Study on the process, thermodynamical isotherm and mechanism of Cr(III) uptake by *Spirulina platensis*

Zhi-Yong Li a,*, Si-Yuan Guo b, Lin Li b

a School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, PR China
b School of Food and Bioengineering, South China University of Technology, Guangzhou 510641, PR China

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Abstract

The process, thermodynamical isotherm and mechanism of Cr(III) uptake by *Spirulina platensis* were investigated in this paper. At the beginning of Cr uptake, Cr is physically adsorbed to the surface of algal cell by electrostatic attraction. Finally, chemical complexation through ion-exchange with K⁺, Mn²⁺, Ca²⁺, Mg²⁺, Na⁺, Fe³⁺, Zn²⁺, H⁺, etc. ions by reversible or irreversible strategies plays the main role in Cr uptake. Most of the absorbed Cr are steadily bound to proteins, polysaccharides, lipids, etc. biological ligands, whereas only a very small part of Cr are loosely adsorbed on the algal surface. Langmuir isotherm model seems to be suitable for describing Cr uptake than Freundlich isotherm model. pH is the most important factor influencing Cr uptake as well as anions, cell concentration, Cr concentration, temperature, light. Scatchard analysis and isotherm analysis indicate multi types binding sites for Cr and multi interactions between Cr and alga. Bioaccumulation strategy is suggested to be involved in Cr uptake besides biosorption e.g. adsorption and chemical complexation. *S. platensis* is of efficiently capable in the uptake of Cr(III), consequently high-value *Spirulina* can be produced.

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Keywords: *Spirulina platensis*; Cr(III); Adsorption; Complexation; Ion exchange; Bioaccumulation

1. Introduction

The toxicity of chromium depends on its chemical form in which the trivalent form has a low toxicity. Chromium (III) is an essential trace element required for normal carbohydrate, protein, nucleic acid and lipid metabolisms, which can activate certain enzymes, stabilize proteins and nucleic acids (Anderson, 1987; Okado, Tsukada, & Ohba, 1984), improve insulin sensitivity, protect against glucose intolerance by taking part in the glucose tolerance factor (GTF) (Anderson, 1998a, 1998b). Chromium also functions in corticosteroid metabolism and preservation of bone density (McCarty, 1995; Ravina, Slezak, Mirsky, Bryden, & Anderson, 1999).

It has been reported that Cr(III) supplementation to diets of animals and human being can normalize blood glucose levels (Anderson, 1994; Anderson, Bry, Polansky, & Gautschi, 1996) and has a positive effect on poultry growth rate (Lien, Hormig, & Yang, 1999; Sahin, Kütcük, Sahin, & Ozbey, 2001). Some authors have reported beneficial effects of dietary supplementation with Cr(III) (Van Cauwenbergh, Hendrix, Robberecht, & Deelstra, 1996). So the intake through the diet is the most important route of Cr(III) entry into human being. According to the US National Research Council (1989) the recommended dietary intake for adults is 50–200 µg/day. Now people are trying to develop some high-value foods enriched with Cr(III) to prevent disease such as diabetes.
Spirulina, a blue green microalga, has been used as a source of health foods since ancient times for its high protein and nutritional components e.g. polyunsaturated fatty acids and phycocyanin which are rare in plant and animal sources. At the present time there are growing demands for various natural products that can be produced from Spirulina on the basis of enhanced nutritional value (Mosulishvili et al., 2002).

Trace elements play an important role in human metabolism. Spirulina platensis enhanced with trace elements will have a strengthen biological value than ordinary one. We have successfully cultured high-value S. platensis rich in organic selenium (Li, Guo, & Li, 2003), which have been proved to have stronger ability for anti-oxidation, anti-aging, resistance to fatigue and enhancement of immunity than ordinary S. platensis.

In general, organic Cr(III) will be absorbed easily by people than inorganic Cr(III), but Cr content in natural Spirulina is very low (e.g. 5.41 mg/kg). Based on the above bioeffects of Cr(III) we think it will strengthen Spirulina’s biological function if Cr content is improved, so we pay our attention to Cr(III) uptake in S. platensis. Cogne, Lehmann, Dussap, and Gros (2003) studied Zn, Mg, Fe, Mn, Cu, K uptake by Spirulina, but Cr was not involved. Up to now, study on Cr uptake by Spirulina is very rare. The objective of the present study was to investigate the process, thermodynamical isotherm and mechanism of Cr(III) uptake by S. platensis. The understanding of Cr(III) uptake will be helpful for us to produce high-value Spirulina enriched with Cr(III) and ensure Cr content in the safe range for people.

