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Biosorption of Ni^{2+} and Fe^{3+} by Fungal Cell Wall Sacchrides*

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Abstract This paper reports on the adsorption characteristic of heavy metal ions ($Ni^{2+}$and Fe^{3+}) using a novel biosorbent, prepared from cell walls of *Rhizopus oryzae*. The optimum operating conditions are investigated in both single ion system and binary system.
Keywords biosorption, *Rhizopus oryzae*, heavy metal ion

1 INTRODUCTION

Removal of heavy metals at low concentration but in large volume is always a big problem in wastewater treatment. Traditional methods such as chemical precipitation, reverse osmosis and ion exchange with resin are not very successful[1]. Adsorptions using all kinds of adsorbents, like activated carbon, residues in nature and microbial materials, are the effective alternatives[2−4]. Biosorption of heavy metals by microbial cells has been recognized as a more potential method[5]. Compared with other microbial biosorbents like bacteria and algae, fungal biomass in particular has shown unique metal adsorption ability[6,7]. *Rhizopus oryzae* was chosen for its high metal adsorption capacity[8]. It has been suggested that two stages be related to the mechanism of metal sorption of living microbial bisorbents[9,10]. The first is rapid and reversible surface adsorption on the cell walls, which is also called passive uptake or extracellular adsorption. The surface of cell wall contains largely polysaccharides that provide amino, hydroxyl, carboxyl, and phosphate and sulfate groups for binding with metal ions[11,12]. The second stage is slow and irreversible metabolic accumulation called active uptake or intracellular adsorption. However for dead microorganism out of any metabolic activities, the mechanism of metal ion sorption is only limited to the first stage. Non-viable microbial materials are superior to viable microbial cells on removal of heavy metal ions[13,14].

Although the biomass is effective to remove heavy metal ion from aqueous solution, its sorption capacity is low. Thus, some researches resort to chitin or chitosan for biosorption of metal ions[15,16]. As chitosan contains more free amino groups than chitin, the results of biosorption using chitosan are more distinguished. However chitosan is unstable in acid solution. The methods of crosslinking and chelation are introduced to modify and improve the stability of chitosan. These chemical modifications are conducted under harsh conditions, leading to a high cost of the biosorbent and partly loss of free amino groups[16,17]. Searching for ideal biosorbent is an urgent task.

This paper reports on a new biosorbent, cell wall skeletal sacchrides prepared from the cell wall of *Rhizopus oryzae*, and its adsorption characteristics on binary metal system of Ni^{2+} and Fe^{3+}. The cell wall of the filamentous fungus mainly consists of a complex of chitosan/chitin in which chitosan is cross-linked with chitin. After a process of physicochemical treatment, a substance of a porous network is obtained, in which free amino groups in addition to hydroxyl groups are the important groups for chelating with heavy metal ions.

Although iron is not considered as a major environmental problem, its ubiquitous presence in solutions makes it an obvious investigation target because of its effect on the uptake of many other metals. Nickel, known for its toxicity, is often accompanied by iron in industrial effluents. The effect of iron on the biosorption of nickel is of interest in the paper.

2 MATERIALS AND METHODS

2.1 Microorganism and growth conditions

Rhizopus oryzae was obtained from Zhejiang Institute of Microbiology (Hangzhou, China). *Rhizopus oryzae* was first cultured in a 250 ml flask containing 100 ml standard potato extract medium at 34℃ on a rotary bed with a speed of 200 $r \cdot min^{-1}$. 54 hours later, mycelia were transferred to a 6-liter stirred-aerated fermenter and cultured for two days. Mycelia are harvested with stainless steel mesh of 0.15 mm.

2.2 Preparation of cell wall sacchrides

Harvested mycelia are disintegrated at a high-speed blender for 6 minutes and then washed with 2 liter distilled water. The broken mycelia are treated

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twice with 0.5 mol·L^{-1} NaOH solution, each at 60℃ for 0.5 h. After washed with distilled water until the pH of cell wall is between 7 and 8, the cell wall skeletal sacchrides are filtered with steel mesh and preserved directly in 2% acetic acid. The cell wall skeletal sacchrides can also be dried and preserved permanently, without loss of adsorption capacity. In this experiment, the wet cell wall skeletal sacchridess were used as biosorbent.

