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Genetic engineering of *E. coli* SE5000 and its potential for Ni^{2+} bioremediation

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Abstract

A genetically engineered *Escherichia coli* SE5000 strain simultaneously expressing nickel transport system and metallothionein was constructed to accumulate Ni^{2+} from aqueous solution. Bioaccumulation was fast and followed linearized Langmuir isotherm. Compared with 1.62 mg/g of Ni^{2+} uptake capacity by original host *E. coli* cells, genetically engineered *E. coli* could bind 7.14 mg/g Ni^{2+}, and it accumulated Ni^{2+} effectively over a broad range of pH (4–10) and the optimal pH was 8.6. The presence of 1000 mg/l Na^{+} and Ca^{2+} or 50 mg/l Cd^{2+} and Pb^{2+} did not decrease Ni^{2+} bioaccumulation significantly, but Mg^{2+}, Hg^{2+}, Cr^{3+} and Cu^{2+} posed severe deleterious influences on Ni^{2+} uptake by genetically engineered *E. coli*. Furthermore, the presence of EDTA inhibited nickel bioaccumulation.

Keywords: *Escherichia coli*; Nickel; Bioaccumulation; Genetic engineering

1. Introduction

With its attractive chemical properties such as high ductility, thermal conductivity, moderate strength and hardness, nickel has been widely used in battery manufacture, steel production, alloy synthesis and electroplating. However, the increase in demand for nickel over the last several decades has resulted in a large amount of industrial wastes containing nickel ions which may pose severe effects on natural environment. Although biosorption is an attractive alternative to existing methods for toxicity reduction and recovery of valuable metals from industrial effluents, the nickel ion is a more recalcitrant pollutant compared with other heavy metal ions. Many metal tolerant microorganisms have proved to have a relatively low Ni-binding capacity [1,2]. This is probably due to the intrinsic chemical properties of nickel ions leading to steric hindrance of biosorption [3]. Furthermore, the biosorption process is sensitive to ambient conditions, e.g. pH and the presence of other inorganic and organic components [4]. In particular, they lack specificity in metal binding, which may cause difficulties in the recovery and recycling of the desired metal(s).

Genetic engineering technique have the potential to improve or redesign microorganisms so that biological metal binding systems have higher intrinsic capability and specificity and are more resistant to ambient conditions. To construct strains that are capable of specifically accumulating Ni^{2+} from dilute solution, *Escherichia coli* SE5000 was genetically engineered to express an Ni^{2+} transport system (the products of *nixA* gene) [5] and overexpress metallothionein (MT) as a glutathione S-transferase fusion protein (GSM-MT) [6]. The *nixA* gene encodes a 37 kDa integral membrane protein consisting of eight transmembrane domains, which has a very high affinity for Ni^{2+}; metallothioneins are a class of low-molecular-weight metal binding proteins rich in cysteine residues, thus capable of binding a variety of heavy metals including Ni^{2+}. Thus, the Ni^{2+} transport system would make the cells specifically bind Ni^{2+}, and the intracellular overexpressed MTs would accumulate Ni^{2+}, which would allow bioaccumulation system to be less sensitive to ambient conditions than biosorption. The present study therefore aimed to investigate the Ni^{2+} bioaccumulation performance of genetically engineered *E. coli* SE5000 from dilute solution, including Ni^{2+} uptake rate, accumulating isotherm, the effects of pH, metal chelator, ion strength and other co-existing metal ions.

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2. Materials and methods

2.1. Plasmids and bacterial strains

This study employed two compatible plasmids, pSUNI (Deng et al., unpublished) and pGPMT3 [7] to express an Ni^{2+} transport system and the GST fusion protein of pea MT (GST-MT), respectively. pSUNI was constructed by inserting a *nixA* coding sequence into pSU39 [8] from pUEF202 [5]. pSUNI and pGPMT3 contained Kan^r and Amp^r sequence, respectively. *E. coli* SE5000 was employed to be the host strain in this study [9].