2. Materials and methods

2.1. Strain and cultivation

S. platensis was obtained from South China Normal University, Guangzhou, P.R. China and was batch cultivated under 35 °C, 315 μE·m⁻²·s⁻¹ for 5 days in Zarrrouk medium (Zarrrouk, 1966), 11 medium consist of 1.00 g NaCl, 0.04 g CaCl₂, 2.50 g NaNO₃, 0.01 g FeSO₄·7H₂O, 0.08 g Na-EDTA, 1.00 g K₂SO₄, 0.20 g MgSO₄·7H₂O, 16.80 g NaHCO₃, 0.50 g K₂HPO₄ and 1 ml trace elements solution A: ZnSO₄·7H₂O (0.22 g/l), CuSO₄·5H₂O (0.08 g/l), MoO₃ (0.015 g/l), H₃BO₃ (2.86 g/l), MnCl₂·4H₂O (1.81 g/l) and 1 ml trace elements solution B: K₂Cr(7H₂O)₂·12H₂O (0.096 g/l), NiSO₄·7H₂O (0.048 g/l), Na₂MoO₄·2H₂O (0.018 g/l), Co(NO₃)₂·6H₂O (0.049 g/l).

Biomass dry weight was determined as follows: 50 ml cultures were filtered onto preweighted filter paper, washed with distilled water to remove medium and dried at 60 °C to constant weight. The cell dry weight was an average of three experimental results.

2.2. Cr(III) uptake and mineral content analysis

Cr(III) will precipitate at pH beyond 7 because of the existence of OH⁻ ions in the solution. Because the pH of Zarrrouk medium is above 8, so all the Cr(III) uptake treatments were carried out using fresh living S. platensis after 5 days cultivation. At the same time of cell dry weight analysis, 50 ml cultures were filtered by filter paper, washed three times with distilled water and resuspended in 50 ml Cr(III) solution of different initial concentration in 150 ml Erlemeyer flask, then mixed in a temperature controlled gyratory shaker. All Cr(III) uptake experiments were done in dark except for light effect study. CrCl₃·6H₂O was used except for anions effect study.

After centrifugation (4000 g, 10 min) Cr content in supernatant was analyzed by AAS (PE3100, USA). The Cr uptake quantity was calculated according to the Cr concentration difference before and after Cr uptake by an equation as follows:

\[ q = \frac{(C_1 - C_2)IM}{W} = \frac{(C_1 - C_2) \times 50 \times 52}{W} \]

where \( q \) is absorbed quantity (mg/g), \( C_1 \) is initial Cr concentration (mM), \( C_2 \) is Cr concentration at equilibrium (mM), \( V \) is treatment volume (ml), \( M \) is atomic weight of Cr(52) and \( W \) is algal cell dry weight (g) of 50 ml algal sample.

In the case of time course and light effect tests, samples were analyzed in an interval from 10 min to 420 min. As for the studies of influencing factors and thermodynamical isotherm, Cr uptake were finished in 60 min.

In complexation formation study, alga was extracted by 200 ml solution of CH₃OH/CHCl₃ (1/3), CH₂OHCH₂OH (72%), NaOH (0.1 M), HCl (1.2 M) for 30 h at 25 °C mainly according to the methods of Kuyucak and Volesky (1989). The residues after centrifugation (4000 g, 10 min) and washing by distilled water were used for Cr uptake using the untreated alga as control.

S. platensis sample with 10.3 mg/g Cr after three times washing by distilled water was used for mineral content comparison by ICP-AES (OPTIMA 3000DL(P-E), USA). All experiments and analysis were done in triplicate, pH was adjusted by 0.01 M HCl and 0.01 M NaOH and analyzed by digital pH reader (pHS-ZQA, China).

2.3. Cr(III) desorption

In order to understand the mechanism of Cr uptake in depth, both continuous and batch desorption experiments were carried out with different eluant solution using S. platensis sample with 10.3 mg/g Cr.
A small chromatographic column (30 cm high and 2 cm in inner diameter) fixed with alga was used for continuous desorption by 0.2 M and 0.4 M Na-EDTA at a stable elution rate of 1 ml/min. Cr content in eluant solution was analyzed by AAS (PE3100, USA), and the yield of Cr desorption was decided by the Cr concentration increase in eluant solution. In the case of batch desorption, *S. platensis* was resuspended in distilled water or different concentration of acids solution and mixed for 1 h at 25 °C. The desorption yield was an average of the results of three parallel treatments.

2.4. Statistical analysis

All experiments were carried out in triplicate. Statistical analysis was carried out using the Microsoft EXCEL 6.0 statistical package calculating mean and standard error. One-way ANOVA was used to determine the significant difference at $P < 0.05$.

