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ORIGINAL ARTICLE

Nickel Biosorption by Immobilized Biomass of *Bacillus Sp.* From Aqueous Solution

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ABSTRACT

Biosorption of Nickel was studied by using free with variation in the parameters of pH, initial metal ion concentration and agitation speed, and immobilized biomass of *Bacillus sp.* strain MGL-75 at pH 7. The aim of this work was to find the biosorption characteristics of selected a biomaterial against to heavy metals for the removal of nickel ions. The obtained results showed that, the alginate calcium, entrapped microorganism (*Bacillus sp.*) is found as a good adsorbing medium for these metal ions and has high adsorption yields for the treatment of wastewater containing nickel ions, as compared to beads without biomass and cell free. Biosorption of metal ions on alginate beads was investigated by using a batch stirred system at pH 6.0, 25°C, in initial metal concentration of 0.4 mmol/l of nickel ions. The equilibrium biosorption level was determined as a function of contact time at several initial metal ion concentrations. All these observations indicate that the nickel biosorption on *sargassum* is mainly based on ion exchange mechanism. Using the single extrapolation method, three kinds of acidic functional groups with three intrinsic pKa were determined at 4.4, 6.9, and 11.2. The effect of adsorbent concentration on the amount adsorbed was also investigated. The desorption of nickel by 1 mM EDTA was very slow, the maximum being after 20 min of elution. The experimental adsorption data were fitted to the Langmuir adsorption model.

Key words: Biosorption, immobilization, Heavy metal

Introduction

Heavy metal pollution is one of the most important environmental problems today. One of the processes used for heavy metal removal from waste water and even their recovery can be biosorption that utilizes various natural materials of biologic origin [1,2,3]. Biosorption process has advantages compared to other processes which include cheap cost of materials, easiness of operation and selectivity over the alkaline metal. Recently, many biopolymers are known to bind metals strongly, and the use of biopolymers as adsorbents for the recovery of valuable metals or the removal of toxic metal contaminants has been studied. Especially, many

studies have been carried out about the application of alginic acid to the aqueous phase separation of heavy metals, and the possibility of alginic acid for the adsorbent material has been suggested.

According to Beveridge (1989) bacteria make excellent biosorbents because of their high surface-to-volume ratios and a high content of potentially active chemisorption sites such as on teichoic acid in their cell walls [4]. Bacterial cell walls are negatively charged under acidic pH conditions and the cell wall chemically functional groups display a high affinity for metal ions in solution [1, 2]. Fein *et al.* [5] used *Bacillus subtilis* to examine the bacterium interaction Cd, Cu Pb and Al.

One of the main characteristics of the genus

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Bacillus is the ability of its members to produce heat-resistant-endospores (5Jen). Members of the genus Bacillus are generally easy to grow to high cell density and do not require expensive growth factors. Uptake of heavy metals, such as Cd, Cr, Fe, U and Zn, has been reported by bacillus species (6). For the industrial application of biosorption, the use of an immobilization biomass in a polymeric matrix or in other supports improves biomass performance and allows its use in many subsequent cycles in the usual unit processes characteristic of chemical engineering. Many workers have investigated methods of immobilization and their application to wastewater treatment. Agar, polyacrylamide, alginate or κ -carrageenan has been used for the entrapment of biosorbent. Alginate is biopolymer extracted from brown seaweeds (*Phaeophyceae*). It is composed by β -1, 4 linked D-mannuric and L-guluronic acids like monomers units. These carboxylic groups are capable of forming complexes with cationic metal [7-8]. Adsorption properties of alginate were investigated for Cu²⁺, Co²⁺, Zn²⁺, Cd²⁺, La³⁺ ions by Jang [9; 10] and [11; 12]. For industrial application of biosorption, it is important to utilize an appropriate immobilization technique to prepare commercial biosorbents. Disadvantage of the free microbial cells used in laboratory conditions is that they are basically small particles, with low density, poor mechanical strength and little rigidity [13, 14, and 15]. In real application they may come up with the solid-liquid separation problems, possible biomass swelling, inability to regenerate/reuse and development of high pressure drop in the column mode [16,17]. High pressures can cause disintegration of free biomass. The immobilization of the biomass in solid structures would create a biosorbent material with the right size, mechanical strength, rigidity and porosity necessary for use in practical processes.

