Biosorption Mechanism of Zn\textsuperscript{2+} and Cd\textsuperscript{2+} by a *Rhodotorula mucilaginosa*

Binhui Jiang\textsuperscript{1}, Qianqian Wang\textsuperscript{2}, Yan Zhao\textsuperscript{1}, Li Li\textsuperscript{1} and Xiaomin Hu\textsuperscript{1}

\textsuperscript{1}College of Resources and Civil Engineering, Northeastern University, Shenyang, Liaoning - 110 819, China.
\textsuperscript{2}School of Mining Engineering, University of Science and Technology Liaoning, Anshan, Liaoning - 114 051, China.

(Received: 10 June 2013; accepted: 16 August 2013)

WT6-5, a *Rhodotorula* sp. isolated from a Tailings Reservoir soil in Liaoning province, might have ability to adsorb heavy metals. Its biosorption ability and mechanism for zinc and cadmium were studied, and the results showed that the removal efficiency of zinc and cadmium were up to 78.0% and 100% separately, with the wet biomass of 18mg/mL and the conditions of Zn\textsuperscript{2+} 10mg/L and Cd\textsuperscript{2+} 1mg/L. In order to identify the main sorption characteristics of WT6-5, biosorption time, wet biomass and pH values were measured. The main biosorption functional groups including hydroxide, acylamide, carbonyl, C-N, carbonyl and sulfur carbonyl were analyzed by IR spectroscopy; moreover, zinc and cadmium ions absorbed by WT6-5 distributed in cell wall after destroying capsule were observed with SEM. The results suggested that WT6-5 was a valuable biosorbent for treating the heavy metal pollution.

**Key words:** *Rhodotorula* sp.; biosorption; zinc ions; cadmium ions.

---

Heavy metal pollution is worsening in China. Dealing with the problem, Bio-adsorption as an ideal method possessing high adsorption rate, high selectivity and no secondary pollution is able to treat wastewater which contain low concentration heavy metal of large amount, with less investment and lower operating costs\textsuperscript{1-2}. There are a various of microorganisms, including fungi, yeast, algae, bacteria, etc, which can afford to the uptake of contaminations\textsuperscript{3}. Previous studies have been performed using bacteria, fungi, algae, and yeasts to remove organism and heavy metals\textsuperscript{4}. Lesage et al. suggested that the plant species that is a submerged aquatic macrophyte *Myriophyllum spicatum* is efficient for the treatment of metal-contaminated industrial wastewater\textsuperscript{5}. Ertugrul et al. also investigated that the removal of Remazol Blue and Reactive Black B by an immobilized *Phormidium* sp. which is a thermophilic cyanobacterial strain\textsuperscript{6}.

Yeast, especially red yeasts are a better raw biosorbent material for the removal of organism or heavy metal ions due to their unicellular nature and high growth rate\textsuperscript{7}. Yeast cells can be easily cultivated into inexpensive growth media\textsuperscript{8}. The red yeasts belonging to genus microzyme, a kind of saprophytic fungi have been reportedly to effectively bioaccumulate and remove organic pollutants or heavy metallic ions by growing cells. Cadmium and lead biosorption by the *Rhodotorula* genus of yeast has been documented\textsuperscript{9}. Falih reported that *Rhodotorula minuta* was able to accumulate copper and nickel\textsuperscript{10}. These studies show that *Rhodotorula* sp. is promising for the treatment of heavy metal contaminated wastewater, however, its research and development are still at initial stage, not having been widely applied.

Above all, in this paper a *Rhodotorula mucilaginosa* WT6-5, usually used for producing lipid\textsuperscript{11} and carotenoids\textsuperscript{12,13} or used as an biological
flotation agent\textsuperscript{14}, was screened out of a certain molybdenum ore tailing soil. Adsorption experiments of Zn\textsuperscript{2+} and Cd\textsuperscript{2+} were carried out by making use of living WT6-5, and adsorption mechanism was analyzed by infrared spectroscopy and scanning electron microscopy, providing a theoretical foundation for further practical application.