Frozen *E. coli* cells expressing simultaneously both nickel transport system and metallothionein were inoculated into Luria broth (LB) containing ampicillin (50 mg/l) and kanamycin (30 mg/l), grown overnight at 37 °C, diluted to an optical density at 600 nm (OD_{600}) of 0.1–0.3 with fresh LB containing ampicillin and kanamycin, and incubated at 37 °C with vigorous shaking (180 rpm). Isopropyl β-D-thiogalactoside (IPTG), 1 M solution in deionized water, was added to 1.0 mM concentration when the OD_{600} reached 0.5–0.7, and after 4 h the cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4 °C. Original host *E. coli* SE5000 cells were also prepared as above, except for the antibiotics adding and IPTG induction. These original host *E. coli* cells were used in Ni^{2+} bioaccumulation experiments as the control.

2.2. Ni^{2+} bioaccumulation

E. coli cells were harvest by centrifugation (10,000 × *g* for 10 min), washed with 10 mM phosphate buffer, and resuspended in Ni^{2+} solutions with the Ni^{2+} concentration varying from 0 to 80 mg/l. After 1 h incubation at 180 rpm and 37 °C, the cells were harvested by centrifugation as above, dried and digested with 70% trace-metal grade nitric acid for nickel analysis. For time-dependent accumulation, the cells were harvested at the different time intervals by filtration of 10 ml samples on a 0.20 μm-pore-diameter nitrocellulose filter (Millipore).

To determine the effect of the presence of other metal ions on the bioaccumulation of Ni^{2+}, *E. coli* cells were suspended in 10 mg/l Ni^{2+} solution containing either Na^+ (0–1000 mg/l), Mg^{2+} (0–1000 mg/l), Ca^{2+} (0–1000 mg/l), Cu^{2+} (0–50 mg/l), Pb^{2+} (0–50 mg/l), Hg^{2+} (0–50 mg/l), Cr^{3+} (0–50 mg/l) or Cd^{2+} (0–50 mg/l), respectively. The pH of the suspensions was adjusted to the desired value with 0.1 M NaOH or HNO_3. EDTA was added (0–2.0 mmol/l) to investigate the effect of metal chelator on Ni^{2+} bioaccumulation. During these experiments, the stirred speed and temperature were kept constant at 180 rpm and 37 °C, respectively, unless otherwise specified. All treatments were in triplicate. All chemicals used in this study were at least of analytical grade. Chloride salts of nickel, sodium, magnesium, calcium, copper or cadmium, nitrate salts of mercury or lead, and chromium sulphate were used to prepare solutions of these metals by molecular-weight calculation providing single metal ion solutions at desired concentrations in deionized water.

To prevent metal contamination, all glassware were soaked in 20% nitric acid overnight and rinsed three times with deionized water before complete drying.

2.3. Analytical techniques

Ni^{2+} was measured by atomic absorption spectrophotometry. The dry weight of cells was determined from the OD_{600} using the value of 0.396 g dry weight per liter of OD 1.0. Nickel binding capacities were expressed as milligrams Ni^{2+} accumulated by gram dry weight of cells.

3. Results and discussion

3.1. Time course of Ni^{2+} bioaccumulation

A time course assay was carried out with induced cells as well as original host *E. coli* cells (as the control) to measure uptake rate of Ni^{2+} by the genetically engineered *E. coli*. The result (Fig. 1) showed that the rates of nickel uptake by these two types of cells were rapid and 90% of the maximum accumulations were reached within the first 5 min. After the rapid initial uptake, further bioaccumulation by both strains occurred slowly and reached an equilibrium after 1 h of treatment. From inspection of Fig. 1, it was also noted that original host *E. coli* cells reached the equilibrium a bit faster than genetically engineered *E. coli* cells. This might be because only surface adsorption, an immediate process, occurred on the surface of the original host *E. coli* cells, while intracellular bioaccumulation process by genetically engineered *E. coli* cells required more time than surface adsorption to reach equilibrium.