The potential use of immobilized bacillus biomass in Ca-alginate beads for removal of nickel ions from aqueous solution was also investigated. The results of the kinetic studies showed that the sorption of nickel ions on gel immobilized beads is the most suitable.

Materials and methods:

2.1 Biomass Production and Biosorption Method:

Bacillus sp. was grown in a 250 ml Erlenmeyer flask containing 50 ml medium GMS glucose mineral salts (yeast extract 3.0, Na₂PO₄ 5.35, NH₄Cl 2.67, Glucose 10.0, FeSO₄.7H₂O 0.4, MnSO₄.7H₂O 0.075, MgSO₄.7H₂O 0.1, CaCl₂.2H₂O 0.1 g/l, pH7.0, at 30±1°C and 150 rpm, for 72 h). Cell suspension was used for inoculums (2%). After 72h, biomass was harvested by centrifugation at 10000 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 (13).

2.2. Preparation of Dry cells:

Washed biomass from a measured amount of whole cell broth was placed in a previously weighed aluminum cup and dried at 70°C over night. It was weighed again and weight of dry cell mass was calculated from the difference.

2.3 Preparation of Calcium Alginate Beads:

Sodium alginate was dissolved in distilled water at a concentration of 3wt. %. After sodium alginate was completely dissolved, the solution was left undisturbed for 30 minutes to eliminate the air bubbles. The solution was then dropped from a height of 20 cm into gelling medium of 0.2M calcium chloride solution using a syringe with a needle. On the principle of simple ion exchange water soluble sodium alginate was converted to water insoluble and stable calcium alginate salt. The formed beads were cured in the gelling medium for 30 min.

Calcium alginate beads were divided to three groups:

Gel beads: fresh prepared calcium alginate beads were washed with distilled water and used for experiments. The average size of the bead was found to be 3.0 mm.

2.4 Immobilization Using Calcium Alginate Gel:

Cell suspension (3ml) 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.

2.5 Nickel Biosorption Experiments:

Immobilized *Bacillus sp* were used for biosorption of nickel ions from solution with initial concentration 140 mg/l. pH value of used solutions was 7.00. Each fraction (beads with bacillus and without bacillus,) of beads at concentration 2 g/l of beads were added into the solutions. These solutions were mixed during 24 hours. At selected time intervals, solution samples were withdrawn for metal analysis. The concentration of nickel ions was measured by atomic absorption spectroscopy (Chem., Tech, Analytical CTA 2000).

The metal uptake q was calculated from the mass balance equation as follows:

$$q = \frac{F(C_0 - C_e)}{m} \quad (\text{Eq.1})$$

where q - the quantity of metal uptake by biomass [mg g⁻¹]

C_0 - the initial metal concentration [mg l⁻¹]

C_e - final (after sorption at equilibrium) metal concentration [mg l⁻¹]

V - the volume of solution [l]

m - dry weight of the biomass added [g]

Desorption experiment:

Efficiency of various eluents (0.1 and 1M) like HNO₃, HCl, EDTA, CaCl₂, KCl, NaHCO₃ and CH₃COOH was examined to recover nickel from biosorbed bacterial cells of the selected strain at 30°C and 150 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 eluents taken in 250 ml Erlenmeyer flask. Each flask containing eluents solution was incubated for 4 h at 30°C and 150 rpm. It was then centrifuged and supernatant was collected.

