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Biosorption and desorption studies of chromium (III) by free and immobilized *Rhizobium* (BJVr 12) cell biomass

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Abstract

Experiments with free cell biomass (cells + exopolysaccharides) of *Rhizobium* BJVr 12 (mungbean isolate) showed that amount of Cr^{3+} ion sorbed is influenced by the amount of biomass to Cr^{3+} concentration ratio and time of contact. A ratio of 0.5 g fresh biomass to 10.0 ml 5.03 ppm Cr^{3+} sorbed 0.0275 mg Cr equivalent to an uptake of 2.86 mg Cr g-^{1} dry biomass and 1.0 g: 10.0 ml sorbed 0.0366 mg Cr equivalent to an uptake of 1.9 mg Cr g-^{1} biomass. Immobilized cell biomass in ceramic beads and in aquacel (a porous cellulose carrier with a charged surface) were more efficient than free cell biomass in adsorbing Cr(III). A reduction of 49.7% of Cr(III) for free cells, 95.6% for cells immobilized in ceramic beads and 94.6% for cells in aquacel was achieved after 48 hours under shaken conditions. Sorption capacities of immobilized cell biomass in ceramic beads and aquacel ranged from 5.01 to 5.06 mg Cr g^{-1} dry cell biomass. The biosorption of Cr^{3+} follows generally the Langmuir and Freundlich models of adsorption at low Cr^{3+} concentrations. The Langmuir constant for immobilized cells in ceramic beads are: Q_0, 0.065 mmol Cr g^{-1} biomass; b (affinity constant), - 694 l $mmol^{-1}$ Cr and for cells in aquacel Q, 0.07 mmol Cr g^{-1} biomass; b, - 694 l mmol Cr g^{-1} Cr. The Freundlich constants are: K, 0.071 mmol Cr g^{-1} biomass; n, 0.13 g^{-1} biomass l^{-1} and for aquacel: K, 0.074 mmol g^{-1} biomass; n, 0.13 g^{-1} biomass. Biotraps made up of immobilized cells in ceramic beads and aquacel were tested for adsorbing Cr(III) using two different flow rates: 0.5 ml/min and 1.5 ml/min. A significantly higher amount of Cr(III) was adsorbed at the lower flow rate of 0.5 ml/min.

Biosorption of Cr^{3+} is competitive. The treatment of a waste water sample containing 6.03 ppm Cr^{3+} and other cations with the biomass reduced the Cr^{3+} concentration to that much lower than for the test solution containing only Cr. Recovery of biosorbed Cr(III) was by treatment at a different pH using dilute HCl solution. Recovery was higher for cells imbibed in ceramic beads than aquacel. Percentage recoveries for cells in aquacel are 46.4% at pH 1.0, 33.0% at pH 3.0 and 6.6% at pH 6.0–7.0. For cells in ceramic beads, percentage recoveries are: 93.1% at pH 1.0, 75.6% at pH 3.0 and 16.4% at pH 6.0–7.0. Biosorption of Cr^{3+} by cells immobilized in ceramic beads is reversible but only partially for cells in aquacel.

Introduction

Bioremediation of toxic metals by bacterial biosorption as an alternative technology to chemical speciation for the metal removal of industrial and mining waste has received much attention recently [1, 2]. The amount of metals biosorbed by microbial biomass may vary from a few micrograms g^{-1} to several percentages of the cell dry weight. Studies on *Zoogloea* sp. which produces extensive capsular polymers showed metal accumulations of 0.25 g Co^{2+}, 0.34 g Cu^{2+} and 0.13 g Ni^{2+} g^{-1} dry weight biomass [3, 4].

Zoogloea ramigera grown in sewage sludge was found to contain 25% by weight of metals [5]. Brown and Lester [6] reviewed the physical entrapment of precipitated metals by the polymer matrix produced by *Zoogloea*.

Nitrogen fixing bacteria which produces capsular exopolysaccharides such as *Azotobacter vinelandii* and *Derxia gummosa* have also been found to sorb metals

from solutions [7]. Rhizobial exopolysaccharides have been studied extensively for their role in plant-host specificity [8] but only recently have their metal sorption capacity been investigated. Douka and Xenoulis [9] reported the significant reduction of radioactive metal concentration of nodulated pasture legumes after the Chernobyl radioactive fallout. Cotoras et al. [7] found that *Rhizobium trifolii* (4 g) can reduce the UO_2^{2+} content by 60% in a 0.4 mM solution. *Rhizobium* isolates from *Leucaena leucocephala* and soybean (*Glycine max*) were able to tolerate and grow in a culture medium containing a concentration Pb^{2+} as high as 30 ppm. Transmission electron micrographs of *Rhizobium* grown in 30 ppm Pb^{2+} revealed the total coverage of the cell surface by a dense dark layer of Pb^{2+} [10].

Thirty-two *Rhizobium* isolates from different legume host plants and trees in Australia, Thailand, Japan, and the Philippines were tested for tolerance to mercury, copper, and cadmium. Two strains from mungbean (*Vigna radiata*), BJVr 12 and BJVr 7 and 3 strains from *Leucaena leucocephala* were found to be quite tolerant to a concentration as high as 50 ppm Hg [11].

In this paper, Cr(III) is chosen because of its industrial importance and the very toxic nature of its oxidized form, Cr(VI). Chromium is a silver-white metal, hard, brittle and rarely found in the free state in nature. The principal source of Cr(III) is the mineral chromite (MgFe) O (Cr, Al, Fe)$_2O_3$. Cr and its compounds are widely used in the manufacture of steel, as an electroplated coating for corrosion control; as a mordant in the textile industries; as anti-corrosive agents in the tanning industry; for the manufacture of catalysts, pigments and paints; for fungicides and wood preservatives; and in anoding aluminum in the aircraft industry. Hexavalent Cr(CrO_4^{-2}, $Cr_2O_7^{-2}$) are strong oxidizing agents.

