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Biosorption of Metal Ions with *Penicillium chrysogenum*

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Abstract

Biosorption of metal ions with *Penicillium chrysogenum* mycelium is described in this article. Alkaline pretreatment was used to remove proteins and nucleic acids from cells, and this treatment increased the adsorption capacities, for Cr^{3+} from 18.6 mg g^{-1} to 27.2 mg g^{-1}, for Ni^{2+} from 13.2 mg g^{-1} to 19.2 mg g^{-1}, for Zn^{2+} from 6.8 mg g^{-1} to 24.5 mg g^{-1}. The adsorption of metal ions was strongly pH dependent. The mycelium could be used for large-scale removal of Cr^{3+} from tannery wastewater. The results show that this inexpensive mycelium adsorbent has potential in industry because of its high adsorption capacity. The main chelating sites are amino groups ($-NH_2$) of chitosan in the mycelium. A new model is established, which describes the relation of adsorption of metal ions on pH according to amino group chelating with metal ions and H^+. The relative errors of simulation for Cu^{2+}, Ni^{2+}, Zn^{2+}, and Cr^{3+} are 4.66%, 5.45%, 11.55%, and 1.69%, respectively.

Index Entries: *Penicillium chrysogenum*; mycelium; adsorption.

Introduction

The use of microorganisms as biosorbents for metal ions offers a potential alternative for existing methods such as ion exchange and precipitation for detoxification or recovery of valuable metals from industrial wastewater *(1)*. The biosorption of metal ions can be obtained by different microorganisms such as yeast and fungi *(2)*. For biosorption, mycelium, a byproduct of fermentation, is inexpensive, easy to manipulate, highly selective, and readily available. The chelating sites in those mycelia include amines, carboxyl groups, and hydroxyl groups, which have high binding ability for metal ions. Many fermentation processes such as penicillin and

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citric acid production produce a large quantity of biomass that has high metal ion adsorption capacity due to the large amounts of chitosan in the cell wall, which accounts for 3–40% of cell wall (dry) *(3)*.

Much work has been done on adsorption of metal ions using different mycelia *(2,4)*. For example, Puranik studied the parameters influencing adsorption of metal ions *(4)*, in which the adsorption was strongly pH dependent. Until now few reports have been made on the adsorption model of metal ions by biomass. Chang used a Langmuir model to describe bioseparation *(5)*. The experimental results of Puranik were in accordance with Freundlich and Langmuir models *(4)*. However, these models cannot explain the adsorption of metal ions influenced by pH. Schiewer suggested a two-binding-site model for adsorption of metal ions on mycelium, in which adsorption of metal ions mainly contributed to carboxyl and thiol groups *(6)*. The adsorption capacity is the sum of the two-site-binding capacities. The model was too complicated due to six constants used in the equation and, in many cases, the thiol groups did not play the key role in adsorption of metal ions. A new model, in which the adsorption of metal ion mainly contributes to the chelating by the amino groups of chitosan in the mycelium, is suggested in this article.

Materials and Methods

Chemicals

The mycelium of *Penicillium chrysogenum* was obtained from North China Pharmaceutical Co. The wastewater was supplied by Shangdong Tanning factory. All other chemicals are of analytical grade.

Pretreatment of Mycelium from Penicillin Production

The proteins and nucleic acid were removed according to the procedure in ref. *7*: 1 kg mycelium (dry) was added into 10 dm^3 0.5 *M* NaOH and was stirred in a water bath at 70°C for 3 h to release proteins and nucleic acid from the cells. The suspension was filtered and the residual mycelium was washed first with tap water and then with deionized water until a pH 7.0–8.0 was achieved. The residual mycelium was dried at 50°C under vacuum. The chitosan in the mycelium was extracted by a 10 vol (w/v) 2% acetic acid solution at 80°C for 10 h. The residual mycelium was obtained by filtration and was dried as described above.

Biosorption Experiment

Twenty-five cubic centimeters of heavy metal ion solution (metal ion concentration: 100 mg dm^{-3}) was added to a 50 cm^3 flask. The pH was adjusted to pH 4.0–5.0 by adding 1 *M* NaOH or 1 *M* HCl; 100-mg dry mycelium was added and the mixture was stirred at 30°C for 12 h. The metal ion concentration in the supernatant was determined, and the adsorption capacity was determined by a mass balance calculation.