2.3 Biosorption of metal ions

The solutions of single metal ion were prepared by dissolving $Fe_2(SO_4)_3$ in distilled water or dissolving metal Ni^{2+} in solution of concentrated HNO_3. The binary metal ion solution was prepared by mixing two kinds of single ion solutions.

A certain amount of cell wall skeletal sacchridess in fresh weight was mixed with 100 ml metal solutions in 250 ml Erlenmeyer flasks. The dry weight of biosorbent was determined simultaneously through drying another biomass at the same fresh weight to constant weight in a 70℃ vacuum oven. 0.1 mol·L^{-1} NaOH or 0.1,mol·L^{-1} HCl was added to alter the pH at which the adsorption took place. The rotation speed of the shaker was controlled at 120 r·min^{-1}. After the biosorption was finished, the solution was separated from cell wall sacchridess by filtering through stainless steel mesh of 0.1 mm. The control experiment was simultaneously carried out under the same conditions except for no addition of the biosorbents to check for the micro-precipitation. The metal concentrations before or after biosorption were analyzed. The adsorption capacity Q_{eq} (mg·g^{-1}) was calculated as follows: $Q_{eq} = (C_0 - C_{eq})/S_0$, where C_0 and C_{eq} are respectively the initial metal concentration and the metal equilibrium concentration, and S_0 (g·L^{-1}) is the biosorbent concentration. All experiments of biosorption were carried out in triplicate.

2.4 Analysis of metal ions

The concentration of Ni^{2+} was determined by dimethylglyoxime spectrophotometric method at wavelength of 530 nm[18]. The concentration of Fe^{3+} was determined by potassium thiocyanate spectrophotometric method at wavelength of 480 nm[18].

3 RESULTS AND DISCUSSION

The characteristics of metal ion adsorption are involved with the operating conditions of pH, temperature, ion concentration and the presence of the other ion if adsorption occurs in the binary metal ion system. To improve the selectivity of one ion, the adsorption should be carried out under the optimum operating conditions for this ion. Thus, in this paper, the sorption performance of the biosorbent will be evaluated by determining the optimum conditions and sorption isotherms for Fe^{3+} and Ni^{2+}, respectively, before examining it in solutions containing both metals. At last, the work will continue to improve the selectivity.

3.1 Adsorption on single ion system

With the initial metal ion concentration C_0 and the biosorbent concentration S_0 fixed on certain values, the effect of pH on the sorption is shown in Fig. 1. It indicates clearly that pH of 6.0 is the optimum pH for adsorption of Ni^{2+} and 4.0 for Fe^{3+}. The precipitation of Ni^{2+} can be observed and influence the adsorption when pH is higher than 6.0; the adsorption capacity of Ni^{2+} decreases rapidly with the drop of pH when pH is lower than 6.0. It is likely that protons compete with metal ions for binding sites. The profile of sorption capacity to pH at different concentration of Ni^{2+} is almost the same (not shown in Fig. 1), just as in sorption of Cu^{2+} using the same biosorbent[19]. The effect of pH on adsorption of Fe^{3+} follows the same regularity but with the optimum value (pH 4.0). Therefore, pH is a considerable factor on adsorption of metal ions just like other biosorbents.

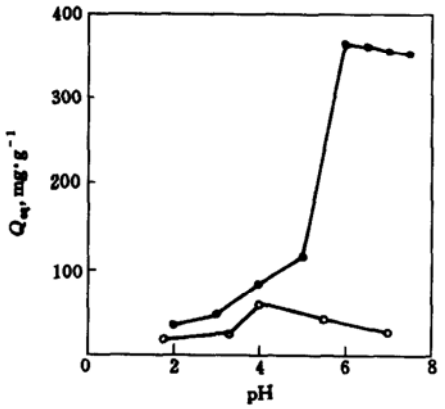

Figure 1 Effect of pH on biosorption(25℃)
● Ni^{2+} (C_0=212 mg·L^{-1}, S_0=0.364 g·L^{-1});
○ Fe^{3+} (C_0=54.7 mg·L^{-1}, S_0=0.903 g·L^{-1})

Fig. 2 shows that temperature has a gentle effect on biosorption of both Fe^{3+} and Ni^{2+}. However the trend for Ni^{2+} is quite different from that for Fe^{3+}, in which the sorption capacity of Ni^{2+} increases a little with the increase of temperature while that of Fe^{3+} decreases a little. Therefore, the temperature of 25℃ is chosen except when the improvement of adsorption selectivity is focused on.