Just like other metals, Cr is beneficial in trace amounts to humans, animals, plants and microorganisms. However, at higher concentrations Cr is detrimental to health. Cr(VI) compounds are more toxic than Cr(III). Cr^0 (metal) is stable and relatively nontoxic. Chronic inhalation and excessive ingestion of Cr(VI) produce respiratory problems including perforated or ulcerated nasal septa and lung tumor, liver and kidney damage and internal hemorrhage. Subchronic and chronic dermal exposure to Cr(VI) may cause contact dermatitis and skin disorders. Cr(VI) is a carcinogen whose mechanism of action may involve the catalysis of free radical reactions and cross linkage of DNA [12]. The acceptable upper limit of Cr(VI) in water is 0.05 mg/l [13].

This study investigates the amount of Cr(III) biosorbed by *Rhizobium* BJVr12, and isolate from mungbean nodules, which produces large amounts of mucilaginous polysaccharides and is tolerant to heavy metals such as Hg and Cd [11]. Studies on the biosorption of Cr(III) by free and immobilized cells in ceramic beads and aquacel, desorption, and percentage recoveries were also conducted.

Materials and methods

Microbial strain

Rhizobium BJVr 12, a nitrogen fixing bacteria isolated from the nodules of mungbeans (*Vigna radiata* w.) which produces copious amounts of viscous exopolysaccharides was used in these studies. This strain can be obtained from the BIOTECH Microbial Culture Collection.

Culture media

Rhizobium BJVr 12 was maintained on slants of Yeast Extract Mannitol Agar (YEMA). Cell biomass production used YEMA and Yeast Extract Mannitol Broth (YEMB) as culture media [14].

Preparation of seed culture and growth curve

250 ml flasks containing 100 ml YEMB were inoculated with a loopful of the *Rhizobium* strain and incubated for three days at ambient room temperature while being regularly shaken. This constituted the seed culture.

A preliminary growth curve was determined for 10 days to determine the period of maximum growth based on the production of cell dry weight, total plate count per day and maximum production of polysaccharides (precipitation with 95% ethanol at 3× the volume of the culture medium) followed by filtration, drying and weighing. Maximum growth and polysaccharide production was after six days of incubation at 28 °C.

Mass production of cell biomass

Flat bottles containing 20 ml YEMA were inoculated with 2 ml of a four day old pre-culture broth. A glass rod spreader was used to facilitate an even cell growth on the flat agar surface. The cell biomass was harvested

after six days of incubation at ambient room temperature using a spatula to scrape off the viscous mass taking precautions that no media material was included. Fresh cell biomass was used in all the experiments. The equivalent dry weight of the fresh biomass was determined (a 1.0000 g fresh cell biomass was oven dried to constant weight at 105 °C and the percentage water was calculated).

The production of cells in broth culture was done in 10 ml of YEMB contained in 18 × 150 mm test tubes and inoculated with 1 ml seed culture, and incubated for 6 days at ambient room temperature while being continually shaken (120 strokes min^{-1}).

Microbial carriers

Porous ceramic beads were used to imbibe the *Rhizobium* cells. The average weight of one bead was about 0.26 g. Preliminary experiments were conducted to determine the weight of dry ceramic beads that can support 0.5 ± 0.01 g cell biomass (fresh weight). 30 beads can hold approximately this amount of weight.

Aquacel was also used as a cell carrier. It has a porous cellulose structure with a charged surface. The CU type aquacel is cubic shaped with a diameter of 5 mm and 100 μm pore size. The average weight of 5 pieces of aquacel, 0.0603 g can immobilize 0.5 ± 0.01 g cell biomass. The aquacel was supplied by Dr. Matsumura of the Fermentation Engineering Department University of Tsukuba, Japan.

Chromium (III) solutions

A 50 ppm Cr(III) stock solution was prepared by dissolving Analytical grade $CrCl_3 \cdot 6H_2O$ crystals in distilled water. Five ppm Cr(III) was prepared from the stock solution by dilution. The actual concentration of the Cr(III) solution used in the experiment was 5.03 ppm.

The waste water sample containing Cr(III) came from the liquid wastes of the Analytical Chemistry Division of the Institute of Chemistry, College of Arts and Sciences, UPLB. The waste water sample was diluted 10, 10^{-1}, 10^{-2}, 10^{-3}. The Cr(III) concentration of the waste water sample used in the experiments was 6.05 ppm.

Cr(III) adsorption isotherm of R. *BJVr 12 exopolysaccharides*

The exopolysaccharides produced by the treatment of the culture suspension with 95% ethanol at 3× the volume was treated with acetone and air dried. The dry polymer was weighed (7 to 15 mg) and treated with 50 μl of water per mg and allowed to swell to form a firm and solid gel and cut into 2 mm^3 cube.

Two ranges of initial Cr^{3+} concentration were used at a low concentration of 10 ppm and at a high concentration of 100 ppm. The gels were equilibrated with 15 ml of the Cr solution in an L-tube (10 cm × 12.5 cm). The L-tube was clipped in place by clamps in the L-tube shaker and immersed in water at 25 °C and agitated gently in a continuous up and down motion for 24 h. After equilibrium at 24 h, the gel-Cr solution was filtered through a disposable syringe filter unit (Dismic 25, cellulose nitrate, 0.20 μm, hydrophilic, pressure limit: 5.3 kg/in^2) before Cr^{3+} analysis.

