in calcium alginate and agar at 30°C at different time intervals up to 280 min. Pseudo second order equation (Fig. 9) was applicable to all sorption data with correlation co-efficient 0.9996 for agar entrapped biomass and 0.9997 for calcium alginate entrapped biomass. Values of Q_e and k_2 obtained by linear regression t/Q_e against t were 13.93 mg/g dry cell, 0.0104

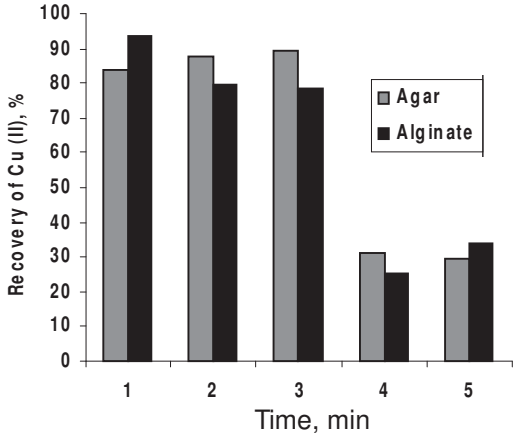

Fig. 6—Desorption capacity of various eluents (HCl-1,HNO_3 –2, H_2SO_4 –3, CH_3COOH –4, HCOOH –5

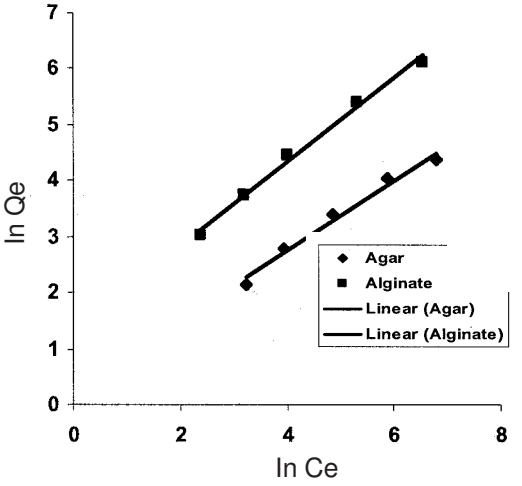

Fig. 7—Freundlich isotherm for adsorption of Cu(II) by calcium alginate entrapped *Bacillus cereus* M^1_{16} and agar immobilized *B.cereus* M^1_{16}

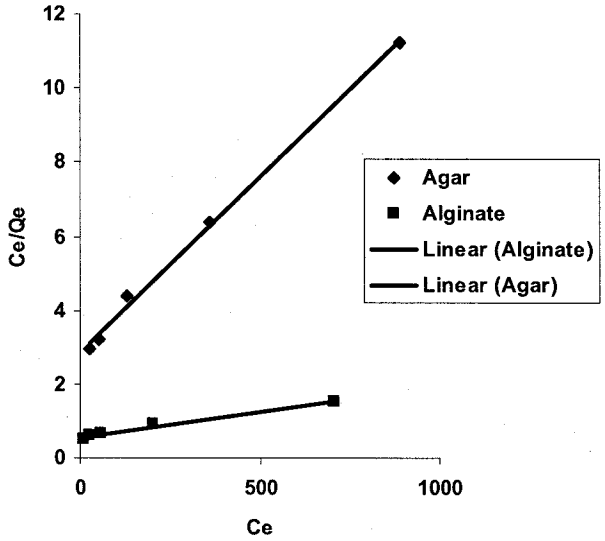

Fig. 8—Langmuir isotherm for biosorption of Cu(II) by *B.cereus* M^1_{16} immobilized in calcium alginate gel and agar entrapped *B.Cereus* M^1_{16}

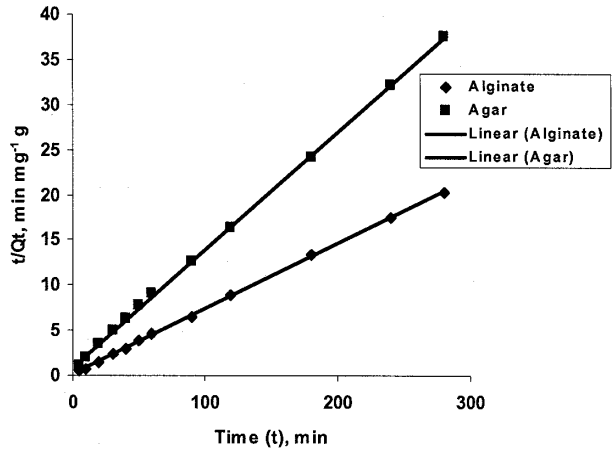

Fig. 9—Pseudo second order adsorption kinetics of Cu(II) on agar immobilized biomass and calcium alginate entrapped biomass

Table 1—Freundlich isotherm parameters for Cu(II) biosorption on *Bacillus cereus* M^1_{16} immobilized in agar agar and calcium alginate matrices

Matrix used	K	1/n	R^2
Agar agar	1.281	0.6265	0.9830
Calcium alginate	3.778	0.7487	0.9891

Table 2—Langmuir isotherm parameters for Cu(II) biosorption on *Bacillus cereus* M^1_{16} immobilized in agar agar and calcium alginate gel

Matrix used	Q_m, mg/g dry wt	b, l/mg	R^2
Agar agar	105.26	3.3×10^{-3}	0.9969
Calcium alginate	714.28	2.47×10^{-3}	0.9896

Table 3—Evaluation of pseudo second order rate equation parameters

Constants	Agar immobilized biomass	Ca-alginate immobilized biomass
Q_e, mg/gm dry cell	13.93	21.37
k_2, g/mg/min	0.0104	0.0200
R^2	0.9996	0.9997

g/mg/min and 21.37 mg/g dry cell, 0.0200g/mg/min when agar and Ca - alginate were used as matrices respectively (Table 3). Second order kinetics were earlier reported for adsorption of Pb(II) on *P. ostreatus* with a rate constant of 0.04583 g/mg/min[20].

Conclusions

Bacterial strain immobilized in calcium alginate gel matrix was found most effective in removing Cu(II) ion from solution. Uptake of metal was very fast initially and equilibrium was attained within 240 min. Overall biosorption process was best described by pseudo second order kinetics. Highest Cu(II) uptake (87.32%) by selected biomass (3.09 g/l, dry wt) immobilized in 3% calcium alginate occurred at 30°C, 120 rpm when initial copper concentration was 50 mg/l.

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