

Removal and Recovery of Chromium(III) from Aqueous Chromium(III) Using *Arthrobacter nicotianae* Cells

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Abstract

The removal of Cr(III) from aqueous Cr(III) using *Arthrobacter nicotianae* cells was examined. Cr(III) removal was strongly affected by the pH of the solution and the amounts of Cr(III) removed increased as the pH (1 - 5) of the solution increased. The removal of Cr(III) using the cells was also strongly affected by the Cr(III) concentration of the solution, and obeyed the Langmuir isotherm. The percentage of Cr increased as the cell quantity increased, whereas the amount of Cr ($\mu\text{mol/g}$ dry wt. cells) decreased. The removal of Cr(III) using the cells was very fast, and reached an equilibrium within 6 h from the supply of Cr(III) in the solution. A small amount of Cr(III) absorbed by immobilized cells was desorbed at 30°C; however, most was desorbed at reflux temperature using diluted HCl. Cr(III) adsorption-desorption cycles can be repeated 5 times using immobilized cells. These results have practical implications for industrial wastewater management.

Keywords

Cr(III) Removal, Cr(III) Recovery, *Arthrobacter nicotianae*, Immobilized Cell, Cr(III) Recycling

1. Introduction

Chromium is used in the textile, leather tanning, electroplating, metal finishing, wood treatment, corrosion control, oxidation, and anodizing industries [1]. High levels of chromium absorbed by the body can generate serious health issues and a concentration of 100 $\mu\text{g/g}$ body wt. can ultimately be lethal [2]. Currently, the main processes for the elimination of chromium are adsorption, reverse osmosis, and chemical reactions that involve reduction and precipitation [1]. Among these processes, adsorption is a feasible method for removing traces of

chromium from wastewater [1] and many adsorbents have been examined for this purpose [2] [3].

Adsorption is the most effective and widely used technique for the removal of toxic heavy metals from wastewater [4]. Although activated carbon has been commonly used for this purpose, it is limited by its high cost and difficult procurement [1]. Accordingly various low-cost substances, like fly ash [5], wood charcoal [6], bituminous coal [7], bagasse and coconut juice [8], rice husk carbon [9], peat [10], red mud [11], used black tea leaves [12], activated carbon from sugar industrial waste [13] and sugarcane bagasse [14] have been examined.

We previously demonstrated that microorganisms are able to remove many toxic and useful metals, such as lithium [15], uranium [16], thorium [17], rare earth metals [18], and gold [19] from aqueous solutions. Additionally, immobilized persimmon tannin gel removes gold(III) from a hydrogen tetrachloroaurate(III) solution [20]. Microorganisms could remove small amounts of chromium from a chromium(VI) solution; however, the amount of chromium removed using persimmon tannin gel is much larger than that using microbial cells [21]. Most Cr(VI) is removed using persimmon tannin gel, but some chromium remains in the form of Cr(III) in the solution.

Despite of the Cr(III) removal using persimmon tannin gel under various experimental conditions, little improvement in the removal efficiency has been observed.

Therefore, the removal of Cr(III) using microorganism, *Arthrobacter nicotianae* which can remove a large amount of metals [15] [16] [18], was examined in this paper.

2. Material and Methods

2.1. Culture of Microorganisms

Microorganisms were grown in medium containing 4 g/L meat extract, 5 g/L peptone, and 5 g/L NaCl in deionized water. The cultures of microorganisms, maintained on agar slants, were grown in 300 mL of the medium in a 500 mL flask with continuous shaking (120 rpm) at 30°C. To ensure a sufficient amount of resting microorganisms after separation from the growth medium, the cultures were grown for 72 h.

Cells were collected by centrifugation (10,000 rpm) at 20°C for 10 min, washed thoroughly with deionized water, and used in subsequent removal experiments.

2.2. Immobilization of Microorganism

Five grams of precultured cells were suspended in 4.5 mL isotonic sodium chloride solution, and 680 mg of acrylamide monomer, 34 mg of *N,N'*-methylene-bis(acrylamide), 0.3 mL of 3-dimethylaminopropionitrile solution (5%), and 0.34 mL of potassium persulfate solution (2.5%) were added to the suspension.