The isotherms for both Ni^{2+} and Fe^{3+} at 25℃ are correspondingly shown in Fig. 3. The well known expression of Langmuir isotherm is $Q_{eq} = aC_{eq}/(1 + bC_{eq})$, where a and b are constants. This equilibrium can also be linearized by plotting C_{eq}/Q_{eq} *vs.* C_{eq} to determine the Langmuir constants from the slope b/a and the intercept $1/a$. The values of maximum

sorption capacity $Q_{max}(= a/b)$ are also listed in Table 1, which are almost an order higher than those reported[12,20]. Fig. 4 shows the linearized Langmuir type isotherm for Ni^{2+} and Fe^{3+}. The linearized Freundlich type isotherm equation can be written as follows: $\lg(Q_{eq}) = 1/n \times \lg(C_{eq}) + \lg K_f$, where n and K_f are constants. Fig. 5 shows the linearized Freundlich isotherm [$\lg(Q_{eq})$ *vs.* $\lg(C_{eq})$], from which the values of n and K_f are determined (listed in Table 1). Therefore, the sorption of nickel and ferric ion onto fungal cell wall skeletal sacchridess individually comply with both Langmuir and Freundlich type isothermal equations, suggesting adsorption by a hybrid mechanism onto a heterogeneous surface.

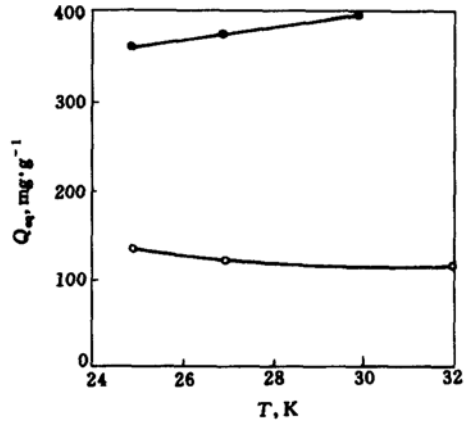

Figure 2 Effect of temperature on biosorption
● Ni^{2+} (C_0=242.5 mg·L^{-1}, S_0=0.364 g·L^{-1});
○ Fe^{3+} (C_0=123.0 mg·L^{-1}, S_0=0.903 g·L^{-1})

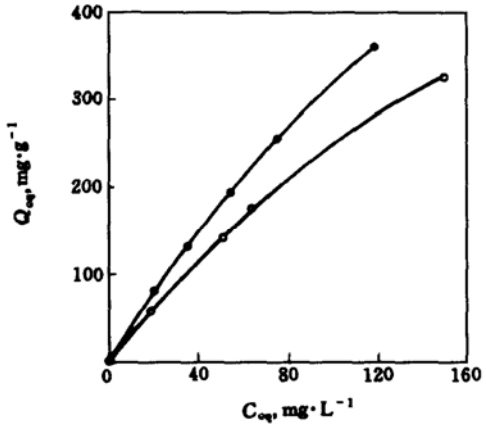

Figure 3 Isothermal curve of biosorption (25℃)
● Ni^{2+}—pH 6.0; ○ Fe^{3+}—pH 4.0

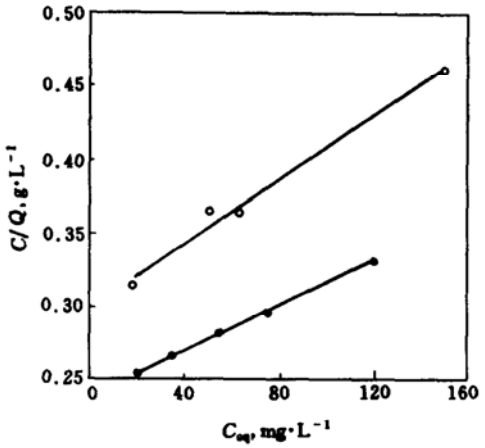

Figure 4 Linearized Langmuir type isotherm for Ni^{2+} and Fe^{3+}
● Ni^{2+}; ○ Fe^{3+}

