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Utilization of an exopolysaccharide produced by *Chryseomonas luteola* TEM05 in alginate beads for adsorption of cadmium and cobalt ions

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Abstract

Cadmium and cobalt adsorption from aqueous solution onto calcium alginate, sodium alginate with an extracellular polysaccharide (EPS) produced by the activated sludge bacterium *Chryseomonas luteola* TEM05 and immobilized *C. luteola* TEM05 was studied. In addition, solutions containing both of these ions were prepared and partial competitive adsorption of these mixtures was investigated. Metal adsorption onto gel beads was carried out at pH 6.0 and 25 °C. The maximum adsorption capacities determined by fitting Langmuir isotherms to the data for calcium alginate, calcium alginate + EPS, calcium alginate + *C. luteola* TEM05 and calcium alginate + EPS + *C. luteola* TEM05 were 45.87, 55.25, 49.26, 51.81 mg g^{-1} for Co(II) and 52.91, 64.10, 62.5, 61.73 mg g^{-1} for Cd(II), respectively.

The biosorption capacity of the carrier for both metal ions together in competition was lower than those obtained when each was present alone.

Keywords: *Chryseomonas luteola*; Biosorption; Cadmium; Cobalt; Extracellular polysaccharide; Alginate

1. Introduction

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

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. It seems likely that incorporating polysaccharides into biofilter technology may provide applications for remediation, although much depends on the economics of such treatments (Gutnick and Bach, 2000).

On the other hand, 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 with desirable size, mechanical strength

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and biosorption 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 dead microbial biomass alone for wastewater treatment (Arica et al., 2003; Sag et al., 1995; Veglio et al., 2002). Most studies involve the removal of only one kind of metal ion by microorganisms from aqueous solutions. However, the presence of only one kind of heavy metal ion is a rare situation either in nature or in wastewaters. When two or more metal ions are present in solution together, they may increase, decrease or leave unchanged the metal ion adsorption capacity of microorganisms and immobilized microorganisms (Nourbakhsh et al., 2002).

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

2. Methods

2.1. Bacterial strain

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

2.2. 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 bacterial culture producing the most EPS was centrifuged at 10,000*g* for 20 min at room temperature and the supernatant liquid was then decanted into 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 and Aida, 1977; Ozdemir et al., 2003).

2.3. 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 with Coomassie blue (Bradford, 1976).

2.4. Alginate and alginate–EPS beads preparation

Calcium alginate and calcium alginate–EPS beads were prepared by using a peristaltic pump to drop 2% (w/v) aqueous solutions of sodium alginate into 5% (w/v) $CaCl_2$ solution under magnetic stirring at 4–7 °C. The pH of these solutions was 7.0. The beads were stirred in the mixed solution for 2 h. 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 was the same as that described above except that 2% sodium alginate was replaced by an aqueous solution of 1.5% sodium alginate and 0.5% crude EPS.

2.5. 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 rpm) at 30 °C. Cells were harvested at the end of exponential phase, i.e. after 48 h incubation and centrifuged at 10,000*g* for 20 min. For inactivation of the cells, the cultures were autoclaved (121 °C, 15 min) before being harvested by centrifugation (10,000*g* for 20 min at room temp.) and finally freeze-dried. Freeze-dried cells were resuspended in 2% Na-alginate or Na-alginate–EPS mixtures at a concentration of 0.67% (w/v). Na-alginate–biomass or Na-alginate–EPS–biomass slurries were then extruded into 5% (w/v) $CaCl_2$ for polymerization and bead formation (Sag et al., 1995; Blanco et al., 1999).

2.6. Biosorption studies

Stock solutions (2000 ppm) of cadmium and cobalt were prepared by dissolving analytical grade salts ($CdCl_2 \cdot H_2O$, $CoNO_3 \cdot 2H_2O$) supplied by Merck (Darmsradt, Germany). The salts were dissolved in distilled deionized water. Sorption experiments were carried out in 250-ml Erlenmeyer flasks. 0.1 g (dry weight) of alginate, alginate–EPS, alginate–cell or alginate–EPS–cell beads were added to 100 ml of cadmium or cobalt solution of known initial concentration and the solution was stirred continuously at 100 rpm, at pH 6.0 and 25 ± 2 °C. 0.1 N HCl or 0.1 N NaOH was used for pH adjustment. Experiments were carried out in an incubator at temperatures ranging from 20 to 40 °C. The time intervals for metal sorption were 2, 5, 15, 30, 60, 90 and 120 min. The effects of the initial concentrations of Cd(II) and Co(II) ions were studied at pH 6.0 as de-