**MATERIAL AND METHODS**

**Microorganism, medium and cultivated conditions**

Strain *Rhodotorula mucilaginosa* WT6-5, a red-pigmented yeast, isolated from a tailings soil, located in Huludao (Liaoning Province, People’s Republic of China), could survive under 20 mg/L and 1 mg/L cadmium. *Rhodotorula* sp. WT6-5 was maintained and activated in YPD medium comprising glucose, 20 g/L; yeast extract, 10 g/L; peptone, 20 g/L. The pH of the medium was adjusted to 2.8 with 1 M NaOH and 1 M HCl. Then culture on an air bath shaking table at 26 °C with a rotation speed for 120r/min. Growth was determined from the optical density at 530 nm (OD\textsubscript{530}). Optical density measurements were carried out for 36 h.

**Adsorption experiment**

Experiment in different adsorption conditions, such as adsorption time lasted, heavy metal concentration of the solution, cell dosage added into the solution, solution’s pH (measure by pH-25 pH meter) and cell’s culture time. Repeat the experiments and take the average. Then make some isotherm curves and adsorption models. The adsorption rate of metal ion ($Q$) would be calculated as equation 1. And the equilibrium uptake of red yeast ($q$) would be calculated as equation 2.

$$Q = \frac{(C_0 - C)}{C_0} \times 100\% \quad \text{ ...(1)}$$

Where $C_0$ is the metal ions concentration of solution before adsorption (mg/L); $C$ is the metal ions concentration of solution after adsorption (mg/L)

$$q (\text{mg/g}) = \frac{(C_0 - C_e)}{C_b} \quad \text{ ...(2)}$$

Where $C_e$ is the metal ions concentration of solution before adsorption (mg/L). $C_b$ is the metal ions equilibrium concentration of solution (mg/L); $C_b$ is the dosage of cells added into the solution (g/L) (wet weight).

**IR determination**

The cells were collected and washed twice with deionized water, then were grinded by agate mortars to certain fineness after drying in an oven at 50°C for 24h. Taking 1mg of grinded cells and about 150 mg of potassium bromide powder together, which grounded and mixed for 4-5 min by agate mortars. Then the samples were pressed by a pressure machine and determined by a FT-IR Fourier infrared spectrometer (Nicolet 380).

**Observation of WT6-3’s cell wall structure**

The yeasts were separated from solution on a centrifuge with speed of 8000 rpm for 3-5min. 2.5% glutaraldehyde were put into the rest cells, then the liquid inhaled into small centrifuge tubes were centrifuged again, and some new 2.5% glutaraldehyde was put in to fix the cells. The disposed liquid was dripped on a clean piece of glass which was placed in a clean environment, waiting for drying in air. Finally, the prepared samples were observed with scanning electron microscope (SEM, SSX-550) after the dried glass was sprayed gold.

**RESULTS AND DISCUSSION**

**Adsorption process**

Prepare two 25mL bottles of solution contained Zn\textsuperscript{2+} 10mg/L and Cd\textsuperscript{2+} 1mg/L separately. Add cultured cells about to the solution and vibrate to make the metals be adsorbed. The adsorption curve of heavy metals by WT6-5 was shown in figure 1. Fig.1. showed that their trend is similar to each other when WT6-5 adsorbing Zn\textsuperscript{2+} and Cd\textsuperscript{2+}. Within the first 15 min, the adsorption rates were very fast. The best adsorption times were both 15 min, at that time the adsorption rate of 10mg/L Zn\textsuperscript{2+} was 78.0%, while the adsorption rate of 1mg/L Cd\textsuperscript{2+} was 100%. 15min later, adsorption rates both declined, because cells flocculated after adsorption, destroying some unstable bonds associated WT6-5 with ions, and released the metal ions into the solution again. Living organisms adsorption process has two phases\textsuperscript{15}: the first stage occurs at the cell surface, with the main adsorption and ion exchange process, and it happens fast; the second stage is initiative to absorb, which is shifting the metal ions adsorbed on the cell surface into the cell interior, and the process is slow. Figure 1 show that the sorption of Zn\textsuperscript{2+} and Cd\textsuperscript{2+} is fast, which indicates surface adsorption, rather than energy metabolism biosorption\textsuperscript{16}.
Relationship between cell dosage and adsorption rate