Desorption capacity is defined as:

(Concentration of desorbed nickel / concentration of adsorbed nickel) / 100%

Results and discussion

3.1 Effect of pH on biosorption:

From batch studies on nickel ions biosorption with bacillus sp. immobilized on calcium alginate, a high adsorption was obtained at pH 7.0 at higher pH (> 7.0), nickel precipitation takes place. At low pH, concentration of proton is high, so metal binding sites become positivity charged and metal cations and proton compete for binding sites, resulting in lower uptake of metal. With increase in pH, bi functional groups on cell wall with negative charge increase, due to deprotonation of metal binding sites, promoting metal uptake. Ionic form of metal in solution and electric charge of biomass depend on solution pH. (Fig 1)

3.2 Effect of Biomass Concentration:

Nickel ion uptake by immobilized *Bacillus sp.*, was studied using biomass bacterial (1.33-5.87 g/l for calcium alginate entrapped) using 50 ml pH 6.8, containing 50 mg/l nickel ion in a 250

Erlenmeyer flask at 30°C and 150 rpm for 4 h. With increase in biomass concentration in immobilized beads, biosorption increased in biomass concentration. Availability of nickel adsorption sites increase with increasing cell mass concentration, but agglomeration of biomass, total adsorption sites are not available and nickel adsorption is decreased (Fig.2).

The release of calcium, initially fixed onto the SA, has been followed in the same time of nickel adsorption (fig 3). This release depends on the initial nickel concentration of the solution, which could lead to a fixation mechanism by ion exchange. Because the isotherms of Ni²⁺ adsorption and Ca²⁺ desorption were practically similar, Ni²⁺ ions seemed to be exclusively adsorbed by an ion exchange mechanism.

Ion-exchange is an important in biosorption, because it explains many of the observations made during heavy metal uptake experiments. Under certain conditions, the ions attracted to a solid surface may be exchanged with other ions in an aqueous solution.

3.3 Desorption Efficiency of Different Desorbents:

Among eluents (0.1 and 1 M each: HNO₃, HCl, EDTA, CaCl₂, KCl, NaHCO₃ and CH₃COOH) tested to recover nickel from biosorbed immobilized bacterial cells of selected strain at 30°C and 150 rpm, EDTA, acetic acid, potassium chloride and HCl my act as better desorbing agents than, NaHCO₃, calcium chloride and nitric acid (Fig4). EDTA showed maximum desorption (69.1%) in 1 M, (49.14%) in 0.1 M, from biosorbed cells immobilized in calcium alginate gel. Figure 5 presents' data on Ni²⁺ desorption by the three most efficient desorbing agents at different times. This figure demonstrates significant increase in desorption with EDTA as a desorbing agent. Of a total of 0.52 mmol /g adsorbed Ni²⁺, 0.28 mmol Ni²⁺ was desorbed by EDTA within the first 20 min of elution time. In contrast to EDTA, the desorption of Ni²⁺ by 0.1 mM KCl was very slow, being 6% at 80 min of elution.

3.4 Intrinsic pKa determination:

To understand the chemical feature of the metal binding process, it necessary to determine which chemical groups on the alga cells could be responsible for the binding. A possible approach is the study of the functional groups present onto the biomass. For this purpose, the calculation of intrinsic pKa using the simple extrapolation method has been proposed by Stumm and Morgan (1996)(18). (Fig.6)

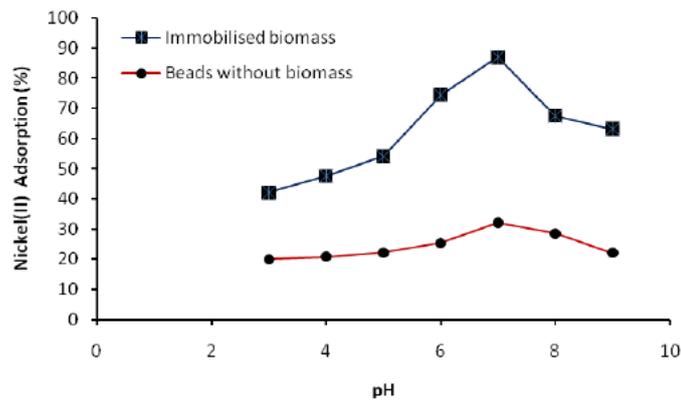


Fig. 1: Effects of initial pH on Ni (II) biosorption, immobilised biomass and calcium alginate bead without biomass