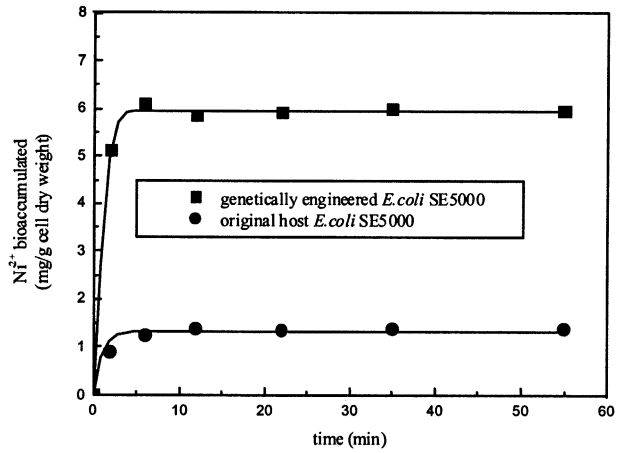

Fig. 1. Time course of Ni^{2+} uptake by genetically engineered *E. coli* SE5000 and original host *E. coli* SE5000 from 10 mg/l Ni^{2+} solutions.

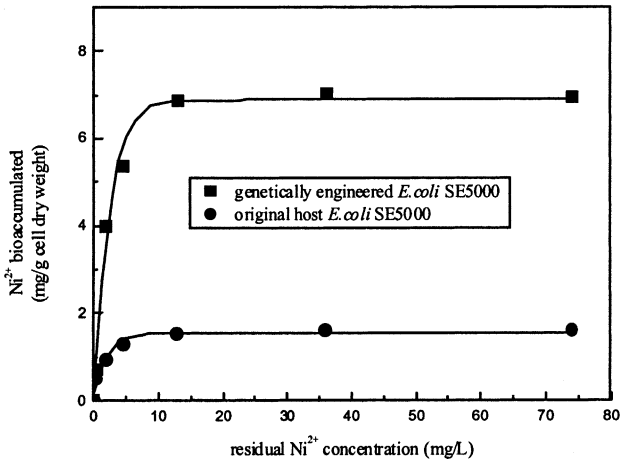

Fig. 2. Ni^{2+} bioaccumulation isotherms by genetically engineered *E. coli* SE5000 and original host *E. coli* SE5000.

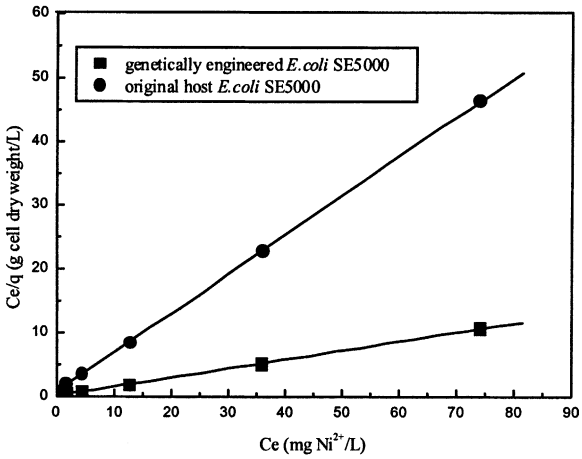

Fig. 3. Linearized Langmuir isotherm plot for Ni^{2+} bioaccumulation by genetically engineered *E. coli* SE5000 and original *E. coli* SE5000.

3.2. Bioaccumulation isotherm

Fig. 2 displays the result of a 1 h incubation of *E. coli* cells with solutions containing different concentrations of Ni^{2+}. It was clear that Ni^{2+} bioaccumulation by the genetically engineered cells expressing simultaneously both the nickel transport system and metallothionein was dramatically enhanced from 1.59 mg/g by original host *E. coli* cells to 7.05 mg/g, showing a more than four-fold increase. Apparently the increase of Ni^{2+} accumulation by genetically engineered *E. coli* cells was attributable to the specific nickel transport system near the cell membrane and metallothionein in cytoplasm.