Table 1
Adsorption Capacities of Metal Ions on Different Mycelium (pH 5.0)

	Q (Cr^{3+}) mg g^{-1}	Q (Ni^{2+}) mg g^{-1}	Q (Zn^{2+}) mg g^{-1}
Mycelium	18.6	13.2	6.8
Mycelium pretreated	27.2	19.2	24.5
Mycelium after separation of chitosan	13.1	8.2	9.6

Scale Up of Biosorption

Fifty kilograms of mycelium was packed into a column 200 cm in length and 30 cm in diameter. The expended bed was obtained at flow rate between 10 and 20 m^3 h^{-1} from the bottom of the column.

Five cubic meters of wastewater from the tanning factory (Cr^{3+} concentration 1500 mg dm^{-3}) was pretreated by an alkaline precipitation, in which the pH was adjusted to pH 7.0–8.0 by adding CaO powder. The precipitate was removed by filtration and the Cr^{3+} concentration in the filtrate was reduced from 1500 to about 49 mg L^{-1}. The filtrate was pumped through the column from the bottom at a flow rate of 1.25 m^3 h^{-1}. The concentration of Cr^{3+} in the elute was measured and was controlled under 1.5 mg L^{-1}.

Analytical Methods

The metal ion Cu^{2+} content was analyzed with an adsorption spectrophotometer according to the method described by Ma *(8)*. Ni^{2+} and Zn^{2+} were determined by the method of Luo and Cen *(9)*. The Cr^{3+} concentration was measured according to *Analytical Method for Water Detection (10)*.

The glucosamine content in the mycelium was analyzed by the method described by Bitter *(11)*. One gram sample was added into a flask with 6 mL 6 *M* HCl and the hydrolysis was carried out at 95°C for 3 h, then the glucosamine content in the supernatant was determined.

Results and Discussion

Adsorption of Metal Ion

An alkaline treatment removed proteins and nucleic acids from cells. As a result, the metal ions in solution could more easily touch the chelating sites and the adsorption capacities were enhanced (Table 1).

The residual mycelium after alkaline treatment mainly contained polysaccharides such as chitosan and cellulose. According to the glucosamine determination, the chitosan in the residual mycelium was about 25%–30%. After the chitosan had been removed by acid extraction, the adsorption capacities of metal ions on the mycelium were reduced significantly. Therefore, the main chelating groups for metal ions were $-NH_2$ groups in the chitosan.

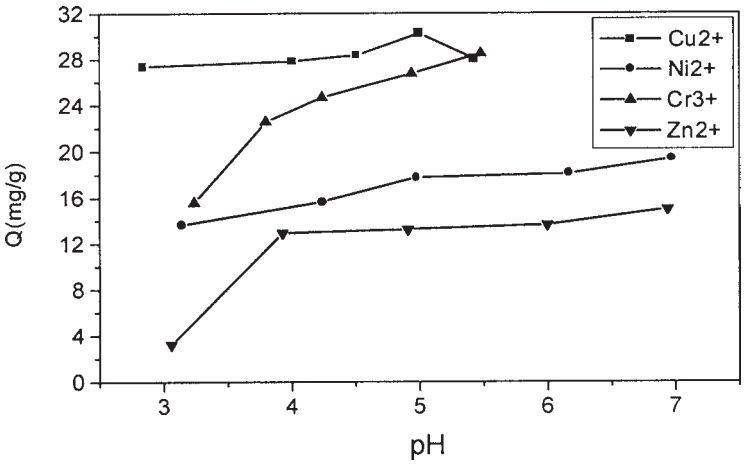

Fig. 1. Effect of pH on adsorption.

Table 2
Desorption of Metal Ion Cr^{3+}

Acid used	Concentration	Desorption (%)
HCl	0.5 *M*	100
HNO_3	0.5 *M*	95
Citric acid	0.5 *M*	86

Table 3
Reuse of Penicillin Mycelium for Biosorption of Cr^{3+}
(Cr^{3+} Concentration 100 mg/L)

Reuse batch	1	2	3	5	6	7
Kinetic adsorption capacity (mg g^{-1})	22.5	24.0	25.3	20.4	21.5	20.0

In chitosan chelating, the pH is an important parameter for the adsorption and its influence is shown in Fig. 1. The optimum pH values for adsorption of different metal ions are Cr^{3+} 5.0–5.5, Cu^{2+} 4.5–5.5, Ni^{2+} 5.0–7.0 and Zn^{2+} 6.0–7.0, respectively.