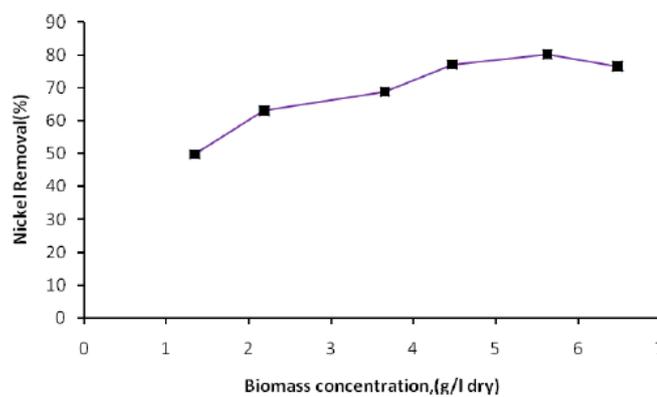


Fig. 2: Effect of biomass concentration on Nickel ion biosorption by *bacillus* sp biomass immobilized in calcium alginate.

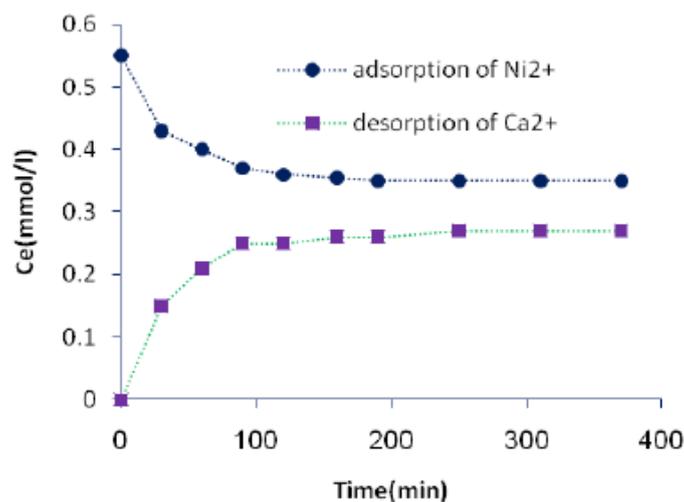


Fig. 3: kinetic of adsorption of Ni²⁺ by bacillus sp and kinetic of desorption of Ca²⁺.

For bacillus sp, three pK_a have been extrapolated:

- (I) $pK_{a1} = 4.4 \pm 0.2$ corresponding to the carboxylic groups.
- (II) $pK_{a2} = 6.9 \pm 0.2$ which can be identified as amine functions.
- (III) $pK_{a3} = 11.2 \pm 0.2$ usually due to phenol groups.

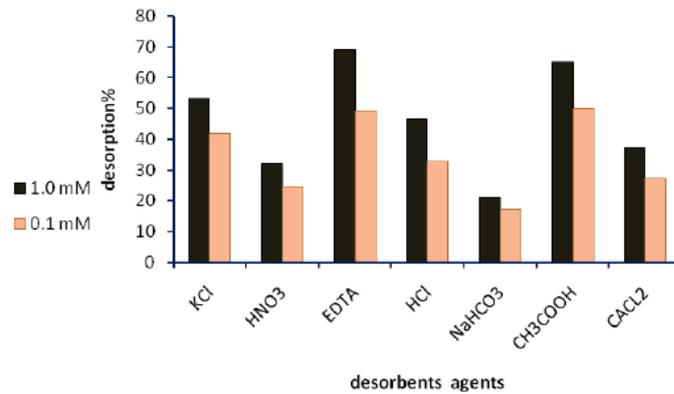


Fig. 4: Effect of different desorbing agents on percent desorption of Ni from *Bacillus sp.* (HNO₃, HCl, EDTA, CaCl₂, KCl, NaHCO₃ and CH₃COOH)