3. Results and discussion

3.1. Cr uptake process

3.1.1. Time course

Time course of Cr uptake is presented in Fig. 1. It is obvious that Cr uptake is rapid, an equilibrium can approximately be established within 60 min between absorbed Cr ions on the algal cell and unabsorbed metal ions in solution. For instance, at 141.96 mg/l initial Cr, 23.4 mg Cr per gram of alga amounting to 95.12% of the total absorbed Cr in 420 min (24.6 mg/g) was obtained in 60 min. The process can be mainly divided into two stages rapid increases at the very beginning of the incubation (e.g. 20 min) followed by slow uptake. In the second period, Cr uptake takes place slowly. The similar two-stage process was observed at 70.98 mg/l initial Cr concentration at the same condition, and the higher is the initial Cr concentration, the larger amount is the Cr taken up. The time course of Cr uptake is in agreement with the result of other metal ions uptaken by alga (Thomas, Bohumil, & Alfonso, 2003).

3.1.2. Uptake isotherms

The contact time of 60 min in dark was chosen for the determination of Cr uptake isotherm (Fig. 2). The isotherm shows that the metal uptake increase as the equilibrium concentration increase and also depends on biomass quantity. Thermodynamical equilibrium can be quickly reached at lower quantity alga.

Scatchard analysis is widely used to investigate the characteristics of the adsorption process. The linearised Scatchard isotherm model is $\frac{q}{C} = \frac{q_m k_b}{q_k b} - \frac{q_m}{q_k b}$, where $q$ and $C$ are the equilibrium ligand adsorption capacity of the resin and the equilibrium ligand concentration in the aqueous solution, respectively. $q_m$ and $K_b$ are the adsorption isotherm parameters. According to Fig. 3, the curvilinear regression equations are $y = -0.3228x + 2.4657 \ (r^2 = 0.7741)$ and $y = -0.2481x + 1.9376 \ (r^2 = 0.8047)$ for 1.20 g/l and 2.45 g/l alga, respectively. Obviously, the Scatchard plot shows an evident deviation from linearity, which suggests the presence of more than one type of binding sites during Cr uptake.

To test the fit of data, firstly the Langmuir isotherm model $\frac{q}{C} = \frac{1}{K q_{\text{max}}} + \frac{1}{q_{\text{max}}} C$ was applied in our study where $q$ is the absorbed Cr per gram of dry alga (mg/g), $K$ is the equilibrium constant, $C$ is the Cr concentration at equilibrium (mg/l) and $q_{\text{max}}$ is absorbed metal at saturation. In Fig. 4 the data provide a better fit to the Langmuir isotherm model with higher correlation coefficients 0.9818 and 0.9296 for 2.45 g/l and 1.20 g/l alga, respectively.

We also examined the adsorption process of Cr in Fig. 5 using the Freundlich equation $q = K C^{1/n}$, where $q$ is the absorbed Cr per gram of dry alga (mg/g), $C$ is the Cr concentration at equilibrium (mg/l) and $K$ is the higher is the initial Cr concentration, the larger amount is the Cr taken up. The time course of Cr uptake is in agreement with the result of other metal ions uptaken by alga (Thomas, Bohumil, & Alfonso, 2003).

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the equilibrium constant. By taking logarithms of both sides of the above equation we obtain \( \lg q = \lg k + \frac{1}{n} \lg C \). Although a plot of \( \lg q \) vs \( \lg C \) is linear over the tested Cr concentration range, the curvilinear regression equations are \( y = 0.2674x + 0.0532 \) with \( R^2 = 0.9818 \) and \( y = 0.1505x + 0.0529 \) with \( R^2 = 0.9296 \) for 2.45 g/l and 1.20 g/l alga, respectively.

In general, when adsorption is the main strategy for metal uptake, if the Scatchard plot shows a deviation from linearity, the Freundlich model should be applied for describing adsorption process, otherwise Langmuir isotherm model will be used (Oztürk, Artan, & Ayar, 2004). Obviously, in our study an evident deviation from linearity was observed in Scatchard analysis, the Langmuir isotherm model was proved to be better than Freundlich isotherm model for describing Cr(III) uptake by \( S. \) platensis, which indicates us a different strategy from adsorption in Cr uptake to some extent.

Subsequently, the saturation capacity of \( S. \) platensis toward Cr(III) was evaluated according to Langmuir isotherm model. When algal concentration is 1.20 g/l the equilibrium constant \( K \) is 0.35, which is greater than 0.20 for 2.45 g/l alga. This means that, at the same Cr initial concentration, Cr uptake is quickly at lower concentration alga because of plentiful Cr for single algal cell. Interestingly, at 141.96 mg/l initial Cr, the \( q_{\text{max}} \) in theory is 18.87 mg/g based on Langmuir isotherm model, which is only 76% of the actual total absorbed Cr 24.6 mg/g. The difference between actual value and the ideal value suggests us a mutimodel interaction for Cr uptake besides adsorption.