Biosorption of Cr(III) by free cells

Two ratios of free cell suspensions were prepared: 0.5 g : 10 ml and 1.0 g : 10 ml 5.03 ppm Cr(III) solution. The cell suspensions were shaken at different periods of time: 0, 2, 24, 72, 96 h. The cells were removed from the suspension by centrifugation (14,000 rpm, 10 °C) for 30 min. The high speed and longer time of centrifugation as compared to the usual 10,000 rpm for 10 minutes were to minimize particulate contamination of the supernatant since no filtration of the supernate was done. The supernatant was then analyzed for its Cr(III) content for each period of contact. The variability of the cells after each treatment period was not monitored. The Cr^{3+} biosorbed was determined by subtracting the Cr concentration of the supernatant from the initial Cr concentration of the contacting test solution. Control experiments used only the Cr test solutions without the cell biomass and were run at the same time as those treated with the cell biomass. All biosorption experiments were run in triplicates.

Biosorption of Cr(III) by immobilized cell biomass

Cells in ceramic beads

The weight of cell biomass used in all experiments was within 0.5 ± 0.01 g. The weight of cell biomass immobilized by the carrier was determined by subtracting the weight of the carrier from the total weight of carri-

er and cell biomass, and some surface adsorbed media components (the immobilized cells were not washed). An average weight of 7.305 g (30 pieces of ceramic beads) and 0.0603 g (5 pieces) were determined by preliminary experiments to immobilize 0.5 ± 0.01 cell biomass.

Thirty pieces of dry beads (7.305 g) were soaked overnight in 10 ml of the cell culture at ambient room temperature to allow the imbibition of the cells into the pores of the beads. The excess culture broth was removed by sorption with a cotton plug. The net weight of cells imbibed was 0.4935 g. The ceramic beads with the imbibed cells were mixed with 10 ml of 5.03 ppm Cr(III) solution and agitated for 0, 2, 24, 48, 72, and 96 hours. The Cr(III) content after each period was analyzed and the percentage Cr(III) reduction calculated.

Cells in aquacel

Five pieces of aquacel cubes (0.0603 g) were soaked in 10 ml of cell culture following the same procedure as that for the imbibition of cells by ceramic beads. The net weight of cells imbibed in aquacel was 0.4940 g.

The same procedure for the biosorption of Cr(III) experiments of cells in beads was followed for experiments of cells in aquacel.

The same volume of 5.03 ppm Cr test solution (experimental conditions of 120 strokes min^{-1}, ambient room temperature, same time intervals) as those for the biosorption experiments with the free cell biomass of 0.5 ± 0.01 g^{-1} were used. All biosorption experiments were run in triplicate.

Control experiments used only beads or aquacel without cell biomass and were run at the same time as the samples.

Biosorption of Cr(III) by immobilized cells in columns (biotraps)

The biotraps were prepared using a plastic column (1.5 cm [diameter] $\times$ 6.0 cm [height]) and contained immobilized cells in beads (0.4935 g cells) and another set containing immobilized cells in aquacel (0.4940 g cells).

30 ml of 5.03 ppm Cr(III) solution were passed through the biotraps at two flow rates: 0.5 ml/min and 1.5 ml/min. Fractions of 3 ml were collected and analyzed for their Cr(III) content. Control experiments used columns containing only beads or aquacel without cell biomass. All experiments were run in triplicate.

Biosorption of Cr(III) from a waste water sample

Waste water containing 6.05 ppm Cr(III) and other metal ions and cations were treated with the same weight of free and immobilized cell biomass under the same experimental conditions as that for the Cr test solution. The Cr(III) content of the waste water sample was analyzed after each biosorption treatment. The Cr test solution had a higher pH of 6.8 and a lower ionic strength than the waste water sample.

Desorption of biosorbed Cr(III)

10 ml of dilute HCl solution of pH 1.0, pH 3.0 and deionized water, pH 6.0–pH 7.0, were passed through the columns of immobilized cells in ceramic beads and in aquacel containing sorbed Cr^{3+} of known amount at a flow rate of 0.5 ml min^{-1}. The eluent from the columns were analyzed for their Cr(III) content.

Determination of Cr(III) concentration of samples

The Cr(III) concentration of the samples were determined by Atomic Absorption Spectroscopy (AAS) using a Model Perkin Elmer 5000 Atomic Absorption Spectrophotometer with a multi-element cathode lamp. The analytical parameters were: wavelength, 357.9 nm; lamp, 130 ma; slit 0.7 nm, program 1A; energy, 67; time, 0.5 sec, oxidant (air), 30 lb./sq.in; fuel (acetylene), 10 lb./sq.in. Direct absorbancies were determined. Cr(III) concentrations of 0, 0.1, 0.3, 0.5, 1.0, 3.0, 6.0 ppm were used to prepare the standard calibration curve. All samples were acidified with concentrated HCl prior to analysis.

The Cr concentration of the adsorption isotherm experiments were analyzed using a Nippon Jarrel-Ash Inductively Coupled Argon Plasma Atomic Emission Spectrophotometer (ICAP-757v).