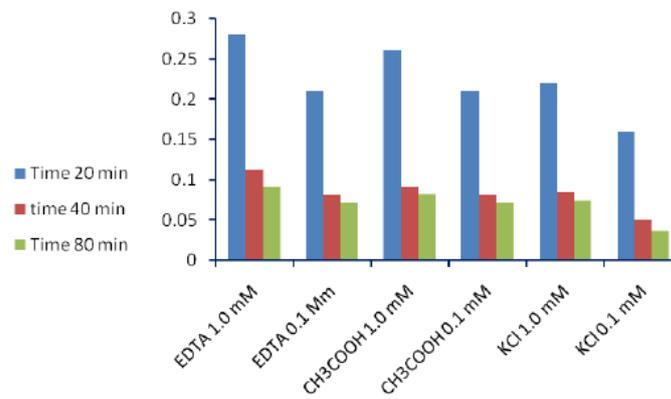


Fig. 5: Desorption of Ni²⁺ from *Bacillus sp.* by selected desorbing agents.

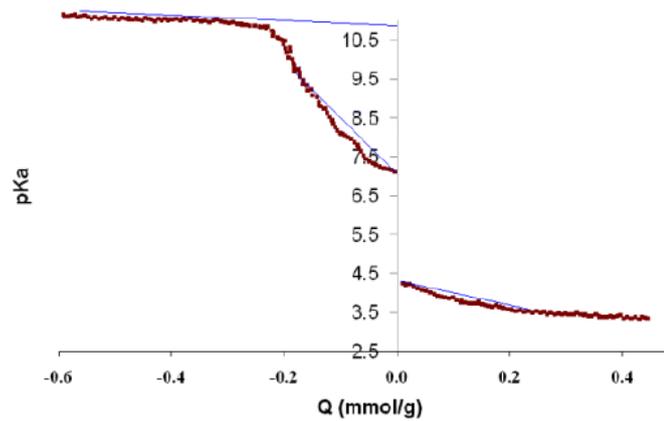


Fig. 6: Determination of pKa intrinsic by simple extrapolation (Stumm and Morgan 1996).

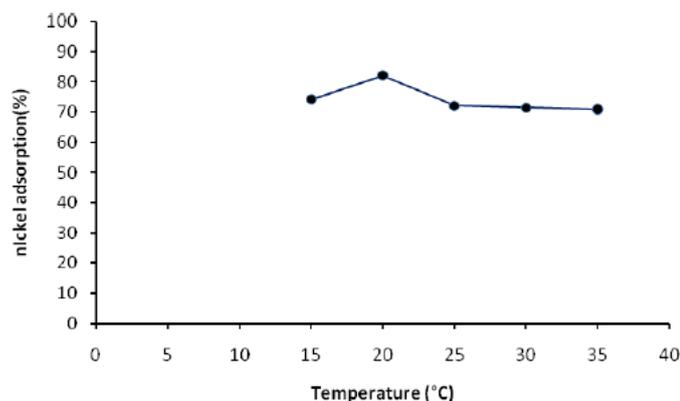


Fig. 7: Effect of temperature on nickel biosorption by *Bacillus sp* entrapped in calcium alginate.

3.5 Effect of Temperature:

Alginate immobilised biomass adsorption decreased with increase temperature (20-35°C) and maximum nickel removal was observed at 20°C, may be cause of physical adsorption, which is normally an exothermic process. (Fig.7)

Conclusions:

Biosorption of heavy metals ions by immobilized bacillus in packed bed column is an economically feasible technology for removing metal ions from solution. The behaves as an ion exchanger. Bacterial strain immobilized in calcium alginate gel matrix was found most effective in removing nickel ion from solution. Highest nickel uptake (88.54%) by selected biomass (3.00 g/l, dry wt) immobilized in 3% calcium alginate occurred at 30°C, 150 rpm when initial nickel concentration was 50 mg/l.

Acknowledgments

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