As shown in Fig. 2, the isotherms for Ni^{2+} accumulation were of Langmuir type, as represented by:

$$\frac{C_e}{q} = \frac{k}{q_m} + \frac{1}{q_m} C_e$$

where C_e is final or equilibrium concentration of Ni^{2+} (mg Ni^{2+}/l), q is the bioaccumulation of Ni^{2+} (mg Ni^{2+}/g cell dry weight), q_m is the maximum bioaccumulation capacity (mg Ni^{2+}/g cell dry weight), and k is the dissociation constant (mg Ni^{2+}/l). Linear plots of C_e/q against C_e (Fig. 3) resulted from the Langmuir equation to the bioaccumulation in Fig. 2, showing that they were good empirical representations of the bioaccumulation data, where q_m values were 7.14 and 1.62 mg/g, respectively for genetically engineered *E. coli* cells and original host *E. coli* cells. Ni^{2+} uptake abilities of some other biomasses are shown in Table 1.

3.3. Effect of pH on Ni^{2+} bioaccumulation

pH variation in contaminated water often affects metal clean-up processes. Uptake of metals by organisms is highly dependent on their bioavailability, and bioavailability is related to the speciation and partitioning of a metal, which are greatly influenced by pH. A high pH may result in the formation of stable metal complexes, e.g. hydroxides, oxides, and carbonates, making the heavy metals less available for removal by ion exchange resins or biosorbents. A low pH may increase the mobility of heavy metals and therefore may enhance their availability. On the other hand, cation competition due to the presence of excess protons often dramatically decreases adsorption of heavy metals to biosorbents.

The effect of pH on the biosorption of Ni^{2+} has been widely investigated. Lopez et al. [16] indicated that 92% of nickel biosorption by free *Pseudomonas fluorescens* 4F39 was lost as the pH of the solution changed from 8 to 6.5, while a 48% drop of Ni^{2+} biosorption by *Azolla filiculoides* occurred as pH varied from 6.5 to 3.0 [17]. Fig. 4 shows the pH profile of Ni^{2+} bioaccumulation by genetically engineered *E. coli* cells and original host *E. coli* cells. Within the pH range from 4 to 10, genetically engineered *E. coli* cells displayed resistance to pH variation to a certain extent by retaining more than 3.92 mg/g of Ni^{2+} bioaccumulation and the maximum reduction was 38% at pH 4. To the contrary, original host *E. coli* cells which did not express nickel transport system and metallothionein showed up to 77% decrease of Ni^{2+} binding capacity within the tested

Table 1
Maximum nickel binding capacities of some biomasses

Biomass	q_{max} (mg/g)	References/ source
Arthrobacter sp.	13.0	[10]
Medicago sativa	4.10	[11]
Aspergillus niger (ABM-1)	19.6	[12]
Chlorella vulgaris	1.282	[13]
Chlorella miniata	2.985	[13]
Kandelia candel	0.472	[14]
Fecus vesiculosus	2.85	[15]
Genetically engineered *E. coli* SE5000 expressing both nickel transport system and metallothionein	7.14	This study
Original host *E. coli* SE5000	1.62	This study