Results and discussions

Biosorption of Cr(III) experiments

For the Cr adsorption isotherm experiments, no Cr was detected in the filtrate after treatment of the test solution with the rhizobial exopolysaccharide after 24 h indicating an almost 100% Cr removal at 10 ppm initial Cr concentration. The Cr biosorbed at 10 ppm was 13.28 mg Cr g^{-1} dry adsorbent or 0.25 mmol g^{-1} dry

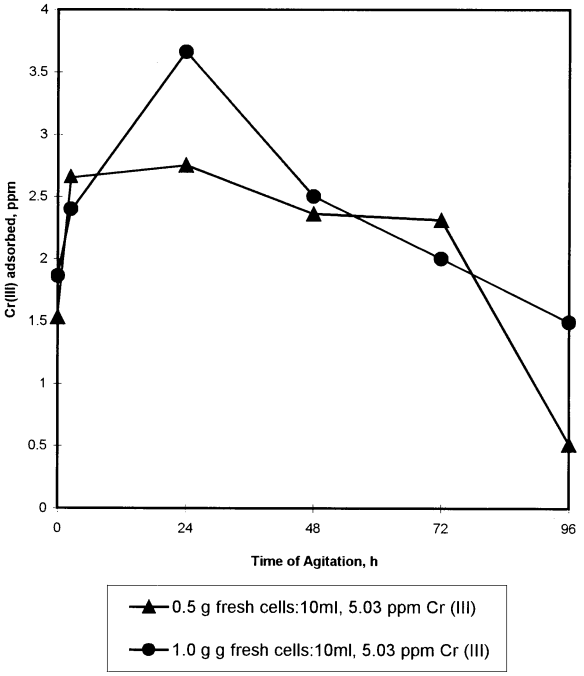

Figure 1. Biosorption of Cr(III) by free *Rhizobium* cells at different periods of time under shaken conditions.

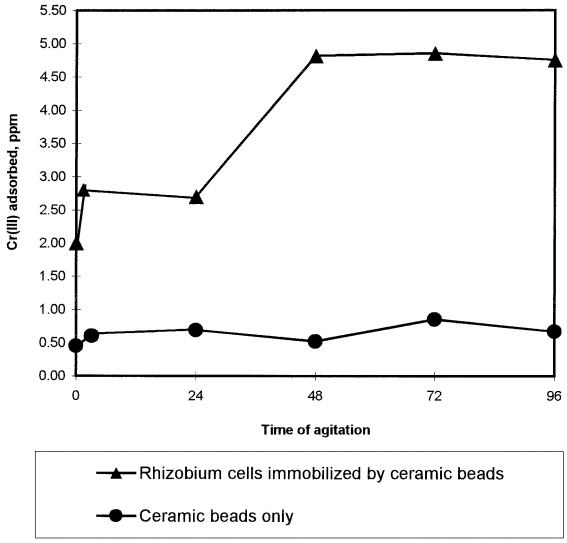

Figure 2. Biosorption of Cr(III) by *Rhizobium* cells immobilized by ceramic beads at different periods of time under shaken conditions.

adsorbent, and at 100 ppm Cr, 16.15 mg Cr g^{-1} dry adsorbent.

The sorption of Cr^{3+} ions at two ratios of fresh cell biomass to a volume of a 5.03 ppm Cr^{3+} solution: 0.5 g : 10 ml and 1.0 g : 10 ml at different time periods under the same experimental conditions are shown in Figure 1. The sorption of Cr^{3+} by free cell biomass was instantaneous upon contact of Cr^{3+} ions with the biomass. Equilibrium at saturation was achieved within 2 h for the lower ratio. Sorbed Cr^{3+} was released with prolonged contact and agitation (after 48 h) gradually until equilibrium between sorption and desorption was stabilized between 48 h and 72 h. The release of sorbed Cr^{3+} after 24 h may have been due to the release of entrapped Cr^{3+} in the spaces of the cell aggregates. With prolonged agitation, the cell aggregates broke up to form smaller and more stable cell aggregates thus a saturation plateau was established until the cell structures deteriorated and weakened with starvation and prolonged mechanical agitation to cause a rapid release of sorbed Cr^{3+} after 96 h. A reduction of 54.7% Cr^{3+} was attained after 24 h, equivalent to 2.86 mg Cr^{3+} g^{-1} biomass.

For the higher ratio, a higher total uptake of Cr was attained due to the higher amount of biomass which offered more binding sites for the Cr^{3+} ions. No saturation equilibrium was established between 2 h and 24 h. Since no additional experimental data was obtained during this period, we can only surmise that the saturation equilibrium may have been achieved within this time frame. The release of sorbed Cr^{3+} was greater than that for the lower ratio which may be due to more cell aggregation which entrapped more Cr^{3+}, and as the cell aggregates broke up they released more trapped Cr^{3+}. Since we are dealing with a biological system, desorption of sorbed Cr^{3+} increases when cell and polymer structure start to break down due to prolonged mechanical agitation and lack of nutrients to promote growth. After 96 h, there was relatively much less sorbed Cr released at the higher mass ratio which indicated less physical degradation: this may be due to the cushioning effect at high cell biomass concentration. Further studies are needed to determine the time at which the saturation equilibrium is established and the gradual release of sorbed Cr^{3+} at higher biomass concentration. A total reduction of 72.8% Cr was obtained and an uptake of 1.9 mg Cr^{3+} g^{-1} biomass for the higher ratio of biomass to volume of Cr test solution. The higher value of Cr^{3+} uptake for the lower ratio may be due to more cell surface exposed for Cr sorption per unit weight biomass.

The possible binding sites for Cr sorption are the anionic groups sticking out of the bacterial cell walls and their associated polysaccharides. The bulk of metal

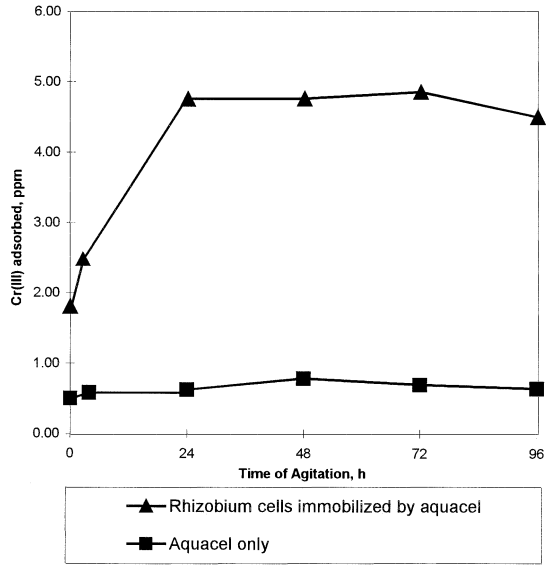

Figure 3. Biosorption of Cr(III) by *Rhizobium* cells immobilized by aquacel at different periods of time under shaken conditions.