### 3.1.3. Effects of Cr associated anions

The effects of anionic ligands \( \text{Cl}^- \), \( \text{SO}_4^{2-} \) and \( \text{NO}_3^- \) on Cr uptake are presented in Table 1. \( \text{NO}_3^- \) exhibits the strongest Cr uptake inhibition because of the strong binding between Cr\(^{3+} \) and \( \text{NO}_3^- \) followed by \( \text{SO}_4^{2-} \), \( \text{Cl}^- \).

### 3.1.4. pH effect

pH effects on Cr uptake are compared in Fig. 6. It was obvious that the uptake of Cr(III) from aqueous solution is pH-dependent with more efficient at higher pH. This result is consistent with the study of Schiewer

### Table 1

<table>
<thead>
<tr>
<th>Cr source</th>
<th>Cr uptake (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CrCl}_3 \cdot 6\text{H}_2\text{O} )</td>
<td>18.20 ± 0.18</td>
</tr>
<tr>
<td>( \text{CrK(SO}_4)_2 \cdot 12\text{H}_2\text{O} )</td>
<td>9.81 ± 0.02</td>
</tr>
<tr>
<td>( \text{Cr(NO}_3)_3 \cdot 9\text{H}_2\text{O} )</td>
<td>6.33 ± 0.05</td>
</tr>
</tbody>
</table>

Note: values are means ±SD (\( n = 3 \)). Alga concentration is 2.45 g/l, Cr is 70.98 mg/l, treatment volume is 50 ml.
and Volesky (1995). The pH-dependent Cr uptake can be largely related to the overall surface charge on the cells. Positive charge at lower pH will inhibit the approach of positively charged metal ions. It is also likely that proton will compete with metal ions for the ligands and thereby decrease the interaction of metal ions with the algal cells, which have been proved by the lower absorbed Cr at lower pH in our experiments.

3.1.5. Light effect

The time course comparison of Cr uptake by S. platensis in dark and under light (315.2 μEm⁻² s⁻¹) at the same condition is shown in Fig. 7. In the further course of incubation a greater increase of Cr uptake under light was observed which indicated an energy-dependent strategy to some extent.

3.1.6. Temperature effect

According to Fig. 8, temperature effect on Cr uptake is relatively smaller than pH. Because adsorption is an exothermic reaction and therefore metal uptake will decrease as temperature increase. Certain Cr uptake increases with temperature suggests a strategy different from adsorption again.

3.2. The mechanism of Cr uptake

3.2.1. Complexation formation

In order to reveal the correlative mechanisms of Cr uptake, several different types of algal pretreatment were applied to elucidate the role of individual cellular component in Cr uptake. A very pronounced decrease in Cr uptake was observed after alga being extracted with 0.1 M NaOH and 1.2 M HCl. Alkali can extract proteins and polysaccharides from algal cells. Concentrated HCl can destroy the structure of alginate chains in algal cells with simultaneous hydrolysis of polysaccharides and may be responsible for the release of ionic species into the solution (Kuyucak & Volesky, 1989). Meanwhile, Cr uptake decrease was found after polysaccharides in the algal cell wall were removed by ethanol pretreatment. The same phenomenon was observed after cellular lipids was extracted with a methanol–chloroform mixture. These results shown that chemical complexation of Cr with algal cell components really take place during Cr uptake, in which proteins, polysaccharides, lipids, etc. act as ligands for Cr binding.

The algal cell walls contain a large number of polysaccharides and proteins. In general, proteins make up to 50–70% of S. platensis cell dry weight. Amino acids in the proteins can provide such functional groups as –NH2, –CONH–, –COO–, imidazole, etc. The polysaccharides of the algal cell wall can also provide the amino and carboxyl group as well as sulphate. The amino and carboxyl groups, imidazole of histidine and nitrogen and oxygen of the peptide bond can be available for bonding with metallic ions like Cr(III) which can also be electrostatically bonded to unprotonated carboxyl oxygen and sulphate residues (Crist, Oberholser, Shank, & Nguyen, 1981). By this way, Cr-ligand compounds can be formed finally by covalent or ionic charge bonding strategy.
3.2.2. Ion exchange

The above results suggest that Cr-ligand compounds can be formed during Cr uptake. Hence, it is very essential to reveal the mechanism of Cr-ligand compounds formation. The comparison of algal mineral contents before and after Cr uptake is given in Table 2.