After solidification, the gel was crushed into small pieces (50 - 100 mesh), washed thoroughly with isotonic sodium chloride solution followed by deionized water, and used for adsorption experiments.

2.3. Cr(III) Removal for Various pH Values, Cr(III) Concentrations, and Cell Amounts

Cr(III) nitrate was used. The pH of the solution was adjusted to the desired value (1.0 - 5.0) using 0.1 M HCl. Resting cells (15 mg dry wt. basis) were suspended in 100 mL solutions containing 100 μ M (5 ppm) Cr(III) (pH 1 - 5) for 1 h at 30°C to examine the effect of pH. Similar experiments containing 19 - 960 μ M (1.0 - 50 ppm) of Cr(III) (pH 5), or resting cells (5.0 - 60 mg dry wt. basis) were also performed to examine the effect of concentration or cell amount, respectively. Microorganisms were then collected by filtration through a nitrocellulose membrane filter (pore size 0.2 μ m). Control studies confirmed that the free metal was not adsorbed on the filter.

The amount of Cr(III) removed by cells was determined by measuring the difference between the initial and final metal content in the filtrate using an atomic absorption spectrometer (AA-6300; Shimadzu Corporation, Kyoto, Japan).

2.4. Time Course of Cell Amount on Cr(III) Removal Using *A. nicotianae* Cells

Resting cells (15 mg dry wt. basis) were suspended in 100 mL solutions containing 5.0 ppm (100 μ M) Cr(III) (pH 5) for 5 min to 24 h at 30°C.

2.5. Quantitative Analysis of the Selective Removal of Seven Metal Ions Using *A. nicotianae* Cells

A. nicotianae cells (15 mg, dry wt. basis) were suspended in 100 mL of a solution (pH 5.0) containing 4×10^{-5} M Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , and Cr^{3+} as nitrates for 1 h at 30°C.

2.6. Chromium Adsorption/Desorption from a Cr(III) Solution Using Immobilized *A. nicotianae* Cells

The extent of Cr adsorption/desorption from a Cr(III) nitrate solution using immobilized *A. nicotianae* cells was investigated. Immobilized *A. nicotianae* cells (15.4 mg dry wt. cells basis) were suspended with 100 mL of Cr(III) (4.68 ppm, pH 5.0) for 1 h at 30°C. Immobilized *A. nicotianae* cells that adsorbed Cr(III) were separated from the suspended solution by filtration through a membrane filter (pore size 0.2 μ m) and suspended with diluted HCl or Na_2CO_3 solution (0.01, 0.1, or 1 M) for 1 h at 30°C.

2.7. Effect of Temperature on Cr(III) Desorption from a Cr(III) Adsorbed Immobilized *A. nicotianae* Cells

Immobilized *A. nicotianae* cells (15.7 mg dry wt. cells basis) were suspended with 100 mL of Cr(III) (5.19 ppm, pH 5.0) for 1 h at 30°C. Immobilized *A. nico-*

*tiana*e cells that adsorbed Cr(III) were separated following the same method described in Section 2.6 and suspended with diluted HCl (0.1 or 1 M) for 1 h at 30°C-(refluxed temperature).

2.8. Recycling of Cr(III) Removal and Recovery

Cr(III) solution (5.26 ppm, pH 5.0, 50 mL) was passed through the immobilized *A. nicotiana*e cells (230 mg dry wt. cells basis) column (diameter 8 mm) at 30°C. Then, immobilized *A. nicotiana*e cells that adsorbed Cr(III) were subjected to desorption by applying 0.1 M HCl at reflux temperature for 1 h using a batch system.

