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Removal of heavy metal Ions in aqueous solution by Exopolysaccharides from *Athelia rolfsii*

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ABSTRACT

This study aimed to evaluate the removal of heavy metal ions in aqueous solution by extracellular polysaccharide (EPS) extracted from *Athelia rolfsii*. The removing mechanism was further analyzed by Fourier transform infrared spectroscopy (FT-IR). Three heavy metal ions (Cd^{2+}, Cu^{2+} and Zn^{2+}) were removed from the aqueous solution. And the effect of pH, contact time and initial EPS concentrations and metal iron concentrations on the metal uptake (q_e) was illustrated. The results showed that the clearance ability of EPS to Cd^{2+}, Cu^{2+} and Zn^{2+} all reached best at 25 °C, pH 5–7 and 60 min reaction time. The biosorption isotherm was also studied. Compared with Freundlich adsorption isotherm, biosorption datas for Cd^{2+}, $Cu^{2+}$and Zn^{2+} all fitted better with the Langmuir adsorption isotherm and according to the q_{max} value in Langmuir adsorption isotherm, the maximal metal uptakes (q_{max}) of EPS were 116.28, 103.09 and 153.85 mg/g for Cd^{2+},Cu^{2+} and Zn^{2+}, respectively. According to FT-IR, Cd^{2+} mainly reacted with –CHO and –NH– in EPS, and $Cu^{2+}$and Zn^{2+} mainly reacted with –NH– and –OH, respectively. The results indicated that EPS could be employed as a promising biosorption for industrial wastewater treatment.

1. Introduction

The accelerating process of industrialization promotes the rapid development of social economy, but causes serious pollution of the environment at the same time, especially water pollution caused by heavy metal. In many developing countries, the problem of heavy metal pollution has existed for a long time and presents growing trend, such as thallium, cadmium pollution in Guangxi Hejiang China in 2013. Nowadays, the removal methods of heavy metal include physical methods (membrane separation (Kanagaraj et al., 2015), surface adsorption (Zhao and He, 2014)), chemical methods (electrochemical processes (Basha et al., 2011), redox processes) and biosorption (Bilal et al., 2013).

As one of fungal polysaccharides, the activities and the mechanisms of *Athelia rolfsii* polysaccharide adsorbing heavy metal ions were few reported. Therefore, this research was carried out to show the potential biosorption of Cd, Cu, Zn to EPS from *A. rolfsii* in this study. In addition, chemical analysis and Fourier transform infrared (FT-IR) were applied to characterize the adsorption mechanisms of EPS to heavy metals.

2. Materials and methods

2.1. Athelia rolfsii cultivation

The strain of *A. rolfsii* was screened out from *Clivia* Plant and identified by Sangon Biocompany (Shanghai, China) (Li et al. 2014). It was preserved in PDA medium covering with liquid paraffin.

A. rolfsii was cultured in potato dextrose agar at 28 °C for 2 days. After being amplificated in seed culture (contained (g/L): glucose, 30; $NaNO_3$, 3.0; yeast extract powder, 1.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; pH 4.5), it was cultivated at 28 °C, 200 rpm in 100 mL optimal medium for 5 days. The optimal culture medium for polysaccharide production contained (g/L): corn soak solution, 5%(v/v); corn starch, 30; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $NaNO_3$, 3.0; citric acid $\cdot H_2O$, 0.5 (adjusted initial pH 4.5) (Miao et al., 2014; Desai et al., 2008; Survase et al., 2006). The following conditions were maintained throughout 5d: stirrer speed, 200 rpm; temperature, 28 °C, pH uncontrolled.

2.2. EPS extraction and purification

Culture broth was neutralized by 1 M NaOH, and 1-fold diluted with distilled water. After being heated at 80 °C for 30 min, culture broth was centrifuged at 10,000 rpm for 30 min to remove mycelium (Francois et al., 2011). Clear supernatant, in which protein was eliminated by Sevag method for 4 times, was collected and

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cooled at 5 °C and precipitated by adding two volume of ethanol absolute. This mixture was allowed to stand at 5 °C for 20 h to complete EPS precipitation. Finally, after being dialyzed, the precipitated polymer was freeze-dried (−50 °C, 2.0 Pa) and used for investigating the potential of biosorption of Cd, Cu, Zn.

2.3. Physical and chemical analysis of EPS

The content of total saccharides was estimated by the phenol-sulfuric acid analysis using glucose as standard.The content of residual protein was estimated by the Coomassie brilliant blue analysis using BSA as standard. The spectra of EPS solution was scanned and recorded from 197–400 nm with a UV–vis spectrophotometer (UV-1700, Shimadzu, Japan) to estimate the existence of protein. The functional group analysis of the EPS was carried out in a Fourier transform infrared (FT-IR) spectrophotometer (IRPrestige-21, Shimadzu, Japan) over a wave number range of 3500–600 cm^{-1}.

2.4. Metal adsorption experiments

Cadmium ions standard stock solutions was prepared by acidolying cadmium powder in 3 mol/L HCl (Norouzian and Lakouraj, 2015) and Copper and Zinc ions standard stock solutions were prepared by dissolving $CuCl_2$ and $ZnCl_2$ into Milli-Q water (Sounthararajah et al., 2015). EPS solution was added to metal solutions at 25 °C for 1 h, then three volumes of cold ethanol (−5 °C) were added to the solution (Farina et al., 2001). After standing for 2 min, the mixed solution was centrifuged at 10,000 rpm for 5 min and then determinate metal content in the supernatant by atomic adsorption spectrophotometer.