The desorption was carried out by different acids (Table 2) and 0.5 *M* HNO_3 should be used.

The mycelium had been used in large-scale treatment for removing Cr^{3+} from tannery wastewater. Reuse of the mycelium for biosorption in industry is shown in Table 3. The treatment capacity reached 13-ton wastewater per cubic meter column bed per hour and the process was carried out continuously for 2 mo. The scale up of the process indicated that the mycelium of penicillin production could be reused several times without

reducing the adsorption capacity and thus had potential for large-scale application.

Adsorption isotherms of metal ions were described with Langmuir model and Freundlich model (Fig. 2), which were in agreement with the results of Puranik *(4)*.

Adsorption Model

The adsorption of metal ions mainly depended on the $-NH_2$ groups of chitosan in the mycelium. The chitosan is simplified to many glucosamine units, each unit is represented as RNH_2. The adsorption of metal ion on glucosamine in chitosan could be expressed in the following forms:

$$RNH_2 + H^+ \overset{K_1}{\longleftrightarrow} RNH_3^+$$

$$RNH_2 + \frac{1}{n} M_e^{n+} \overset{K_2}{\longleftrightarrow} (RNH_2)\left(\frac{1}{n} M_e^{n+}\right)$$

$$K_1 = \frac{[RNH_3^+]}{[RNH_2]_{eq}[H^+]} \tag{1}$$

$$K_2 = \frac{\left[(RNH_2)\left(\frac{1}{n} M_e^{n+}\right)\right]}{[RNH_2]_{eq}[M_e^{n+}]_{eq}^{1/n}} \tag{2}$$

where K_1 and K_2 are equilibrium constants. According to the mass balance, total glucosamine concentration $[RNH_2]$ (mol dm^{-3}) can be given by the following equation:

$$[RNH_2]_0 = [RNH_2]_{eq} + [RNH_3^+] + \left[(RNH_2)\left(\frac{1}{n} M_e^{n+}\right)\right] \tag{3}$$

For the metal ion balance:

$$[M_e^{n+}]_0 = [M_e^{n+}]_{eq} + \frac{1}{n}\left[(RNH_2)\left(\frac{1}{n} M_e^{n+}\right)\right] \tag{4}$$

$[M_e^{n+}]_0$ and $[M_e^{n+}]_{eq}$ is initial concentration and equilibrium concentration of metal ion (mol dm^{-3}), respectively.

The adsorption capacity Q(mg g^{-1}) can be calculated from the following equation:

$$Q = \frac{([M_e^{n+}]_0 - [M_e^{n+}]_{eq})VW_m}{W_s} \tag{5}$$

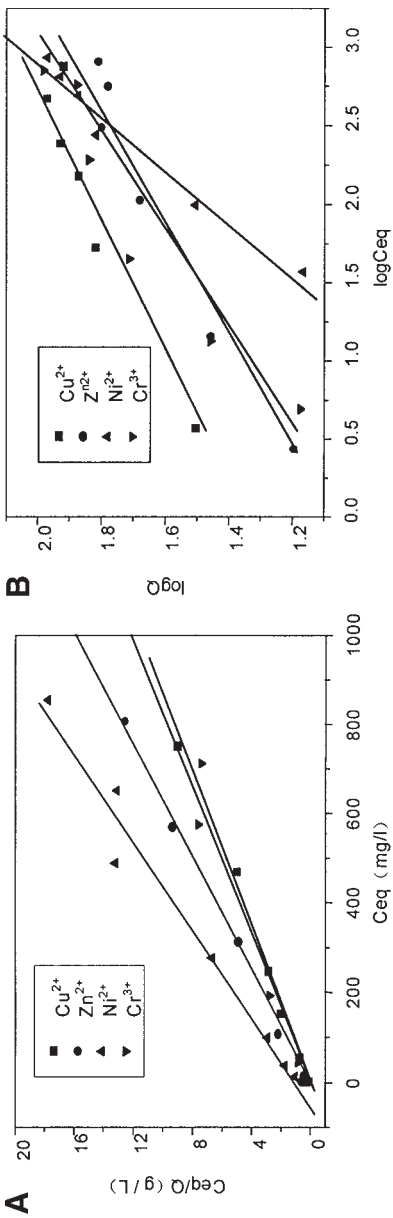

Fig. 2. Adsorption of isotherms of metal ions. **(A)** Langmuir model; **(B)** Freundlich model.

where V is the volume of solution (dm^{-3}), W_m and W_s are metal molecular weight (g mol^{-1}) and sample weight (g), respectively.