3. Results and Discussion

3.1. Effect of pH on Cr(III) Removal from Aqueous Cr(III) Solution Using *A. nicotiana*e Cells

The effect of pH on Cr(III) removal from aqueous Cr(III) as $\text{Cr}(\text{NO}_3)_3$, using *A. nicotiana*e cells was examined. As shown in **Figure 1**, the percentage of Cr(III) removed from the solution was maximal (approximately 90%) at pH 5. For 2.5 ppm, compared with Cr(VI) using persimmon tannin gel [21], the amount of Cr(III) removed using *A. nicotiana*e was six times higher.

In contrast, the zeta potential of *A. nicotiana*e was decreased as the pH of the solution increased [22]. These results indicates that Cr(III) removed (%) depends on the charge of the surface of *A. nicotiana*e cells and solution.

3.2. Effect of Cr(III) Concentration on Cr(III) Removal from Aqueous Cr(III) Using *A. nicotiana*e Cells

The effect of Cr(III) concentration on Cr(III) removal was examined. As shown

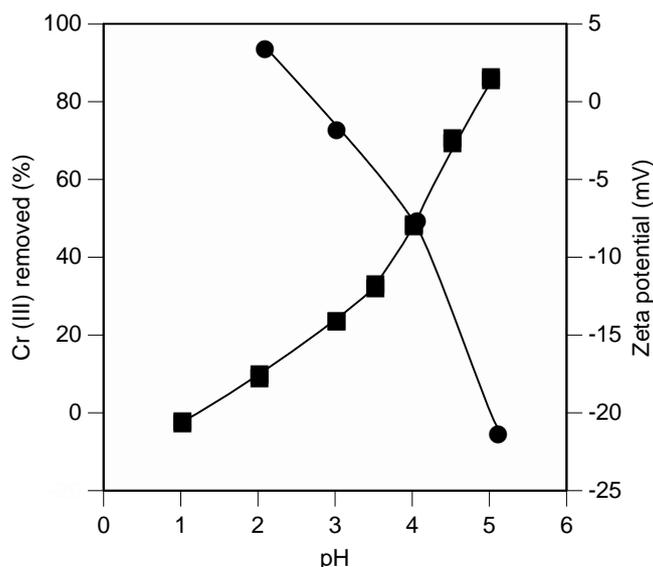


Figure 1. Effect of pH on the removal of Cr(III) by *A. nicotiana*e cells. Symbols: Squares, Cr(III) removed (%), Circles, Zeta potential of *A. nicotiana*e cells (mV) [22].

in **Figure 2**, Cr(III) removal ($\mu\text{mol/g}$ dry wt. cells) increased as the Cr(III) concentration increased, while the Cr(III) removed (%) decreased. Cr(III) removed was almost quantitatively for a low Cr(III) concentration, such as 1.0 ppm (19 μM) Cr(III) solution. The amount of Cr(III) removed for a high Cr(III) concentration, such as a 960 μM (50 ppm) Cr(III) solution (equilibrium concentration, 860 μM (45 ppm)) was approximately about 720 μmol Cr(III)/g dry wt. cells.

The relationship between the residual Cr(III) concentration in the solution and the amount of Cr(III) removed is shown in **Figure 2**. The line for Cr(III) removed ($\mu\text{mol/g}$ dry wt. cells) represents the *Langmuir* isotherm. It is evident from this figure that Cr(III) removal using *A. nicotianae* cells obeys the following *Langmuir* isotherm over the whole range of concentrations tested:

$$C_e/Q = mC_e + n \quad (1)$$

where Q indicates the amount of Cr(III) removed (μmol Cr(III)/g dry wt. cells), C_e is the residual Cr(III) in the solution (μM Cr(III)), and m and n are *Langmuir* constants. It was estimated that $C_e/Q = 1.38 \times 10^{-3} C_e + 2.25 \times 10^{-2}$. The maximum amount of Cr(III) removed (μmol Cr(III)/g dry wt. cells) estimated from the slope of the line was 724 μmol Cr(III)/g dry wt. cells.