As seen in Table 2, a lot of metal ions e.g. K\(^+\), Mn\(^{2+}\), Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\), Fe\(^{3+}\), Zn\(^{2+}\), etc. were released during Cr(III) uptake by *S. platensis*, for instance, 99.78% K\(^+\), 82.75% Mn\(^{2+}\), 81.52% Ca\(^{2+}\), 80.22% Mg\(^{2+}\), 63.11% Na\(^+\), 52.52% Fe\(^{3+}\) and 15.79% Zn\(^{2+}\) were displaced by Cr(III), respectively. This indicates us that Cr(III) is able to bind with algal cell components instead of correlative metal ions there. At the same time, pH decrease after Cr uptake was observed, which also suggested the exchange of proton by Cr(III). So, we think Cr(III) is able to bind with algal cell components instead of correlative metal ions there. At the same time, pH decrease after Cr uptake was observed, which also suggested the exchange of proton by Cr(III). So, we think Cr(III) can bind to biologic ligands such as proteins, polysaccharides and lipids in *S. platensis* to form complexation compounds by displacing K\(^+\), Mn\(^{2+}\), Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\), Fe\(^{3+}\), Zn\(^{2+}\), H\(^+\), etc. ions. Based on our results, Cr binding to ligands in algal cells with ion exchange mechanism maybe the main strategy for complexation. Earlier studies on other biomass have shown that metal uptake by biological materials normally involve ion exchange with correlative ions present on cell walls (Thomas et al., 2003). To our knowledge, it is the first time to observe the phenomenon of ion exchange during metal uptake by *Spirulina*.

3.2.3. Stability of Cr binding

Up to now, we get some information about Cr uptake process and mechanism, but whether Cr binding with algal ligands is steady or not is still a task. In the following experiments, we attempt to evaluate the stability of Cr binding by elution. In our experiment, about 8.13% Cr could be removed easily from alga which meant that a small part of Cr was loosely adsorbed on the surface of alga by physical adsorption. On the other hand, this indicates us that most of the physical adsorbed Cr are transformed into algal cells by chemical strategy finally.

The result of Cr elution by sodium ethylene diamine tetra acetic acid (Na-EDTA) in a continuous way is shown in Fig. 9. Na-EDTA shows a lower desorption capacity for absorbed Cr, for instance, only 12.6% and 18.8% Cr was obtained in 225 ml 0.2 M and 0.4 M Na-EDTA elution, respectively. Interestingly, it was found that the alga could be used again for Cr uptake after Cr desorption and the amount of absorbed Cr corresponded to the recovered Cr by Na-EDTA elution, which meant that a part of Cr could be absorbed by *S. platensis* through a reversible approach.

The result of Cr elution with strongly acidic HCl (pH < 1.7) is presented in Fig. 10. We can find that
acids such as HCl–HNO₃ (1/1) was used, which means HCl. But only 92% Cr was recovered even if stronger tion. 54.4% absorbed Cr could be removed by 12 M HCl exhibits better desorption capacity than Na-EDTA as well as anions, cell concentration, Cr(III) concentra-
tion, temperature, light. Bioaccumulation approach is
suggested in Cr uptake besides biosorption strategies adsorption and chemical complexation.

4. Conclusions

S. platensis is of efficiently capable in Cr(III) uptake, so it is possible to improve Cr content, consequently the algal bioeffects and nutrition can be enhanced. Of course, on the other hand, suitable Cr initial concentra-
tion should be taken into consideration to ensure Cr amount in the safe range for human being.

At the beginning of Cr uptake, Cr is physically adsorbed to the unoccupied, negative sites on the sur-
face of algal cell walls by electrostatic attraction. Synchronously or subsequently, chemical complexation, which plays the main role in Cr uptake, take place through ion exchange strategy instead of K⁺, Mn²⁺,
Ca²⁺, Mg²⁺, Na⁺, Fe³⁺, Zn²⁺, H⁺, etc. ions. As a result, most of the adsorbed Cr are bound to algal cell compo-
nents such as proteins, polysaccharides, lipids, etc. final-
ly, whereas only a very small part of Cr is still adsorbed by physical adsorption. The complexation of Cr with biological ligands can be formed through reversible or irreversible strategies and most of the bound Cr are re-
latively steady.

Scatchard analysis and isotherm analysis indicate multi types binding sites for Cr and multi interactions between Cr and alga. Langmuir isotherm model seems to be suitable for describing Cr(III) uptake than Freundlich isotherm model. As for the factors for Cr uptake, pH is the most important factor influencing Cr uptake

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