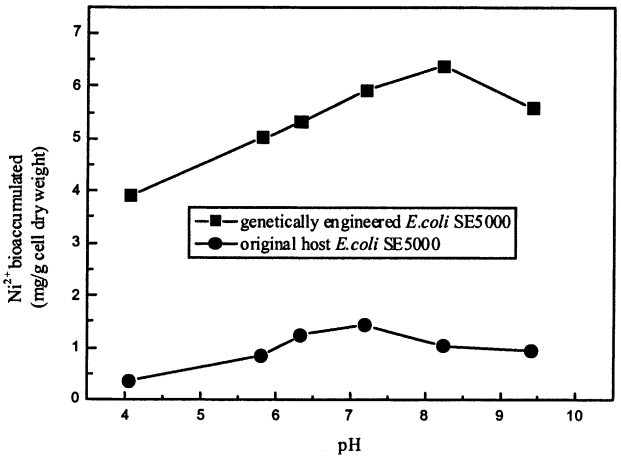

Fig. 4. pH profile of Ni^{2+} bioaccumulation from 10 mg/l Ni^{2+} solutions by genetically engineered *E. coli* SE5000 and original host *E. coli* SE5000.

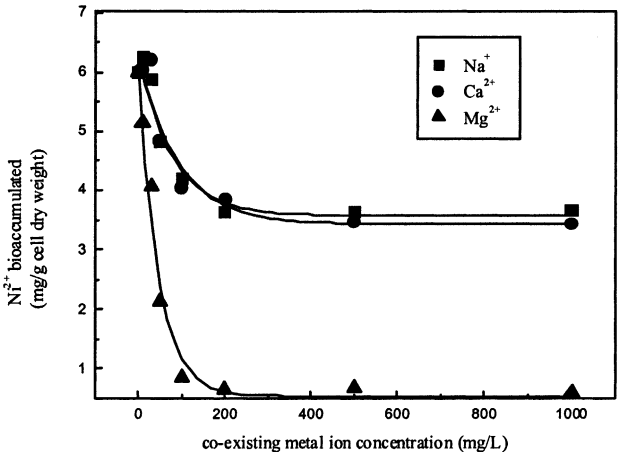

Fig. 5. Effect of Na^{+}, Ca^{2+}, Mg^{2+} on Ni^{2+} bioaccumulation from 10 mg/l Ni^{2+} solutions by genetically engineered *E. coli* SE5000.

pH range, indicating a highly pH-dependent biosorption performance which was in accord with previous studies. The optimal pH for genetically engineered *E. coli* was 8.6 with Ni^{2+} binding capacity up to 6.38 mg/g, while 7.2 was the best pH for original host *E. coli* cells. No precipitation was observed when pH reached 9.0 in our study.

3.4. Effect of Na^{+}, Mg^{2+}, Ca^{2+} on Ni^{2+} bioaccumulation

Sodium, magnesium, and calcium are often found in contaminated waters. The presence of these cations may reduce the efficiency of ion exchange resins or biosorbents by elevating the ionic strength and/or by their competitive binding to the active sites of the ion exchange resins or biosorbents [18]. Chang and Hong [4] reported the mercury biosorption capacity of *Pseudomonas aeruginosa* PU21 (Rip64) was reduced by over 90% in the presence of 150 mM sodium chloride. To determine the effect of these cations on Ni^{2+} bioaccumulation by the genetically engineered *E. coli* cell, bioaccumulations were performed at various concentrations of Na^{+}, Ca^{2+} or Mg^{2+}, respectively. Fig. 5 shows that increases in Na^{+} and Ca^{2+} concentrations diminished Ni^{2+} accumulation to the similar extent. 39 and 42% of Ni^{2+} uptake reductions occurred, respectively as the concentrations of Na^{+} and Ca^{2+} reached 1000 mg/l, indicating that the presence of Na^{+} and Ca^{2+} posed a negative effect on Ni^{2+} bioaccumulation by genetically engineered *E. coli* cells. However, 61 and 58% of Ni^{2+} binding capacity having been retained still suggested the higher selectivity against these two cations by genetically engineered *E. coli* cells compared with biosorbents and ion exchange resins.

