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Utilization in alginate beads for Cu(II) and Ni(II) adsorption of an exopolysaccharide produced by *Chryseomonas luteola* TEM05

Guven Ozdemir[1,*], N. Ceyhan[2] and E. Manav[3]
[1]*Faculty of Science, Department of Biology, Ege University, 35100 Izmir, Turkey*
[2]*Faculty of Science and Letters, Department of Biology, Mugla University, 48147 Mugla, Turkey*
[3] *Faculty of Engineering, Department of Bioengineering, Ege University, 35100 Izmir, Turkey*
**Author for correspondence: Tel.: +90-232-3884000-1519, Fax: +90-232-3881036, E-mail: gozdemir@sci.ege.edu.tr*

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Summary

Copper and nickel adsorption onto calcium alginate, sodium alginate with an extracellular polysaccharide (EPS) produced by the activated sludge bacterium *Chryseomonas luteola* TEM05 and the immobilized *C. luteola* TEM05 from aqueous solutions were studied. After that, the multi metal ions containing these ions together were prepared and partial competitive adsorptions of these mixtures were also investigated. The metal adsorption of gel beads were carried out at pH 6.0, 25 °C. The maximum adsorption capacities in Langmuir isotherm for calcium alginate, calcium alginate + EPS, calcium alginate + *C. luteola* TEM05 and calcium alginate + EPS + *C. luteola* TEM05 were 1.505, 1.989, 1.976, 1.937 mmol/g dry weight for Cu(II) and 0.996, 1.224, 1.078, 1.219 mol/g dry weight for Ni(II), respectively.

The competitive biosorption capacities of the carrier for all metal ions were lower than single conditions.

Introduction

Biosorption may be a suitable wastewater technology to remove heavy metals as demonstrated by several researchers because it is possible the use cheap adsorption materials that can be competitive with the conventional technologies (Volesky 1989; Kratochvil & Volesky 1998).

Biopolymers, which can be used for entrapping microorganisms, are known to bind metal ions (Lazaro *et al*. 2003). Thus far, isolated biopolymers for heavy metal remediation have not been applied on a large scale, although synthetic polymers have been used for various precipitation treatments. However, polyelectrolyte complexes formed by mixing polysaccharides of opposite charge, have recently attracted considerable attention because of their potential in various biotechnological applications (Hugerth *et al*. 1997; Jianlong & Yi 1999). It seems likely that incorporating exocellular polysaccharides or polyelectrolyte complexes into biofilter technology may provide applications for remediation, although much depends on the economics of such treatments (Gutnick & Bach 2000).

The main difficulty in using dead microbial biomass as a biosorbent is the small particle size and the low mechanical strength of the native biomass. It has been reported that biomass immobilisation into particles of a desirable size, mechanical strength and biosorptive characteristics is the best way to apply the process of biosorption for metal value recovery from process or waste solutions (Ferguson *et al*. 1989; Tsezos *et al*. 1989). Therefore, polysaccharide gel immobilized microorganisms can be used to remove heavy metal ions from aqueous solutions, providing an alternative to single dead microbial biomass for wastewater treatment (Sag *et al*. 1995; Veglio *et al*. 2002; Arıca *et al*. 2003). Natural polymers such as alginate, chitosan, chitin and cellulose derivates have been mostly used as the matrix for the immobilization of microbial cells via an entrapment technique. These polymers are also known to bind metal ions strongly. Entrapment of microbial cells in these polymer supports could also enhance microbial cell performance and adsorptive capacity of the biosorbent system for the heavy metal ions (Christ *et al*. 1994; Sag *et al*. 1995; Jianlong *et al*. 2000).

In this study, the use together with alginate of EPS as a new biomaterial for metal adsorbent was investigated. This choice has been dictated by the ability of microorganism to form a polysaccharide capsule, which is responsible for the binding and accumulation metal ions in the form of superficial mucilage layer. Since exocellular polysaccharide (EPS) plays a role in the increase of heavy metal adsorption capacity, the EPS, biomass and alginate mixtures were made into a gel bead and the adsorption of metal ions by these EPS-alginate gel beads was analyzed.