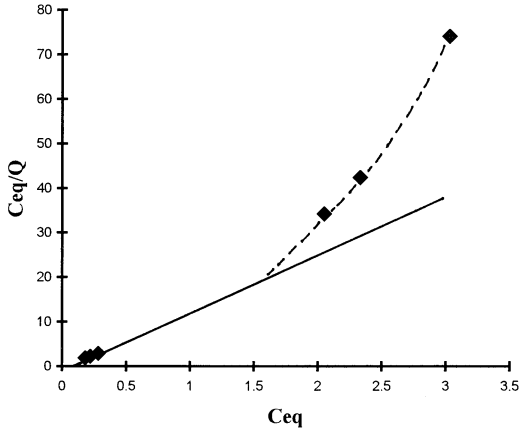

Figure 4. Langmuir model for the adsorption of Cr(III) ions by *Rhizobium* cells immobilized by ceramic beads (ambient room temperature).

removal is through biosorption by extracellular polymers. These polymers have anionic sites which hold the metals by chelation and electrostatic forces. The major binding sites in the cell wall surface are generally carboxyl (COO^-), hydroxyl (OH^-), sulfhydryl (SH^-), and amino (NH_2^-) and phosphate (PO_4^{3-}), (HPO_4^{2}-), ($H_2PO_4^-$) groups which are exposed to the metal solution [16].

Other *Rhizobium* strains have been reported to produce both neutral and anionic exopolysaccharides [8, 17]. Infra-red analysis of the exopolysaccharides of *R.* BJVr 12 showed the presence of carboxyl, hydroxyl and amino groups in the polymer structure (unpublished data of J.C. Mamaril).

Biosorption of Cr(III) by immobilized cell biomass

The biosorption of Cr^{3+} by the *R.* BJVr 12 cell biomass immobilized in ceramic beads (Figure 2) and in aquacel (Figure 3) were significantly higher than that of free cell biomass (Figure 1) for 0.5 ± 0.1 g biomass under the same experimental conditions of cell culture, age, volume of 5.03 ppm Cr^{3+} test solution, pH, ionic strength, temperature, and rate of agitation. Maximum sorption of Cr^{3+} ions by immobilized biomass in ceramic beads occurred after 48 h which caused a 96.4% Cr reduction of 5.03 ppm Cr^{3+} test solution. The equilibrium between sorption and desorption of Cr^{3+} (saturation plateau) was maintained even after 96 h indicating greater stability and resistance to the chemical environment of the immobilized cells.

At the start of the experiment, the sudden jarring and agitation of the beads with the immobilized cell biomass may have loosened the adhesion of the weakly bound biomass. A period of destabilization occurred in the initial hours of contact with the Cr test solution. Adhesion of the loosely bound cells to the beads needs some time for stabilization and this may have caused the lag phase for sorption and desorption of Cr^{3+} to come to equilibrium. Entrapped Cr^{3+} by cell aggregates may have also been released at this time. After this period, the anchorage of the biomass to the ceramic beads and cell aggregation stabilized leading to an equilibrium of Cr^{3+} sorption and desorption.

The lag phase for reaching an equilibrium between sorption and desorption was lower for the immobilized biomass in aquacel. The saturation equilibrium of Cr sorption by the cell biomass may have been attained between 2 h and 24 h. The maximum Cr sorption was at 96.4% reduction of Cr concentration of the test solution. The saturation plateau was maintained for 48 h. A small net release of sorbed Cr was noted after 96 h which may be due to the deterioration of the cell, polysaccharide and carrier structure at prolonged agitation. The lower phase lag of immobilized biomass in aquacel may be due to the organic nature of the aquacel carrier compared to the inorganic ceramic bead carrier which caused more cell biomass adhesion and stronger bonding per unit weight of cell biomass. The greater number of cells in aquacel than in beads may be due to differences in their structures. Aquacel

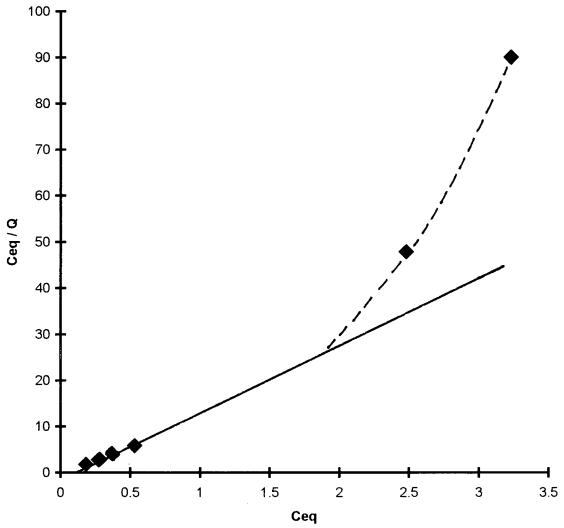

Figure 5. Langmuir model for the adsorption of Cr(III) ions by *Rhizobium* cells immobilized by aquacel (ambient room temperature).