3.3. Effect of Cell Amounts on Cr(III) Removal from Aqueous Cr(III) Using *A. nicotianae* Cells

The effect of cell amounts on Cr(III) removal from aqueous Cr(III) solution using *A. nicotianae* cells was examined. As shown in **Figure 3**, the percentage of Cr(III) removed increased as the cell amounts increased whereas the Cr(III) removed ($\mu\text{mol/g}$ dry wt. cells) decreased. The Cr(III) removed from the 200 μM (10 ppm) solution using over 40 mg (dry wt. basis) of *A. nicotianae* cells was

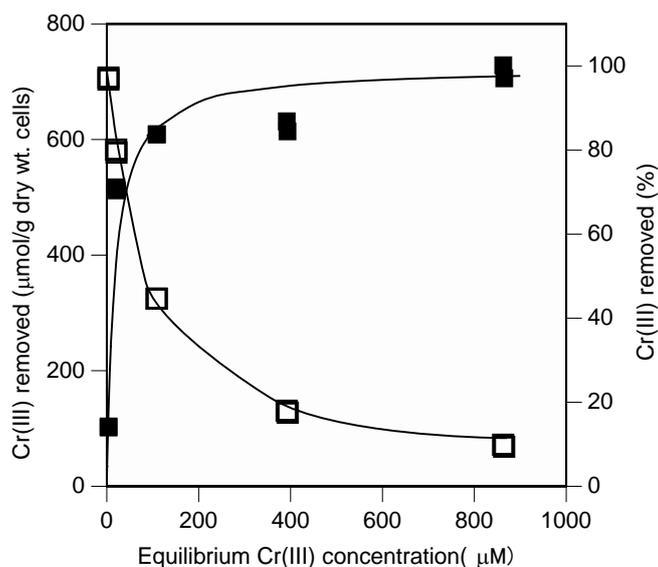


Figure 2. Effect of Cr(III) concentration on the removal of Cr(III) by *A. nicotianae* cells. Symbols: closed squares, Cr(III) removed ($\mu\text{mol/g}$ dry wt cells); open squares, Cr(III) removed (%).

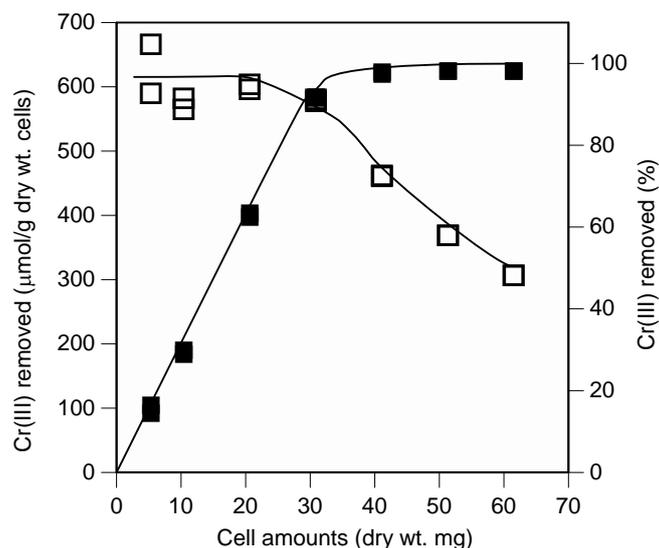


Figure 3. Effect of cell amounts on the removal of Cr(III) by *A. nicotianae* cells. Symbols: closed squares, Cr(III) removed (%), open squares, Cr(III) removed ($\mu\text{mol/g}$ dry wt. cells).

quantitatively reduced, and the amount of Cr(III) ($\mu\text{mol/g}$ dry wt. cells) using less than 20 mg of the cells was approximately $600 \mu\text{mol Cr(III)/g}$ dry wt. cells.

3.4. Time Course of Cr(III) Removal from Aqueous Cr(III) Using *A. nicotianae* Cells

The removal of Cr(III) using *A. nicotianae* cells was examined in a time course analysis. These results are summarized in **Figure 4**. The amount of Cr(III) removed (%) using *A. nicotianae* cells increased very rapidly, and 76% of the Cr(III) in the solution was removed during the first 5 min following the supply of Cr(III). The removal of Cr(III) reached an equilibrium within 6 h.