From Eqs. 3–5:

$$Q = \frac{\left[(RNH_2)\left(\frac{1}{n}M_e^{n+}\right)\right]VW_m}{nW_s} = \frac{K_2[RNH_2]_{eq}[M_e^{n+}]_{eq}^{1/n}VW_m}{nW_s} \quad (6)$$

$$[RNH_2]_0 = [RNH_2]_{eq} + K_1[RNH_2]_{eq}[H^+] + K_2[RNH_2]_{eq}[M_e^{n+}]_{eq}^{1/n} \quad (7)$$

rearranging Eq. 7 gives

$$[RNH_2]_{eq} = \frac{[RNH_2]_0}{1 + K_1[H^+] + K_2[M_e^{n+}]_{eq}^{1/n}} \quad (8)$$

From Eqs. 8 and 7

$$Q = \frac{K_2[RNH_2]_0[M_e^{n+}]_{eq}^{1/n}V}{nW_s/W_m\,(1 + K_1[H^+] + K_2[M_e^{n+}]_{eq}^{1/n})} \quad (9)$$

For divalent metal ions such as Cu^{2+}, Ni^{2+}, and Zn^{2+}, $n = 2$:

$$Q = \frac{K_2[RNH_2]_0\sqrt{[M_e^{2+}]_{eq}}V}{2W_s/W_m\,(1 + K_1[H^+] + K_2\sqrt{[M_e^{2+}]_{eq}})} \quad (10)$$

For trivalent metal ions such as Cr^{3+}, $n = 3$:

$$Q = \frac{K_2[RNH_2]_0\sqrt[3]{[M_e^{3+}]_{eq}}V}{3W_s/W_m\,(1 + K_1[H^+] + K_2\sqrt[3]{[M_e^{3+}]_{eq}})} \quad (11)$$

For comparison of the capacity for adsorption of different metal ions, molar adsorption capacity Q' (mmol/g) was used. The results, simulated for different metal ions such as Cu^{2+}, Zn^{2+}, and Ni^{2+} at pH 5.0 and that of Cr^{3+} at pH 4.5 are shown in Fig. 3 and Table 4. The results at other pH values are similar. The relative errors calculated for Cu^{2+}, Zn^{2+}, Ni^{2+}, and Cr^{3+} at different pH values are 4.66%, 11.55%, 5.45%, and 1.69%, respectively.

The K_1 calculated, which represents the equilibrium constants of H^+ with RNH_2 for Cu^{2+} and Ni^{2+} as well as Zn^{2+}, are similar and in the range of 750–888, but these K_1s show great difference from that of Cr^{3+} (559). This indicated that Cr^{3+} chelating differs from that of Cu^{2+}, Ni^{2+}, and Zn^{2+} considerably. The glucosamine contents calculated from the above model for Cu^{2+}, Ni^{2+}, Zn^{2+} adsorption are in the range of 44–52% and higher than that from glucosamine determination (about 30%). However, the glucosamine content calculated for Cr^{3+} adsorption is 81%, which is significantly higher than that of Cu^{2+}, Ni^{2+}, and Zn^{2+}. As the calculated glucosamine