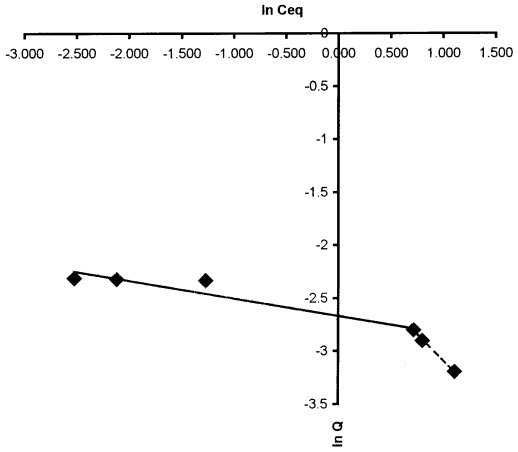

Figure 6. Freundlich model for the adsorption of Cr(III) by *Rhizobium* cells immobilized by ceramic beads (ambient room temperature).

has a 3-dimensional structure which provides a larger surface with continuous pores to maximize the potential of immobilized cells. It also has a higher affinity for organisms than ceramic beads due to its natural fiber composition.

The sorption capacity of the immobilized cell biomass in ceramic beads and aquacel ranged from 5.01 to 5.6 mg Cr^{3+} g^{-1} dry cell biomass.

The beads and aquacel without cells adsorb Cr(III) at about 10–15%. These carriers adsorb Cr(III) by electrostatic forces which attract the positively charged metal ions to negatively charged surfaces and by complexation reactions. However, the greater portion of

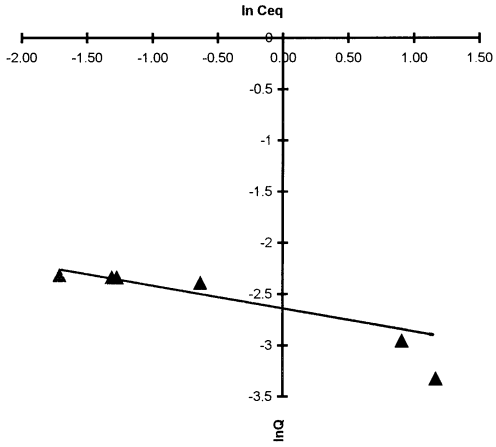

Figure 7. Freundlich model for the adsorption of Cr(III) ions by *Rhizobium* cells immobilized by aquacel (ambient room temperature).

Cr(III) removal is due to the immobilized cell biomass. The higher results of Cr(III) biosorption by the cell biomass may be due to increased cell wall permeability and substantial reduction in metal toxicity due to immobilization.

The greater efficiency of Cr^{3+} sorption by the immobilized cell biomass confirmed the findings of other researchers who reported greater metal sorption by immobilized biomass. Rai and Mallick [18] reported a higher uptake of Cu^{2+} and Fe^{2+} ions by immobilized *Anabaena dodiolum* and *Chlorelle vulgaris*. Immobilization restricts the movement of the cell biomass in space and a greater cell density is increased per unit space [19]. In a study by Zhou and Kiff [20] of immobilized filamentous fungus *Rhizopus arrhizus* in semi-rigid reticulated polyester foam biomass support particles, they reported that immobilization increased the mechanical strength, density and resistance of the fungal mass to the chemical environment.

Application of Langmuir and Freundlich adsorption isotherm models

The linearized Langmuir and Freundlich isotherm model of monolayer adsorption was applied to the biosorption of Cr^{3+} by immobilized *Rhizobium* biomass. A general fit of the data was obtained for both models at a low Cr^{3+} concentration. The Langmuir equation is defined as

$$Ceq/Q = 1/bQo + Ceq/Qo$$

where b is the constant related to energy or net enthalpy of the adsorption, Qo is the number of moles of solute

Table 1. Reduction of Cr(III) concentration of a Cr^{3+} test solution and a waste water sample containing Cr^{3+} and other cations by *Rhizobium* BJVr 12 cell biomass

Adsorbent	Test solution 5.03 ppm Cr^{3+}			Waste water sample 6.05 ppm Cr^{3+} + other cations		
	[Cr^{3+}] after treatment*, ppm	Cr^{3+} uptake, mg g^{-1} dry adsorbent	Reduction of Cr^{3+}, %	[Cr^{3+}] after treatment*, ppm	Cr^{3+} uptake, mg g^{-1} dry adsorbent	Reduction of Cr^{3+}, %
Free cell biomass	2.67	2.46	46.9	3.93	2.23	35.04
Immobilized cell biomass in ceramic beads	0.22	5.06	95.6	3.02	3.19	50.08
Ceramic beads	4.51	0.0007	10.3	5.93	0.0002	2
Immobilized cell biomass in aquacel	0.27	5.01	94.6	3.02	3.19	50.08
Aquacel	4.25	1.29	15.5	5.90	0.025	2.48

* Volume of Cr^{3+} test solution and waste water sample: 10 ml.
Weight of fresh cell biomass: 0.5 ± 0.01 g.
Weight of immobilized cell biomass (ceramic beads): 0.4935 g.
Weight of immobilized cell biomass (aquacel): 0.4940 g.
Weight of ceramic beads: 7.305 g; weight of aquacel: 0.0603 g.
Time of contact: 48 h; rate of agitation: 120 strokes min^{-1}.
Temperature: ambient room temperature (28 ± 2 °C).
Average % water of fresh cell biomass: 98.07%.

adsorbed per unit of adsorbent in forming a monolayer on the surface, Q is the Cr(III) uptake in mg/g cell and Ceq is the concentration of Cr(III) in the liquid phase.