Prepare two kinds of solution contained Zn$^{2+}$ 10mg/L and Cd$^{2+}$ 1mg/L separately. Make each of them in a series of volume, 5mL, 10mL, 15mL, 25mL, 40mL and 50mL. Add the same dosage cells to the solution and vibrate for 15min. Sample and calculate the adsorption rate (Fig.2.). Fig.2. showed that with the increasing dosage of WT6-5, the adsorption rate of Zn$^{2+}$ and Cd$^{2+}$ both rose, then descended slightly after increasing to some extent. Adsorption rate did not proportionally increase with the increasing of the cell dosage, which indicates that the cell surface is not saturated by metal ions, but has different equilibrium conditions according to a different ratio of the metal / cell concentration. When cell dosage was 18mg/mL, the adsorption rates were highest, 85.7% and 96.8% respectively. But when cell dosage rose to 26mg/mL, their adsorption rates were lower than in the case of 18mg/mL, the reason might be that great dosage made discrete cells into conglobation and the surface area of cells was decreased, so the adsorption rates were decreased.

Relationship between concentration and sorption rate

The experiments were carried out just as the method of 3.1. The results were shown as Fig.3 and Fig.4.

Fig.3 and Fig.4 showed that when using WT6-5 to adsorb different concentration of Zn$^{2+}$ and Cd$^{2+}$, with the increase of concentration, the adsorption rate decreased. In the test of Zn$^{2+}$ adsorption, when the concentration was below 20 mg/L, the adsorption rate was up to 40%. While the concentration was higher than 20 mg/L, its adsorption rate reduced rapidly. When the Zn$^{2+}$ concentration was 50mg/L, the rate was only 23.5%. The higher the initial concentration of the metal ion, the greater the adsorption capacity, this might be when the ion concentration was high, there was a larger concentration gradient, therefore the driving force of adsorption was stronger. Although the cell surface adsorption sites increased saturation, the adsorption rate was still low, because the high concentrations of heavy metal ions increased the toxicity to the cell and the original structure of the cell surface might be destroyed. In the test of Cd$^{2+}$ adsorption, when
the concentration was below 15 mg/L, the adsorption rate was up to 70%. When the Cd²⁺ concentration was 20 mg/L, the rate was declined to 61.7%.

Above all, it can be concluded that biosorption method is much more appropriate to treat wastewater with heavy metals of low concentration. When the concentration of metal ions were controlled in a certain range, the effects of removing Zn²⁺ and Cd²⁺ from wastewater by WT6-5 could be expected.

**Relationship between pH and adsorption rate**

The experiments were carried out just as the method mentioned above. The results were shown as Fig. 5.

From Fig. 5, it was found that once pH < 3, the absorption rates on Zn²⁺ and Cd²⁺ were both very low; When pH > 3, the adsorption rate increased sharply; when the pH = 9, the adsorption rate of Zn²⁺ was largest and up to 85.06%. But the largest adsorption rate of Cd²⁺ was almost at pH = 7, which was 95.13%. These results showed that the best absorption condition of WT6-5 was on alkali conditions at pH 7-9. Consequently, adjusting pH value can acquire better absorption effect.

**Adsorption and biosorption isotherm**

The adsorption isotherms of Zn²⁺ and Cd²⁺ on red yeast WT6-5 were fitted by linear regression based on Langmuir equations which are usually used to analyze adsorption isotherm.

The results were shown as followed. The biosorption of Zn²⁺ and Cd²⁺ on red yeast WT6-5 followed the Langmuir adsorption isotherms and was monolayer coverage. This conclusion was the same as others, which using other yeasts adsorbed Cd²⁺, Cu²⁺, and Ag⁺.

To Zn²⁺ \( \frac{1}{q}=7.9107/C_e + 0.2310 \quad R^2: 0.97 \), To Cd²⁺ \( \frac{1}{q}=0.7779/C_e + 1.2148, \quad R^2: 0.92 \).

**FT-IR analysis**

The images of infrared absorption spectrum of WT6-5 before and after absorbing heavy metallic ions were shown as Fig. 6.