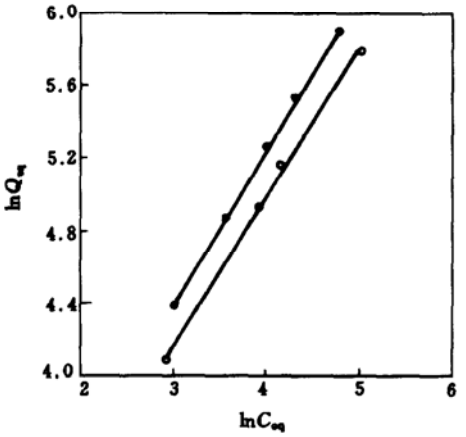

Figure 5 Linearized Freundlich type isotherm for Ni^{2+} and Fe^{3+}
● Ni^{2+}; ○ Fe^{3+}

3.2 Competitive adsorption in binary heavy metal ion system

To determine adsorption performance of Ni^{2+} in the binary solution (Fe^{3+} and Ni^{2+}), the initial Ni^{2+} concentration varies from 0 to 125 mg·L^{-1} while the initial Fe^{3+} concentration fixes on 0, 58.4 mg·L^{-1} and 146 mg·L^{-1}, respectively. The effect of different levels of Fe^{3+} on the adsorption of Ni^{2+} is quantitatively demonstrated in Fig. 6, showing how the biosorption uptake of Ni decreases in the presence of Fe. With the initial concentration of Fe^{3+} increasing, the adsorption capacity in equilibrium decreases rapidly. The presence of Fe^{3+} inhibits the adsorption of Ni^{2+}, which can be explained by Fe^{3+} competition with

Table 1 Isothermal adsorption equation for Fe^{3+} and Ni^{2+} (25℃)

Metal ion	pH	Langmuir type isotherm	Freundlich type isotherm
Fe^{3+}	4.0	$Q_{eq} = 3.023C_{eq}/(1 + 0.00206C_{eq})$	$Q_{eq} = 8.260C_{eq}^{0.715}$
		Q max: 1467.5 mg·g^{-1}	
Ni^{2+}	6.0	$Q_{eq} = 4.110C_{eq}/(1 + 0.00291C_{eq})$	$Q_{eq} = 7.721C_{eq}^{0.796}$
		Q max: 1412.4 mg·g^{-1}	

Ni^{2+} for binding sites. For example, whereas in one-metal Ni system the Ni sorption capacity is $80.0\,mg\cdot g^{-1}$ at equilibrium Ni^{2+} concentration of $20.0\,mg\cdot L^{-1}$, when the initial Fe^{3+} concentration is $146\,mg\cdot L^{-1}$, the sorption capacity of Ni^{2+} decreases to $11.9\,mg\cdot g^{-1}$ or 14.9% of the original value. To reach 81.1% Ni sorption reduction at the same Ni equilibrium concentration, only a low initial Fe presence of $58.4\,mg\cdot L^{-1}$ is required. With the presence of Fe^{3+}, the significant reduction in the Ni^{2+} sorption is observed.

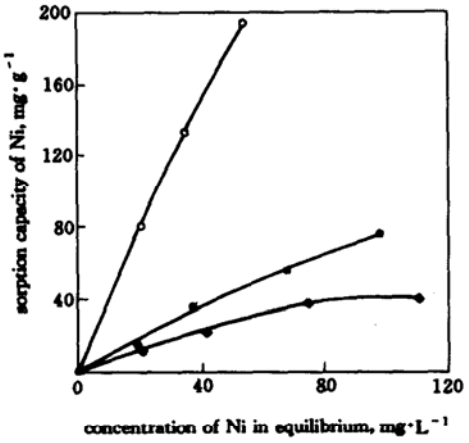

Figure 6 Effect of Fe^{3+} concentration on the sorption capacity of Ni^{2+} in equilibrium
(pH 6.0, 25℃)
○ $C_{Fe,0} = 0$; ● $C_{Fe,0} = 58.4\,mg\cdot ml^{-1}$;
◆ $C_{Fe,0} = 146.0\,mg\cdot ml^{-1}$

Similarly, the summary of the Ni interference in the Fe sorption is presented in Fig. 7. The result indicates that although the presence of Ni^{2+} also interferes with the sorption of Fe^{3+}, the effect of Ni is less pronounced than *vice versa*. For instance, the adsorption capacity of Fe^{3+} at the equlibrium Fe^{3+} concentration of $20.0\,mg\cdot L^{-1}$ is $37.0\,mg\cdot g^{-1}$ or 61.7% of the original value under the equilibrium Ni^{2+} concentration of $125\,mg\cdot L^{-1}$. At the initial Ni concentration of $50\,mg\cdot L^{-1}$, only 17.5% of Fe sorption capacity is affected for the same equilibrium Fe concentration of $20.0\,mg\cdot L^{-1}$.