The Langmuir model assumes the formation of a monolayer of solute molecules on the adsorbent surface and that there is a constant adsorption energy and no migration of adsorbate molecules on the surface plane. The values of Qo and b can be calculated from the slope and intercept of the plot of Ceq/Q vs. Ceq. The higher the Qo value, the higher the number of available binding sites for the solute molecules in the bacterial surface. A high b value shows a high affinity of the biomass for the solute molecules. The Langmuir constants for immobilized cells in ceramic beads are: Qo = 0.065 mmol Cr^{3+} g^{-1} dry cell biomass, b = - 694 l $mmol^{-1}$ and for immobilized cells in aquacel are: Qo = 0.07 mmol Cr^{3+} g^{-1} cell biomass, b = - 694 l $mmol^{-1}$.

These Qo values are relatively low compared to the values of *A. vinelandii* of 0.25 mmol $UO_2{}^{2+}$ g^{-1} biomass and 0.19 mmol $UO_2{}^{2+}$ of *D. gummosa* [7].

The low Qo value of *R.* BJVr 12 may indicate a relatively lower anionic density of the cell biomass. However the affinity constant b of - 694 l $mmol^{-1}$ is relatively much greater than that of *A. vinelandii* of 333 l $mmol^{-1}$ and *D. gummosa* of 100 l $mmol^{-1}$ for $UO_2{}^{2+}$ [7]. The high negative b value of the biosorption of Cr^{3+} by *R.* BJVr 12 cell biomass represents a high spontaneous affinity of the Cr^{3+} ion for the cell biomass.

The Freundlich equation is:

$$InQ = InK + 1/nInCeq$$

where Q and Ceq are the same quantities as in the Langmuir equation. K and n are Freundlich constants. The Freundlich equation assumes a heterogeneous adsorption. The Freundlich constants for *R.* BJVr 12 biomass immobilized in ceramic beads are: K = 0.071 mmol Cr^{3+} g^{-1} dry biomass, n = 0.13 g biomass l^{-1} and in aquacel are: K = 0.074 mmol Cr^{3+} g^{-1} biomass, n = 0.13 g biomass l^{-1}.

The relatively higher value for the Langmuir constant Qo and Freundlich K for immobilized cells in aquacel compared to those for ceramic beads indicated that there are more anionic groups available for binding to Cr^{3+} in aquacel which may be directed towards compexation with the metal. The similar values of the affinity constants of b and n for both immobilized cells in ceramic beads and aquacel indicated that the attraction of Cr^{3+} for the cell biomass was not altered by the two types of carrier used.

The general agreement of the experimental data of the biosorption of Cr^{3+} by immobilized cells in ceramic beads and aquacel implies that Cr^{3+} involves monolayer adsorption and a constant adsorption energy under conditions of low Cr^{3+} concentrations. Deviations from linearity may be caused by fluctuations in environmental factors such as temperature and stability of the adsorbing surface.

Table 2. Effect of rate of flow on biosorption of Cr(III) by immobilized *Rhizobium* cell biomass[a]

Fraction number	Flow rate I: 0.5 ml/min		Flow rate II: 1.5 ml/min	
	Cr(III) sorbed (ppm)	% reduction	Cr(III) sorbed (ppm)	% reduction
Cells immobilized in ceramic beads				
2	2.50 ± 0.01	49.7	1.20 ± 0.03	23.9
4	2.30 ± 0.01	45.7	1.15 ± 0.03	22.9
6	2.50 ± 0.02	49.7	1.10 ± 0.11	21.9
8	2.60 ± 0.03	51.7	0.85 ± 0.01	16.9
10	2.64 ± 0.04	52.5	0.80 ± 0.01	15.9
Ceramic beads[b]				
2	1.41 ± 0.06	28.1	0.09 ± 0.03	1.8
4	0.86 ± 0.04	17.2	0.11 ± 0.03	2.3
6	0.74 ± 0.17	14.7	0.13 ± 0.05	2.7
8	0.64 ± 0.10	12.8	0.10 ± 0.01	2.0
10	0.75 ± 0.16	15.4	0.09 ± 0.01	1.8
Cells immobilized in aquacel				
2	2.35 ± 0.03	46.7	1.10 ± 0.02	21.9
4	2.00 ± 0.03	39.8	1.00 ± 0.04	19.9
6	2.60 ± 0.02	51.7	0.95 ± 0.02	18.9
8	2.40 ± 0.05	47.7	0.78 ± 0.05	15.9
10	2.40 ± 0.01	47.7	0.80 ± 0.01	15.9
Aquacel[c]				
2	1.37 ± 0.04	27.1	0.06 ± 0.01	1.2
4	0.72 ± 0.11	16.8	0.10 ± 0.01	2.0
6	0.70 ± 0.13	13.9	0.12 ± 0.02	2.3
8	0.60 ± 0.08	12.2	0.09 ± 0.01	1.8
10	0.70 ± 0.07	13.9	0.08 ± 0.01	1.6

[a] Weight of fresh biomass: 0.5 ± 0.01 g.
Weight of immobilized cell biomass (ceramic beads): 0.4935 g.
Weight of immobilized cell biomass (aquacel): 0.4940 g.
Weight of ceramic beads: 7.305 g; weight of aquacel: 0.0603 g.
[b] 30 pcs ceramic beads, 7.305 g.
[c] 5 pcs aquacel, 0.0603 g.

Removal of Cr(III) from a laboratory liquid waste sample

The percentage reduction of Cr(III) in a waste water sample containing other cations by free cells and immobilized cells in ceramic beads and aquacel after 48 h are given in Table 1. Under the same experimental conditions of biomass weight, and other parameters except for pH and ionic strength of the test samples, immobilized cells removed a higher amount of Cr at 50.08% reduction for both beads and aquacel, while free cells removed only 35.04% Cr.