Materials and methods

Bacterial strain

A floc-forming bacterium used in this work was *C. luteola*. This strain was previously described by Ozdemir & Baysal (2004) and deposited in the Microbial Culture Collection of the Basic and Industrial Microbiology Section, in Department of Biology, Ege University, Turkey (Izmir), with the code TEM05.

Isolation of crude exocellular polysaccharide (EPS)

The strain was cultivated aerobically in 500 ml conical flasks containing sterile nutrient broth (Difco) on a rotary shaker (100 rev min^{-1}) at 30 °C. Cells were harvested at the end of exponential phase, i.e. after 48 h incubation. After cultivation, the culture of the most EPS producing bacterium was centrifuged at 10,000 g for 20 min at room temperature (25 °C) and supernatant liquid were then decanted in to three volumes of propan-2-ol, shaken vigorously and held at 4 °C for 4 h. Precipitated polysaccharide was freeze-dried to obtain a crude EPS preparation (Tago & Aida 1977; Ozdemir *et al*. 2003)

Sugar and protein contents of EPS

The sugar content was determined by phenol-H_2SO_4 method (Dubois *et al*. 1956); the protein content was determined by the Bradford method (Bolling & Edelstein 1991).

Alginate and alginate-EPS beads preparation and their characterization

Calcium alginate and calcium alginate-EPS beads were prepared by dropping 2% (w/v) aqueous solutions of sodium alginate by a peristaltic pump into 5% (w/v) $CaCl_2$ solution under magnetic stirring at 4–7 °C. The beads were stirred in this solution for 2 h. Successively; they were then collected by filtration, washed three times with distilled water and stored in a 2% (w/v) $CaCl_2$ solution at 4 °C. The second procedure is the same as described above except that 2% sodium alginate was replaced by mixture solution of 1.5% sodium alginate and 0.5% crude EPS.

Preparation of the microorganisms for biosorption

In this study, *C. luteola* TEM05 was cultivated aerobically in 500 ml conical flasks containing sterile nutrient broth (Difco) on a rotary shaker (100 revolutions per min) at 30 °C. Cells were harvested at the end of exponential phase, i.e. after 48 h incubation. After cultivation, the cells were centrifuged at 10,000 × *g* for 20 min for inactivation of the cells, the cultures were autoclaved (121 °C, 15 min) before harvested by centrifugation (10,000 g for 20 min at room temp.) and finally freeze-dried. 0.67% (w/v) of freeze-dried cells was resuspended in 2% Na-alginate or Na-alginate-EPS mixtures. Na-alginate-biomass or Na-alginate-EPS-biomass slurries were then extruded into 5% (w/v) $CaCl_2$ for polymerization and bead formation.

Biosorption studies

The biosorption of the metal ions on the alginate, alginate-EPS beads and on the immobilized *C. luteola* TEM05 from aqueous solutions was investigated in batch biosorption-equilibrium experiments. The biosorption time and the initial concentrations of heavy metal ions on the biosorption rate and capacity were studied.

The effect of the initial metal ions concentration on the biosorption was studied at pH 6.0 as described above except that the concentration of each metal ion in the adsorption medium was varied between 0.393 and 4.721 mM for Cu(II); 0.426 and 5.112 mM for Ni(II).

Competition in metal ion uptake

In order to ascertain whether there was any competition between the different metal ions for uptake by a particular biomass, multiple metal ion solutions were prepared. The experiment was experienced the almost same molar ratio (1.574 and 1.704 mM for Cu and Ni, respectively) of two competitive metal ions; so that where two metals were present the total metal concentration was 3.278 mM. The abilities of the biomasses to adsorb one metal were compared with the adsorption of that same metal where one was present in the same solution.

Analysis of metal ions

The concentration of unadsorbed Ni(II) and Cu(II) ions in the biosorption medium were determined by using a Varian SpectrAA-220 FS atomic absorption spectrometer (in flame mode). Air-acetylene flame was employed and the working currents/wavelengths for Ni(II) and Cu(II) ions were 4 mA/351.5 nm and 4 mA/249.2 nm respectively. Deuterium background correction was used.