3.5. Selective Removal of Cr(III) Using *A. nicotianae* Cells

To determine which heavy metal ion can be most readily removed using *A. nicotianae* cells at pH 5, the selective removal of heavy metal ions from a solution containing $4 \times 10^{-5} \text{ M}$ Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , and Cr^{3+} was examined. As shown in **Figure 5**, the relative degree of heavy metal ion adsorption by *A. nicotianae* cells was $\text{Cr}^{3+} = \text{Cu}^{2+} \gg$ others, indicating that *A. nicotianae* cells can remove equal amounts of Cr(III) and Cu(II) more readily than other heavy metal ions.

3.6. Adsorption and Desorption of Cr(III) from Cr(III) Solutions Using *A. nicotianae* Cells

The amount of Cr(III) removed using *A. nicotianae* cells was strongly affected by the pH of the solution. The amount of Cr(III) removed (%) increased as pH of the solution increased. Therefore, the adsorption of Cr(III) was examined at pH 5 and desorption was examined using both acidic and alkaline conditions by a batch system. As shown in **Table 1**, most of the Cr(III) ($333 - 343 \mu\text{g}$, 71.2% -

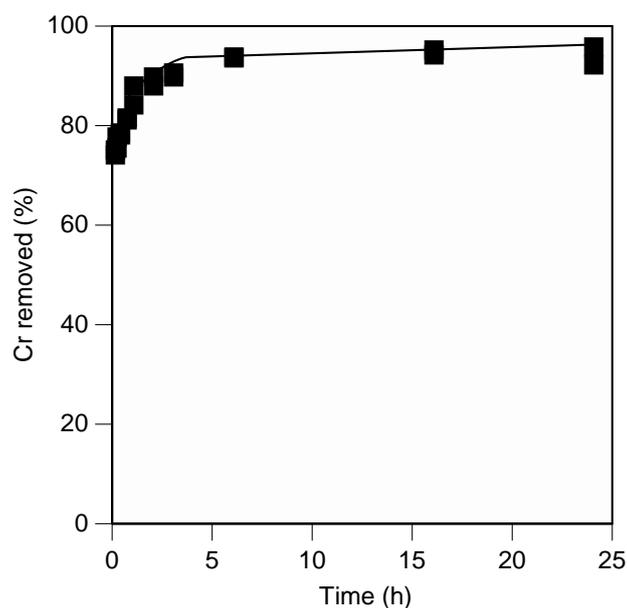


Figure 4. Time course of the removal of Cr(III) by *A. nicotiana* cells.

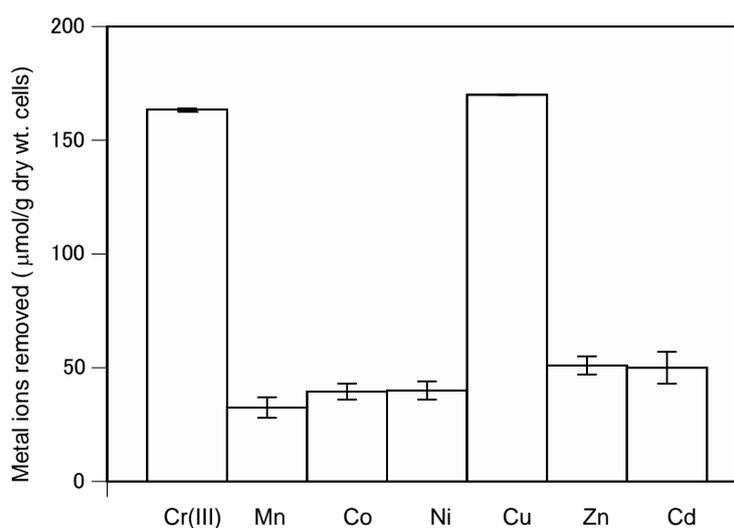


Figure 5. Selective removal of heavy metals by *A. nicotiana* cells.