The percentage of Cr(III) removal in the Cr test solution was 95.6% in ceramic beads and 94.6% in aquacel after 48 h under shaken conditions. The lower values obtained for the waste water sample containing other metal ions suggest that the biosorption process is competitive and therefore Cr^{3+} ions will have to compete with the other cations for the available negatively charged binding sites. Mattushka and Straube [21] reported the sorption capacity of *Streptomyces noursei* for metals in the treatment of waste biomass to follow the order of:

$$Ag^{+} > Cr^{3+} > Pb^{2+} > Cu^{2+} >> gZN^{2} + > Co^{2+} >= Ni.$$

This order may explain the lower Cr(III) reduction of the waste sample since an appreciable amount of Ag^{+} and other cations were also present in the laboratory waste. Cr(III) ions however, are more competitive than the divalent and monovalent metal ions considering

Table 3. Desorption of biosorbed Cr(III) using dilute HCl at different pH

pH	Average percentage desorption of biosorbed Cr(III)
Cells immobilized in ceramic beads	
1	93.1
3	75.6
6.0–7.0	16.4
Cells immobilized in aquacel	
1	46.4
3	33.0
6.0–7.0	6.6

the high affinity constants of the cell biomass for Cr^{3+} ions.

Effect of flow rates on Cr(III) biosorption in columns

Table 2 shows the effect of flow rates on the biosorption of Cr(III) by a column packed with cells immobilized in ceramic beads and in aquacel. The slower flow rate of 0.5 ml/min showed greater Cr(III) sorption than 1.5 ml/min. Since the biosorption process is a phase equilibrium involving adsorption and desorption, a longer residence time allows the system to equilibrate and maximize adsorption. There is a time lag for the Cr^{3+} ions to reach and interact with the binding sites on the cell surface. However, too long a residence time may cause desorption to take place. There is thus, an optimum retention time to achieve higher performance in the shortest possible time.

Desorption of biosorbed Cr(III)

Experimental data for desorption of a measured amount of biosorbed Cr at a different pH using HCl at a flow rate of 0.5 ml min^{-1} are shown in Table 3. The release of sorbed Cr^{3+} ions was highest at 93.1% at pH 1.0, 75.6% at pH 3.0 and 16.4% at pH 6.0–7.0 for immobilized cell biomass in ceramic beads. These results suggest that Cr^{3+} biosorption by immobilized cells in ceramic beads is a reversible equilibrium process similar to ion exchange processes. The H^+ ions in the solution displaced the sorbed counter ions which are Cr^{3+} in this case, in the bed of the same electrical charge depending on the charges and concentration of the displacing ion (H^+ ions) [22].

For the immobilized cell biomass in aquacel, the release of sorbed Cr was non-quantitative at 46.4% at pH 1.0, 33.3% at pH 3.0 and 6.6% at pH 6.0–7.0. These results suggest partial reversibility of the biosorption process. The sorption of Cr^{3+} by immobilized biomass in aquacel may involve, besides electrostatic attraction, stronger physico-chemical complexation between Cr^{3+} and the charged components of the cell biomass and aquacel. To quantitatively release sorbed Cr^{3+} ions which are specifically coordinated to the ligands may require a different approach than pH adjustment only. A strong complexing agent such as EDTA as an eluent following pH adjustment may be tried in further studies to quantitatively release sorbed Cr^{3+} ions in organic carriers such as aquacel.

Conclusions

Rhizobium BJVr 12 which forms copious amounts of viscous exopolysaccharides is capable of reducing the Cr(III) levels in dilute solutions of Cr. The biosorption of Cr^{3+} ions is influenced by the ratio of cell biomass to volume and concentration of Cr^{3+} solutions, time of contact of biomass with Cr^{3+} ions, presence of competing cations, and the state of mobility of the cell biomass.

Immobilization of the cell biomass in ceramic beads and aquacel increased Cr(III) sorption and resistance to the chemical environment and mechanical agitation. Biosorption of Cr(III) by the immobilized biomass generally follow both the Langmuir and Freundlich adsorption isotherm model at low Cr concentration. Langmuir constants, Qo and Freundlich constants, K are relatively lower when compared to values obtained for metal sorption by other N fixing bacteria [7]. However, its relatively much higher affinity constant makes it a good and specific sorbent for Cr^{3+} ions in metal polluted aqueous systems.

The biosorption of Cr^{3+} ions is competitive in the presence of other cations. Sorption of Cr^{3+} ions involves a phase equilibrium between sorption and desorption, and therefore, an optimum flow rate for immobilized cell biomass in biofilters/biotraps must be established for maximum sorption. Biosorption of Cr^{3+} ions is reversible for immobilized biomass in ceramic beads and only partially for biomass in aquacel.

The quantitative release of sorbed Cr^{3+} ions by H^+ ions from immobilized cell biomass in ceramic beads should seriously be considered in designing a metal detoxification process for acidic environments. *Rhizobial* biotraps can not be recommended for acid

industrial and mining effluents since high H^+ ion concentrations can displace sorbed Cr^{3+} ions. However, *Rhizobial* biotraps can be very effective in neutral or basic environments considering its high affinity for Cr^{3+} ions. The Cr^{3+} loaded traps can easily and economically be divested of its Cr load by pH adjustment to 1.0 or below in manageable volumes. The ceramic beads, because of their mechanical and chemical stability, can then be recycled for another load of fresh *Rhizobial* biomass. This cost effective technology can be useful as Cr(III) scrubbers for point sources of Cr^{3+} ion pollution and can be integrated into a comprehensive waste water treatment strategy.

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