From image (a), it was seen that WT6-5 had apparent absorption in several waves in the whole range. While assigning the adsorption bands, the strong wide peak 3419.35 cm⁻¹ is hydroxyl stretching vibration peak combined with –NH stretching vibration peak; 2925.81 cm⁻¹ is –CH stretching vibration peak belonging to aliphatic -CH₂; 1648.39 cm⁻¹ is C=O stretching vibration peak belonging to amide I; 1544.70 cm⁻¹ is N-H bending vibration peak belonging to amide II; the peaks belonging to amide are characteristic absorption peak of protein; 1465.90 cm⁻¹ is C-H asymmetry bending vibration peak belonging to -CH₃; 1403.69 cm⁻¹ is C-O stretching vibration belonging to carboxyl acid; 1241.94 cm⁻¹ is -CN stretching vibration peak; 1084.33 cm⁻¹ is C-O stretching vibration belonging to esters combined with C=S stretching vibration peak belonging to sulfur carbonyl; 876.96 cm⁻¹ is HPO₄²⁻ absorption peak; 528.57 cm⁻¹ is PO₄³⁻ absorption peak. Thus it suggested that WT6-5 has -OH, -CH, -CN, C=O, NH, and C=S groups, which are almost the features of protein and carbohydrate groups. By comparing before and after adsorbing metallic ions of WT6-5’s infrared images, it was found that the adsorption peaks of WT6-5 after absorbing metallic ions haven’t changed significantly, but only a little deviation. No apparent new bands and peaks appeared which indicated that after adsorption of metallic ions, the structure of WT6-5 has not changed. From the differences between the IR images, it can be inferred that in the process of adsorbing Zn²⁺ and Cd²⁺ on WT6-5, hydroxyl, amide, carboxyl, cyan, carboxyl, sulfur carbonyl, HPO₄²⁻ and PO₄³⁻ are primary adsorption functional groups.

**Observation of WT6-5 structure with SEM**

The SEM images of WT6-5 before and
after absorbing heavy metallic ions were shown as Fig. 7.

Fig. 7. showed that after Zn$^{2+}$ was adsorbed on WT6-5, the micro-capsule outside cells was destructed and disappeared. The major component of micro-capsule was polysaccharide. Heavy metallic ions might possibly be combined with the polysaccharides with some chemical bonds, damaging the structure of the microcapsule. After absorption of Zn$^{2+}$ the cells became close, the metallic ions perhaps played a role of connecting the cells. Some cell bodies were squeezed to deformation. There were some flocculated sediments on the cell surface. These sediments might result in inorganic micro-precipitation, and also destroy microcapsule that adsorbed in the cell wall. After WT6-5 adsorbing Cd$^{2+}$, the microcapsule of cells was damaged too; cells lost their order and their surface became rough and a large number of flocculation sediments.

![Fig. 6. FT-IR images of WT6-5 before and after absorbing heavy metallic ions](image1)

![Fig. 7. Characteristics of WT6-6 with SEM](image2)
appeared. In some literature the concentration of Cd\(^{2+}\) adsorbed by cells in solution was significantly less than Zn\(^{2+}\), and the biosorption on WT6-5 also was the same case, which further confirmed that the toxicity and mutagenicity of Cd\(^{2+}\) was significant as reported. It was concluded that when WT6-5 adsorbing Zn\(^{2+}\) and Cd\(^{2+}\), firstly cell’s microcapsule was damaged by heavy metallic ions, and then the cell wall adsorbing the ions had a significant difference comparing with using dead beer yeast to directly absorb materials.

**CONCLUSIONS**

1. The largest adsorption rate of 10mg/L Zn\(^{2+}\) by WT6-5 was 78.0%, while for 1mg/L Cd\(^{2+}\) was 100%. With the increase of concentration, the adsorption rate decreased. The best absorption time was 15min, the best dosage was 18mg/mL, the best culture time was three days and the best absorption pH value was 7-9, alkali conditions. Langmuir equation is more suitable for an adsorption isotherm model of Zn\(^{2+}\) and Cd\(^{2+}\) metallic ions by WT6-5.

2. Through analysis by IR of WT6-5, in the process of adsorption on Zn\(^{2+}\) and Cd\(^{2+}\), hydroxyl, amide, carboxyl, CN\(^{-}\), carbonyl, sulfur carbonyl, HPO\(_4\)\(^{2-}\) and PO\(_4\)\(^{3-}\) are the primary adsorption groups. From the SEM images, the cell’s microcapsule was damaged by heavy metallic ions, and then the ions were adsorbed in the cell wall.

3. Biosorption method using red yeast as an adsorbent can be applied to treat wastewater with low concentration of Zn\(^{2+}\) and Cd\(^{2+}\).

**ACKNOWLEDGMENTS**

This work was supported in part by NSFC under Grant Nos. 51178088 and 51278090.

**REFERENCES**