Table 1. Desorption of Cr(III) from *A. nicotiana* cells after Cr(III) adsorption using diluted HCl or Na₂CO₃.

Desorbent	Adsorbed Cr(III) (mg)	Adsorbed Cr(III) (%)	Desorbed Cr(III) (mg)	Desorbed Cr(III) (%)
0.01 M-HCl	343 ± 3	73.3 ± 0.6	122 ± 2	35.4 ± 0.8
0.1 M-HCl	334 ± 2	71.4 ± 0.5	144 ± 3	43.1 ± 0.5
1 M-HCl	335 ± 3	71.6 ± 0.6	151 ± 2	45.2 ± 0.2
0.01 M-Na ₂ CO ₃	333 ± 3	71.2 ± 0.6	14 ± 1	4.2 ± 0.2
0.1 M-Na ₂ CO ₃	334 ± 2	71.4 ± 0.5	46 ± 1	13.6 ± 0.3
1 M-Na ₂ CO ₃	334 ± 3	71.4 ± 0.5	80 ± 1	23.8 ± 0.1

73.3%) was adsorbed on *A. nicotianae* cells. However, only a small amount of adsorbed Cr(III) was desorbed in acidic conditions (*i.e.*, 35% using 0.01 M- and 45% using 1 M HCl at 30°C) and alkaline conditions (*i.e.*, 4% using 0.01 M- and 24% using 1 M Na₂CO₃ at 30°C).

3.7. Effect of Temperature on the Desorption of Adsorbed Cr(III) Using Immobilized *A. nicotianae* Cell

The amount of Cr(III) desorbed was very low using *A. nicotianae* cells at 30°C. Therefore, the effect of temperature on the desorption of adsorbed Cr(III) was examined. As shown in **Figure 6**, Cr(III) desorbed (%) increased as the temperature increased above 60°C. Most (99.1%) of the adsorbed Cr(III) was desorbed at reflux temperature.

3.8. Cycling of Cr(III) Removal and Recovery

To obtain basic information on the recovery of Cr(III) using immobilized *A. nicotianae* cells, cycles of Cr(III) adsorption and desorption was repeated five times.

As shown in **Figure 7**, Cr(III) was quantitatively adsorbed on the immobilized *A. nicotianae* cells using a column system and approximately 90% of adsorbed Cr(III) was desorbed with a 0.1 M HCl solution at reflux temperature by a batch system. Most of the Cr(III) adsorbed was desorbed after 5 repetitions of the adsorption-desorption cycle in a column (adsorption)-batch (desorption) system.

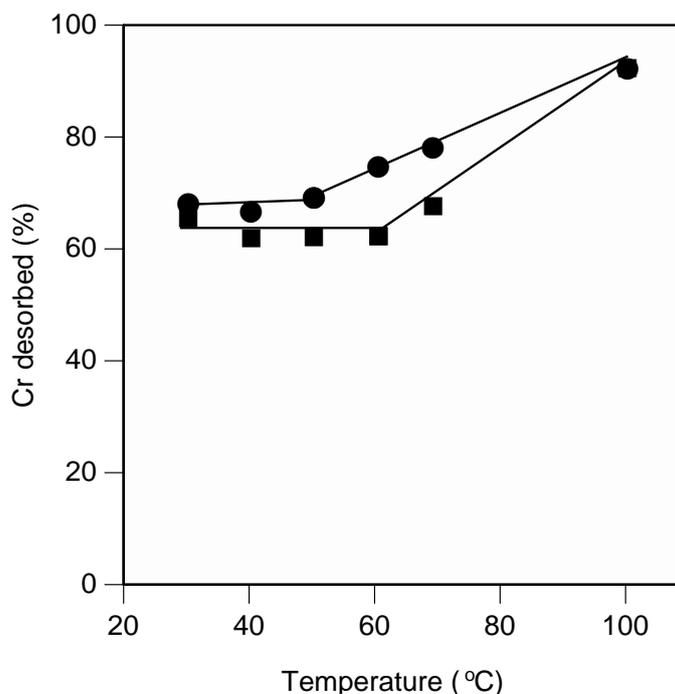


Figure 6. Effect of temperature on the desorption of Cr(III) adsorbed using *A. nicotianae* cells by a diluted HCl solution. Symbols: Squares, 0.1 M HCl, Circles, 1 M HCl.