Data treatment

During the biosorption, a rapid equilibrium is established between adsorbed metal ions on biosorbent (q_{eq}) and unadsorbed metal ions in solution (C_{eq}). This equilibrium can be represented by the Langmuir adsorption isotherm, which is widely used to analyze data for water and wastewater treatment applications.

The Langmuir equation which is valid for monolayer sorption onto a surface a finite number of identical sites and is given by Equation (1)

$$q_{eq} = \frac{Q^0 b C_{eq}}{1 + b C_{eq}} \quad (1)$$

where Q^0 is the maximum amount of the metal ion per unit weight of various preparations to form a complete monolayer on the surface bound at high C_{eq} (mmol g^{-1}) and b is a constant related to the affinity of the binding sites, Q^0(mmol g^{-1} dry weight) represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach its full saturation in experiments. Q^0 and b can be determined from the linear plot of C_{eq}/q_{eq} vs. C_{eq}.

A known quantity of wet Ca-alginate and Ca-alginate-EPS preparations was used in the adsorption test. After the adsorption process, the beads were dried in an oven at 50 °C overnight and the dry weight of the preparations was used in the above equation. Each experiment was repeated three times and the results given are the average values.

Results

Selection of microorganism

The most EPS producing bacterium selected for this study was a gram-negative bacterium, which was further identified as *Chryseomonas luteola* TEM05, which produces a non-diffusible yellow pigment.

Characteristics of extracellular polysaccharide and properties of the beads

Total sugar and total protein analysis indicated that was composed of 33% total sugar and 26% EPS total protein.

Alginate is a natural polymer and may be converted into hydrogels via crosslinking with divalent calcium ions. It was preferred over other materials because of advantages including biodegradability, hydrophilicity, presence of carboxylic groups, and natural origin. One of the most important disadvantages of cell immobilization is the increase in mass transfer resistance due to the polymeric matrix. From this point of view, alginate beads have advantages when compared with support materials such as polyvinyl alcohol and 2-hydroxylethylmethacrylate, because the presence of carboxylic groups in the alginate structure enhances heavy metal ions adsorption.

All beads were spherical shaped with approx. 2.8–3 mm.

Effect of pH on the biosorption capacity

PH of the biosorption studies was selected as 6.0 because the maximum biosorption of Cu(II) and Ni(II) on the immobilized biomass was observed at around pH 6.0. (Al-Saraj *et al.* 1999; Blanco *et al.* 1999; Yan & Viraraghavan 2001).

Biosorption time

The effect of the time of exposure of the beads to metals on the biosorption characteristics was investigated in a batch system. The measured concentrations were plotted as a function of time, as shown in Figure 1. Biosorption of metal ions was rapid during the first 30 min for Ni(II) and the first 80 min for Cu(II) and continued at a slower rate for the following several minutes and reached a saturation value after 90 min. After this period, the concentration of adsorbed metal ions did not significantly change further with time.

Statistically, there was no significant difference between preparations in case Cu(II) or Ni(II) uptake (ANOVA, $P > 0.05$). However, a significant difference was estimated between Cu(II) and Ni(II) uptake of these preparations ($P < 0.005$). From the Figure 1, it is evident that alginate beads adsorbed Cu(II) and Ni(II) ions far less than that of EPS-alginate, alginate-biomass and alginate-EPS-biomass beads. Note that in such a biosorption process, there are several parameters that determine the biosorption rate, including structural properties biosorbent (e.g. protein and carbohydrate composition and surface charge density, topography and surface area). All these studies published in the literature have been carried out under different experimental conditions (Sag *et al.* 1995; Saglam *et al.* 1999; Say *et al.* 2001).

Effects of initial concentration of metals ions on the biosorption capacity

The biosorption capacities of all the beads are given in Figure 2 as a function of the initial concentrations of Cu(II) and Ni(II) ions within the aqueous phase, respectively. The biosorption capacities of alginate beads were near to that of the other beads. The amount of adsorbed metal increased with the initial metal concentration in the solution and reached a saturation value (3.147 mmol for copper (as an exception, alginate-copper; 2.36 mmol) and 3.408 mmol for nickel).