The metal uptake (q_e) (Zewail and Yousef, 2015; Omorogie, et al., 2012; Zhao et al., 2015) was determined as follows:

$$q_e = \frac{V(C_i - C_f)}{W} \quad (1)$$

where V is the volume of mixture solution (L), C_i is the initial concentration of metal in mixture solution (mg/L), C_f is the equilibrium concentration of metal in mixture solution (mg/L), W is the dry weight of EPS (g).

2.5. Modeling

Langmuir (2) and Freundlich (3) model (Duddridge and Wainwright, 1981) was used to describe adsorption isotherms

$$q = q_{\max} \cdot \frac{bC_f}{1 + bC_f} \quad (2)$$

$$q = K_f \cdot C_f^{1/n} \quad (3)$$

They are linearized to the form (4) and (5), respectively.

$$\frac{1}{q} = \frac{1}{q_{\max} \cdot b} \cdot \frac{1}{C_f} + \frac{1}{q_{\max}} \quad (4)$$

$$\lg q = \frac{1}{n} \lg C_f + \lg K_f \quad (5)$$

where q is the metal uptake by EPS (mg/g), C_f is the equilibrium concentration of metal in solution (mg/L), (b, q_{max}) and(K_f, n) are empirical constants of Langmuir and Freundlich isotherms, respectively.

2.6. Analytic methods of metal adsorption

Cd^{2+}, Cu^{2+} and Zn^{2+} were measured by atomic adsorption spectrometer (AA-6300, Shimadzu, Japan). Extracted EPS and heavy metal loaded EPS were characterized by FT-IR spectroscopy (IRPrestige-21, Shimadzu, Japan).

3. Results and discussion

3.1. Physical and chemical analysis of EPS

The contents of total saccharides and residual protein of EPS were 72.54% and 0.34%, respectively.

The UV spectra of EPS was showed in Fig. 1. The UV spectra of EPS showed that an adsorption peak at 197 nm and a faint adsorption peak around 280 nm was measured. As the above-mentioned result of residual protein content, the protein content is too low to dentified on the UV spectra (Lv et al., 2009).

FTIR spectra of EPS was shown in Fig. 2. The broad IR band centering around 3340 cm^{-1} suggested stretching vibration of OH in EPS. A weaker peak at 3000–2800 cm^{-1} suggested the stretching vibration characteristic of C–H in EPS. The strong sharp peak at 1643 cm^{-1} was assigned to C=O (amide) which indicated probably the presence of chitin, a minor component of the *A. rolfsii* cell wall, and some products of protein degradation. A few small peaks at the range of 1575–1500 cm^{-1} suggested the stretching characteristic of –NH– in EPS. The weak adsorption bands at 1430–1200 cm^{-1} were mainly CH and OH bending in-plane ring deformation. Adsorption peak at 890 cm^{-1} was C–H in-plane bending vibration which indicated EPS was β-configuration (Wang et al., 2014; Oves et al., 2013).

3.2. Adsorption of Cd^{2+},Cu^{2+} and Zn^{2+} on EPS

3.2.1. Effects of pH on adsorption of Cd^{2+},Cu^{2+} and Zn^{2+} on EPS

Fig. 3 shows that the effect of initial solution pH on the metal uptake (q_e, mg/g) of EPS to Cd^{2+}, Cu^{2+}, Zn^{2+}. For Cu^{2+},the metal

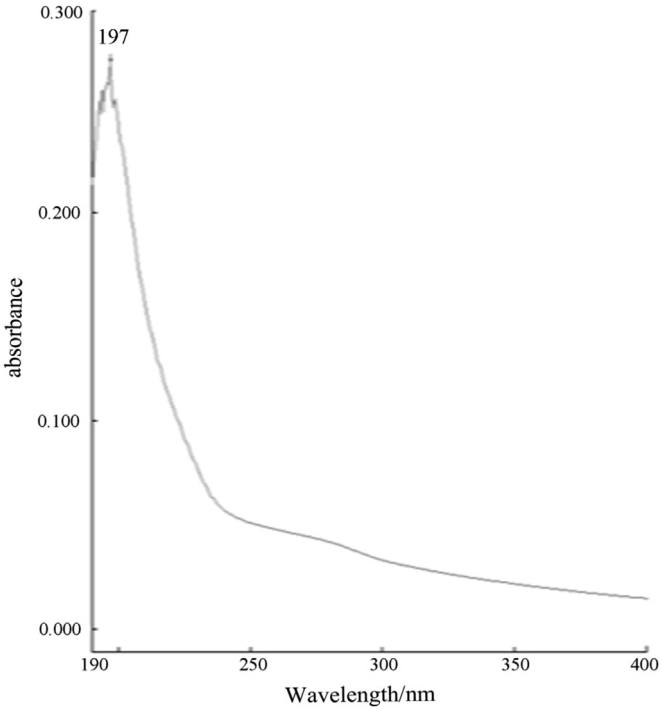

Fig. 1. UV spectra of EPS.