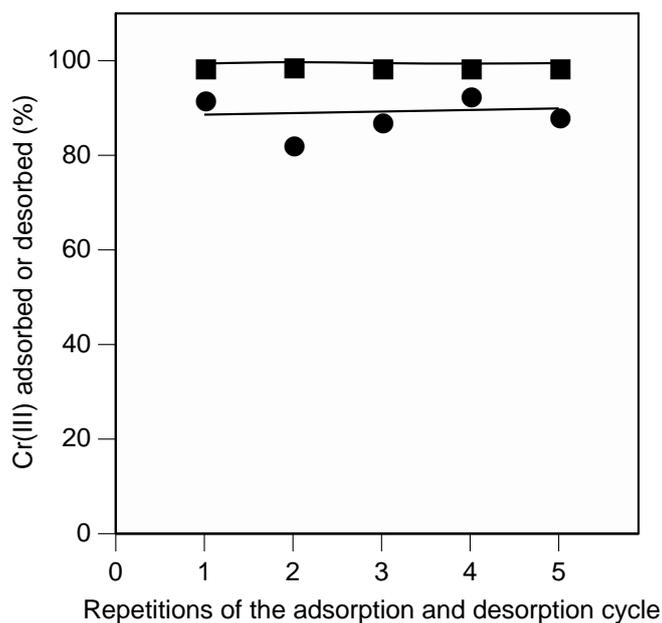


Figure 7. Repetition of Cr(III) adsorption-desorption cycles using immobilized *A. nicotianae* cells. Symbols: Squares, Cr(III) adsorbed (%), Circles, Cr(III) desorbed (%).

4. Conclusions

The removal of Cr(III) using *A. nicotianae* cells was strongly affected by the pH of the solution. The amount of Cr(III) removed increased as the pH of the solution increased. The maximum Cr(III) removed (%) of approximately 90% was observed at pH 5. As the removal of 2.5 ppm Cr(III) using persimmon tannin gel was about 30% (21), the amount of Cr(III) removed using *A. nicotianae* was six times higher than that using persimmon tannin gel.

The Cr(III) removed ($\mu\text{mol/g}$ dry wt. cells) increased as the Cr(III) concentration increased, whereas the Cr(III) removed (%) decreased.

The removal of Cr(III) using *A. nicotianae* cells obeyed the *Langmuir isotherm* over all concentrations examined.

Cr removed (%) increased as the cell amount increased, whereas the Cr(III) removed ($\mu\text{mol/g}$ dry wt. cells) decreased. The amount of Cr(III) removed using *A. nicotianae* cells increased very rapidly and 76% of the Cr(III) in the solution was removed during the first 5 min following the supply of Cr(III). The removal of Cr(III) reached an equilibrium within 6 h. The selective removal of 7 kinds of metal ions was examined using *A. nicotianae* cells; Cr(III) and Cu(II) were removed at much higher rates than the other metal ions. Immobilized *A. nicotianae* cells can also adsorb Cr(III) at 30°C and can desorb most Cr(III) with 0.1 M hydrochloric acid at reflux temperature. Repetition of adsorption (column) and desorption (batch) cycles using immobilized *A. nicotianae* cells can be repeated 5 times.

We observed substantial toxic Cr(VI) removal using persimmon gel; however, some (~20%) Cr(III) was produced, and only a low amount of Cr(III) removed [21]. Based on the results of this paper and recent results, the removals of Cr(VI

and III) from the Cr(VI) wastewater system and Cr(III) products are possible using persimmon gel and *A. nicotiana*, respectively.

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