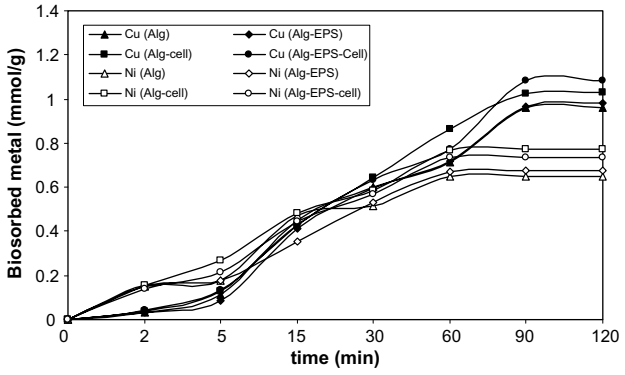

Figure 1. Biosorption times of Cu(II) and Ni(II) ions on biosorbents from aqueous solutions at pH 6.0, at 100 rev/min, at 25 °C and initial concentration of 1.57 mM of Cu(II) and 1.70 mM Ni(II).

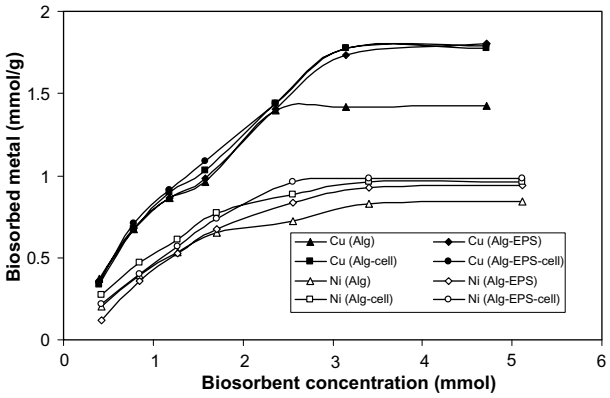

Figure 2. Biosorption was carried out at pH 6.0, at various concentrations of Cu(II) (0.39–4.72 mM) and Ni(II) (0.43–5.11 mM), at 100 rev/min and at 25° for 120 min.

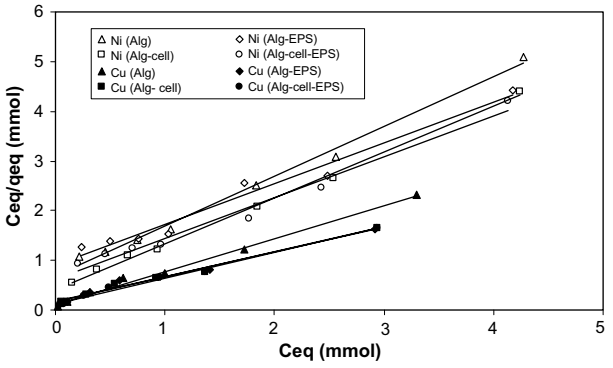

Figure 3. The linearized Langmuir adsorption isotherms of Cu(II) and Ni(II) by various preparations.

It probably means that the cross-linking of potential metal binding-sites among microbial biomass, EPS and alginate gel occurred.

Bioremediation studies have been conducted using microbial polysaccharides and inactivated microbial cell systems from contaminated waters by several researchers (Fourest & Volesky 1997). In the systems of natural polysaccharides used for removal of heavy metal ions from industrial wastewater, the metal removal process is based on solid-liquid contacting and separation process. Such preparations offer advantages in terms of mechanical strength and durability, handling and ease of scale up. On the other hand, all these adsorbents are expensive and required several preparation steps.

The metal removal were 1.427, 1.801, 1.777, 1.787 mmol for 4.721 mmol copper; 0.838, 0.941, 0.961, 0.982 mmol for 5.112 mmol of nickel per g alginate, alginate-EPS, alginate-biomass and alginate-EPS-biomass, respectively. The magnitude of changes in metal ion binding capacity of *C. luteola* TEM05 may due to the properties of the metal sorbates (e.g. ionic size, atomic weight, or reduction potential of the metal) and the properties of the bacterium (e.g. structure, functional groups, and surface area). Capsules and slime layers of bacteria contain polysaccharides as basic building blocks, which have ion exchange properties, and also proteins and lipids and therefore offer a host of functional groups capable of binding to heavy meals. These functional groups such as amino, carboxylic, sulphydryl and phosphate groups differ in their affinity and specificity for metal binding (Nourbakhsh *et al.* 1994).