In the binary metal ion system of Ni^{2+} and Fe^{3+}, the two ions compete with each other for binding sites, although the inhibitory effect of Fe^{3+} on the adsorption of Ni^{2+} is more significant than that reverse.

3.3 Improvement of adsorption selectivity

As the above statements that the optimum operating conditions of Ni^{2+} are quite different from those of Fe^{3+}, choosing different conditions can be expected to improve the selectivity in binary solution (Ni^{2+}-Fe^{3+}). pH and temperature are two important factors on biosorption of heavy metal ion. Thus, pH and temperature are adjusted between the optimum value for Ni^{2+} and Fe^{3+}. The results are listed in Table 2, indicating the profound effects of operating conditions on the selectivity ($K_{Ni^{2+}/Fe^{3+}}$). pH is superior to temperature in improving the selectivity. pH of 4.0, the optimum value for Fe^{3+}, is adverse to the selectivity of Ni^{2+} but good to that of Fe^{3+}; on the contrary, pH of 6.0, the optimum pH for Ni^{2+} , does much good to the selectivity of Fe^{3+}. The effect of temperature on the selectivity is less important, that is to say enhancement of temperature is reluctant to improve the selectivity of Ni^{2+}.

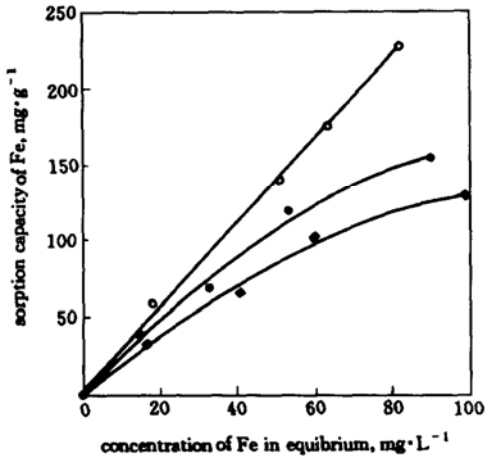

Figure 7 Effect of Ni^{2+} concentration on the sorption capacity of Fe^{3+} in equilibrium
(pH 4.0, 25℃)
○ $C_{Ni,0} = 0$; ● $C_{Ni,0} = 50.0\,mg\cdot ml^{-1}$;
◆ $C_{Ni,0} = 125.0\,mg\cdot ml^{-1}$

Table 2 Selectivity ($K_{Ni^{2+}/Fe^{3+}}$) under different conditions (C_0, $mg\cdot L^{-1}$: Ni^{2+} 131.5; Fe^{3+} 58.032)

Condition of biosorption		$Q_{Ni^{2+}}$	$Q_{Fe^{3+}}$	$K_{Ni^{2+}/Fe^{3+}}$
pH	T, ℃	$mg\cdot g^{-1}$	$mg\cdot g^{-1}$	$(Q_{Ni^{2+}}/Q_{Fe^{3+}})$
4.0	32	14.68	74.21	0.198
6.0	32	90.40	23.99	3.770
4.0	40	16.44	41.85	0.392
6.0	40	98.62	25.11	3.924

4 CONCLUSIONS

The fungal cell wall skeletal sacchrides were found to be excellent in removing Ni^{2+} and Fe^{3+} from aqueous solutions whether in single ion system or binary ion system. In the single ion situation, the optimum pH was investigated, indicating that pH 6.0 is best for Ni^{2+} and pH 4.0 for Fe^{3+}, and both the Langmuir and Freundlich type adsorption models show good fit for the cases. In the binary situation (Fe^{3+} and Ni^{2+} , the antagonistic interactions occur between the two metal ions and the presence of Fe^{3+} has more significant effect on the biosorption of Ni^{2+} than that in reverse; and pH is superior to temperature in improving the selectivity.

Further work in progress is to design a novel reactor and evaluate the biosorption of Ni^{2+} and Fe^{3+} in real wastewater using fungal cell wall skeletal sacchrides.

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