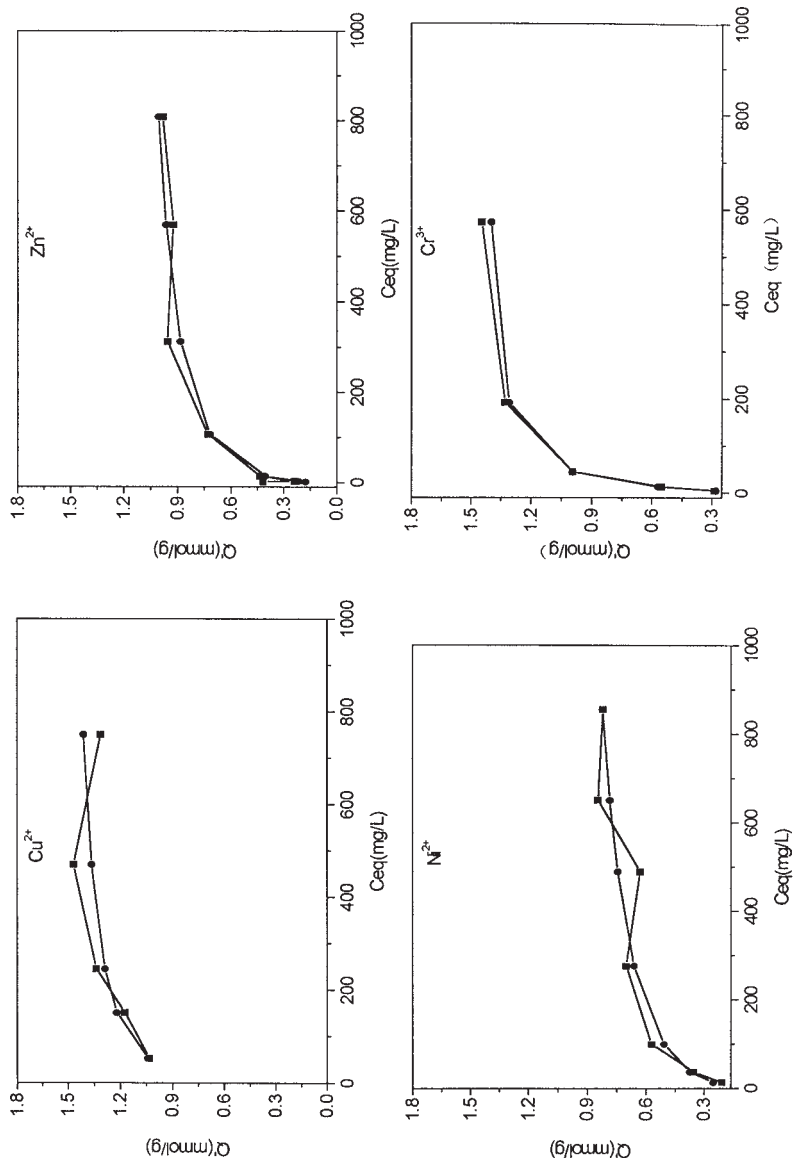

Fig. 3. Comparison of experimental result and simulated result. Square-experimental results, Cu^{2+}, Zn^{2+}, Ni^{2+} at pH 5.0. Cycle-simulated results, Cr^{3+} at pH 4.5.

Table 4
The Simulated Results

Metal ion	Cu^{2+}	Ni^{2+}	Zn^{2+}	Cr^{3+}
pH used	3.0–6.0	3.0–7.0	3.0–7.0	3.0–5.0
K_1 calculated	750	888	826	559
K_2 calculated	17	5.5	9.0	15
Glucosamine content calculated (%)	52	44	49	81
Relative error (%)	4.66	11.55	5.45	1.69

contents in the biomass are higher than that found by analytical determination (30%), this means that beside amino groups in chitosan of mycelium, some other groups such as carboxyl and hydroxyl groups also take part in the adsorption. In fact, the results of acid titration showed that other amino groups also exist in the mycelium. Inoue *(12)* and our work *(13)* for metal ion chelating on chitosan resin demonstrate that the hydroxyl and carboxyl groups in biomass also take part in adsorption of metal ions.

Conclusion

Biosorption of heavy metal ions with the mycelium, a byproduct of penicillin fermentation, is a low cost and easily regenerated method in wastewater treatment. Alkaline treatment enhances the adsorption capacities of metal ions due to removing proteins and nucleic acids from the cells. The pH influenced the biosorption of metal ions. The main chelating sites are amino groups in chitosan and carboxyl groups in the mycelium. A new chelating model, which is based on the interaction of H^+ and amino groups as well as metal ions, was established. The model successfully describes the adsorption of metal ions on *Penicillium chrysogenum* mycelium.

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Notation

K_1 and K_2 = equilibrium constants
$[M_e^{n+}]_0$ = initial concentration of metal ion (mol dm^{-3})
$[M_e^{n+}]_{eq}$ = equilibrium concentration of metal ion (mol dm^{-3})
n = valent number for metal ion
Q = adsorption capacity of metal ion (mg g^{-1})
Q' = molar adsorption capacity of metal ion (mmol g^{-1})
(RNH_2) = concentration of glucosamine at equilibrium (mol dm^{-3})

(RNH_3^+) = concentration of protonlized glucosamine (mol dm^{-3})
(H^+) = concentration of H^+ (mol dm^{-3})
$[RNH_2(1/n)Me]$ = concentration of glucosamine with metal ion complex (mol dm^{-3})
V = volume of solution (dm^{-3})
W_m = metal formula weight (g mol^{-1})
W_s = mycelium sample weight (g)

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