Table 1. Isotherm model constants for adsorption of copper and nickel on *C. luteola* TEM05.

Metal ion Biosorbent type	Langmuir adsorption isotherm		
	Q^0	b	R^2
Cu(II) (Alg)	1.505	5.926	0.993
Cu(II) (Alg-EPS)	1.989	2.001	0.977
Cu(II) (Alg-cell)	1.976	3.261	0.989
Cu(II) (Alg-EPS-cell)	1.937	4.180	0.991
Ni(II) (Alg)	0.996	1.470	0.993
Ni(II) (Alg-EPS)	1.224	0.895	0.972
Ni(II) (Alg-cell)	1.078	2.295	0.997
Ni(II) (Alg-EPS-cell)	1.219	1.309	0.980

Langmuir adsorption isotherms

Figure 3 shows the Langmuir plots for Cu(II) and Ni(II) biosorption by beads of Ca-alginate, Ca-alginate-EPS, Ca-alginate-cell and Ca-alginate-EPS-cell. The Langmuir constants (Q^0 and b) along with correlation coefficients (R^2) have been the plots (Figure 3) for biosorption of Cu(II) and Ni(II) on the biosorbents and the results are presented in Table 1.

Competitive biosorption

Competitive biosorption of Cu(II) and Ni(II) ions were also studied. The medium containing 1.573 mmol Cu(II) and 1.704 mmol of Ni(II) was incubated with the biomass in batch fashion. The Competitive biosorption capacities were 0.551, 0.618, 0.605, 0.628 for Cu(II); 0.356, 0.369, 0.374, 0.389 mmol for Ni(II) per g alginate, alginate-EPS, alginate-biomass and alginate-EPS-biomass, respectively. As seen in Figure 4, the Competitive

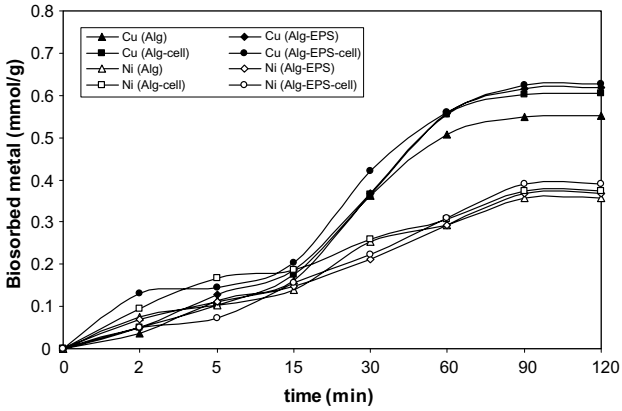

Figure 4. Competitive biosorption times of Cu(II) and Ni(II) ions on biosorbents from aqueous solutions at pH 6.0; at 100 rev/min, at 25 °C and at initial concentration of 1.57 mM of Cu(II) and 1.70 mM Ni(II).

biosorption capacities of the beads for all metal ions were lower than non-competitive conditions. Observation of the figure confirms that copper ions are the most effectively sequestered by the four biomass compared with nickel ions. The order of affinity for competitive conditions was as follows (based on a μmol accumulation): Cu(II) > Ni(II). This order is the same as in the non-competitive condition.

Conclusions

The aim of this work was to find the biosorption characteristics of selected a new biomaterial against to heavy metals for the removal of copper and nickel ions. Experiments were performed as a function of pH, initial metal ion concentration and time. The obtained results showed that the Ca-alginate-EPS beads improved performance in the batch system for the treatment of wastewater containing copper and nickel ions.

The equilibrium was well described by the Langmuir adsortion isotherm for the mathematical description of the biosorption of copper and nickel ions to EPS and the isotherms constants were evaluated to compare the biosorptive capacity of EPS for metal ions.

Consequently, the use of biomaterial biosorption then may provide an attractive alternative to use of conventional ion exchange resins. However, biomaterial biosorption technologies are still being developed and much more work is required.

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