The presence of Mg^{2+} however accounted for a severe detrimental effect on Ni^{2+} bioaccumulation by genetically engineered *E. coli*. A rapid decrease of Ni^{2+} uptake from 6 to 5.15 mg/g, and further to 4.06 mg/g occurred as Mg^{2+} concentration reached only 10 and 30 mg/l, respectively, and genetically engineered *E. coli* cells lost almost all the Ni^{2+} accumulating capacity with Mg^{2+} concentration up to 200 mg/l. Such a serious deleterious effect of Mg^{2+} on Ni^{2+} uptake might be due to the interaction of Mg^{2+} to Ni^{2+} uptake system of microbes. Different from other heavy metals, nickel is an essential nutrient at low levels, therefore microbes have to absorb nickel from environment. Transport systems capable of nickel uptake have been identified and characterized in some microorganisms including *E. coli* [19], and some of them are magnesium-dependent systems. Webb [20] reported that under conditions of magnesium starvation, transport of Ni^{2+} in *E. coli* was through a magnesium transporter; as Mg^{2+} concentration increased, the transport system delivered Mg^{2+} instead of Ni^{2+}. Therefore, Mg^{2+} might inhibit Ni^{2+} uptake not only through endogenous transporters as previously studied, but also through the high Ni^{2+} affinity system expressed by *nixA* gene in genetically engineered *E. coli* cells.

3.5. Influence of other heavy metals on Ni^{2+} bioaccumulation

Compared with some other heavy metal ions, the Ni-binding capacity of many metal tolerant microorganisms was relatively low as mentioned above. An investigation of the accumulation of heavy metals by a variety of microorganisms showed that among the 83 microorganisms tested [21], *Streptomyces albus* HUT6047 was the best strain to accumulate Ni^{2+} with a maximum biosorption capacity of 1.057 mg/g from a solution containing 8 mg/l nickel and eight other heavy metal ions, much less than copper, mercury, lead, cobalt, and uranium. To evaluate Ni^{2+} selectivity over other heavy metals by genetically engineered *E. coli*, bioaccumulation experiments were processed in the presence of various concentrations of Hg^{2+}, Pd^{2+}, Cd^{2+}, Cr^{3+} or Cu^{2+}, respectively. As illustrated in Fig. 6, the genetically engineered *E. coli* cells still retained more than 55% of Ni^{2+} bioaccumulation activity as the concentra-

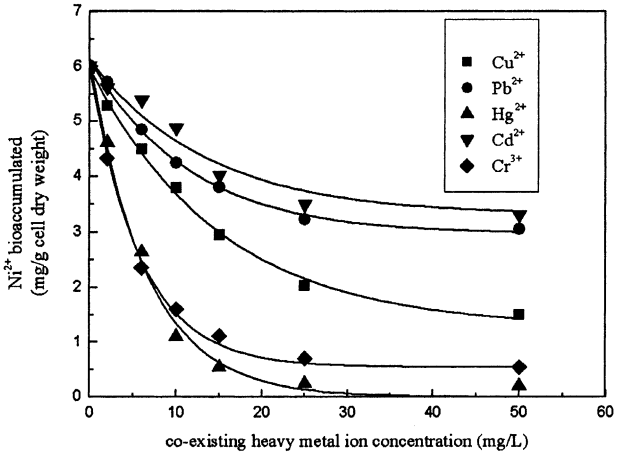

Fig. 6. Effect of Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} and Cr^{3+} on Ni^{2+} bioaccumulation from 10 mg/l Ni^{2+} solution by genetically engineered *E. coli* SE5000.

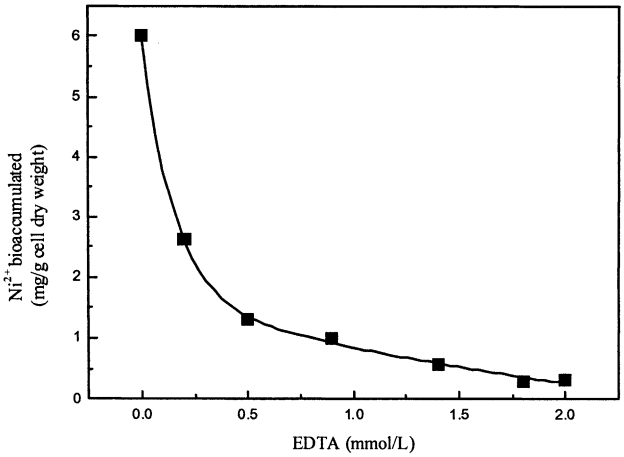

Fig. 7. Effect of EDTA on Ni^{2+} bioaccumulation from 10 mg/l Ni^{2+} solution by genetically engineered *E. coli* SE5000.

tions of lead and cadmium reached 50 mg/l, whereas 90 and 75% reductions of Ni^{2+} uptake capacities were shown with the concentrations of chromium (III) and copper up to 25 and 50 mg/l, respectively, indicating that chromium (III) and copper inhibited Ni^{2+} bioaccumulation by genetically engineered *E. coli* cells to a larger extent than lead and cadmium. Mercury posed the most serious inhibition on Ni^{2+} uptake with 97% reduction as Hg^{2+} concentration was 25 mg/l. The difference of inhibition by these heavy metals might somewhat correspond to the affinities of heavy metals to metallothionein. Winge and Nielson [22] reported that the order in terms of decreasing affinity of heavy metal to metallothionein was Hg > Cu > Cd > Zn > Ni > Co. However, it is still unknown why the inhibition of cadmium was not as severe as expected in our study.

3.6. Effect of EDTA on Ni^{2+} bioaccumulation

Metal chelators or complexing agents are frequently involved in metal contamination. The presence of these substances may form tight complexes with metals, which is thought to decrease their bioavailability and therefore cause difficulty in metal recovery by common treatments such as ion exchange and biosorption. To investigate whether Ni^{2+} bioaccumulation system is resistant to the presence of metal chelator, genetically engineered *E. coli* SE5000 cells were suspended in 10 mg/l Ni^{2+} solution with EDTA from 0 to 2 mmol/l. The result (Fig. 7) showed that addition of EDTA inhibited Ni^{2+} accumulating seriously. As the concentration of EDTA reached 2 mmol/l, only 5% Ni^{2+} binding capacity of the induced cells was retained. It indicated that the tight complex formed between EDTA and nickel was not available to the genetically engineered cells. However, Chen and Wilson [6] found genetically engineered bacteria which express metallothionein and an Hg^{2+} transport system could accumulate Hg^{2+} effectively in the presence of EDTA. The differences between the Ni^{2+} accumulating strain and the Hg^{2+} accumulating strain may be due to the forms in which the metal and metal–EDTA complex are transported. Although the *nixA* protein has a much higher affinity for Ni^{2+} than the merT–merP system has for mercury [23], the transport in the Hg^{2+} accumulating strain might involve uptake of both Hg^{2+} and Hg^{2+}–EDTA complex, while the Ni^{2+} accumulating strain was only able to transport free Ni^{2+} in aqueous solutions.

4. Conclusions

The following conclusions were drawn from the present study.

1. Genetically engineered *E. coli* SE5000 could bioaccumulate Ni^{2+} rapidly from aqueous solution, and bioaccumulation process followed linearized Langmuir isotherm.
2. The recombinant *E. coli* SE5000 accumulated Ni^{2+} effectively over a broad pH range (4–10), suggesting that the Ni^{2+} uptake process was resistant to pH variations.
3. 1000 mg/l of Na^{+} or Ca^{2+}, and 50 mg/l of Cd^{2+} or Pb^{2+} did not decrease Ni^{2+} uptake significantly, while 200 mg/l of Mg^{2+}, and 50 mg/l of Cu^{2+}, Cr^{3+} or Hg^{2+} inhibited Ni^{2+} uptake to a large extent.
4. The presence of EDTA largely reduced Ni^{2+} uptake by recombinant *E. coli* SE5000.

Acknowledgements

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