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Effect of saponins on cell surface properties of *Penicillium simplicissimum*: Performance on adsorption of cadmium(II)

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ABSTRACT

Previous studies about the effect of biosurfactants on cell surface properties mainly focus on cell surface hydrophobicity. In the present study, the effects of plant-derived biosurfactants saponins on cell surface charge and the adsorption of cadmium(II) by *Penicillium simplicissimum* were studied. The pretreatment of saponins changed the optimal pH from 6 to 5 for Cd(II) adsorption. All the adsorption processes by the intact and saponins-pretreated biomasses followed the Langmuir isotherms better than the Freundlich isotherms. According to the Langmuir isotherms, the maximum adsorption of Cd(II) (q_{max}) was increased from 51.6 to 74.6 mg/l by the pretreatment of 0.025% saponins. The mechanisms were also analyzed by Fourier transform infrared spectrometer (FTIR), energy dispersive X-ray (EDAX), and scanning electron microscope (SEM) analysis. The results indicated that the pretreatment of saponins changed the cell surface charge of *P. simplicissimum* and therefore influenced the adsorption of cadmium(II).

1. Introduction

Surface electrical charge and hydrophobicity are both important properties of microbial cells in environmental remediation. They may affect the interaction of microbial cells with soil surfaces, hydrocarbons, and heavy metals [1]. Cell wall of microorganisms, consisting mainly of polysaccharides, proteins and lipids, offers many negatively charged functional groups (such as carboxylate, hydroxyl, thiol, sulphonate, phosphate, amino, and imidazole groups) to bind metal ions [2]. Hence, many microorganisms, including fungi, yeasts, and bacteria, can be used as biosorbents for heavy metal adsorption [2]. For example, *Penicillium simplicissimum* is a well-known species of fungus which can adsorb heavy metals [3].

Recently, biosurfactants have obtained more and more interests for their potential applications in environments due to high biodegradability, low toxicity, and great diversity [4,5]. Several studies have found that biosurfactants can change the cell surface properties. The mechanisms include their adsorption to cellular envelope [1,6] and/or that they can cause the chemical components, such as lipopolysaccharide and protein, to be released from cell surface [4,6–8]. However, previous investigations about the effect of biosurfactants on cell surface properties mainly focus on cell surface hydrophobicity which plays important roles in the interaction of cells with hydrophobic substrates [1,6–9]. It lacks in-depth investigations on other fields such as cell surface charges. Several studies also have found that the presence of biosurfactants can change the cell surface charges. For example, Hua et al. [10] found that biosurfactant produced by *Candida antarctica* can increase the cell surface charge of the yeast itself. Several studies also have investigated the influence of biosurfactants on the interaction of bacterial cells with heavy metal ions. For example, Sandrin et al. [11] found that the presence of rhamnolipids can change the cell surface charge of *Burkholderia* sp. and thus reduce cadmium uptake. However, the changed cell surface charge is dependent on the characteristics of biosurfactants and microorganism species. As a result, the insight into the mechanism of how biosurfactants influence the cell surface charge is desirable, yet heretofore it has not been fully disclosed.

Biosurfactant saponins are glycosides, with pentose and hexose as hydrophilic groups and triterpenes such as quillaic acid and gypsogenic acid as hydrophobic moieties [1]. These compounds have strong microbial activity and can change the cell surface and biomembrane properties [7]. Our work was initiated to determine the effect of saponins on cell surface charges, which may be demonstrated by the differences of Cd(II) adsorption between the intact and saponins-pretreated biomasses of *P. simplicissimum*.

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2. Methods and materials

2.1. Microorganism and biosurfactants

The strain *P. simplicissimum* used in this study was isolated from the soil samples of Yuelu Mountain (Changsha, China) in our laboratory [12]. The cells were maintained on potato dextrose agar and stored at 4 °C. Saponins isolated from tea seeds was purchased from Ningbo United Biotechnology Co. Ltd. (Zhejiang, China). It was a mixture of 76.1% triterpenoids saponins, and the molecular weight was 1222.54. Its critical micelle concentration was 540 mg/l, at which the surface tension of water was reduced to 42.6 mN/m.

2.2. Biosorbents preparation

100 ml mineral salt medium (MSM) containing 1.0 g peptone (10 g/l) was added into a 500-ml Erlenmeyer flask and sterilized at 115 °C for 30 min. The composition of MSM was as follows: $FeSO_4$, 0.005 g/l; $MgSO_4{\cdot}7H_2O$, 0.25 g/l; $NaHCO_3$, 0.05 g/l; KH_2PO_4, 0.5 g/l; $CaCl_2$, 0.1 g/l; NH_4Cl, 2.0 g/l; KCl, 0.1 g/l; NaCl, 0.2 g/l. Then 1.0 ml fungal suspension with 1.0×10^6 spores was added into each Erlenmeyer flask. The culture was incubated at 30 °C, 150 rpm for 3 days with 20 g/l glucose as carbon source. Saponins were also added into the culture medium to achieve the final concentrations of 0, 0.005%, 0.025% and 0.1%, separately. Both glucose and saponins were filtered through 0.22-μm membrane before addition into the culture medium.

After incubation, the fungal mycelium was collected and washed twice with MSM. Then it was freeze-dried to constant weight. The mycelium was grinded to pass through a 180-mesh sieve. The biomass powder was marked separately, laid in the desiccator, and used in further experiments.

2.3. Cd(II) adsorption

All the adsorption batch experiments were carried out at 28 °C, 120 rpm for 4 h. Cd(II) (Cd $(NO_3)_2{\cdot}4H_2O$) was added into 50 ml Erlenmeyer flasks with 20 ml ultrapure water. Then the pH was adjusted with 1 M NaOH or HNO_3 at the beginning of the experiments and not controlled afterward. Then the biosorbents were added into the medium to achieve the final concentration of 0.2 g/l. The effects of pH on the adsorption of Cd(II) were performed at pH 1.0–7.0 with 20 mg/l Cd(II). In the adsorption isotherm studies, batch experiments were carried out at pH 5.0 with various initial concentrations (20–400 mg/l) of Cd(II). The group of the medium without biosorbents was performed as the blank experiments. The biomass from the culture medium without saponins was used in the control experiment as the intact biosorbents.

After adsorption, the medium was centrifuged at 10,000 rpm for 10 min. The concentrations of residual Cd(II) in supernatant were determined using an atomic adsorption spectrometer (Agilent 3510, USA). All the adsorption experiments were performed in triplicate, and the means were used in the data analysis. The amount of adsorbed Cd(II) per gram biomass was obtained by using the general equation:

$$q = \frac{(C_0 - C)}{W} \quad (1)$$

where q (mg/g) is the amount of Cd(II) adsorbed onto the unit amount of biosorbents; C_0 and C (mg/l) are the concentrations of Cd(II) in the medium before and after adsorption, respectively; W (g/l) is the concentration of the biosorbents.

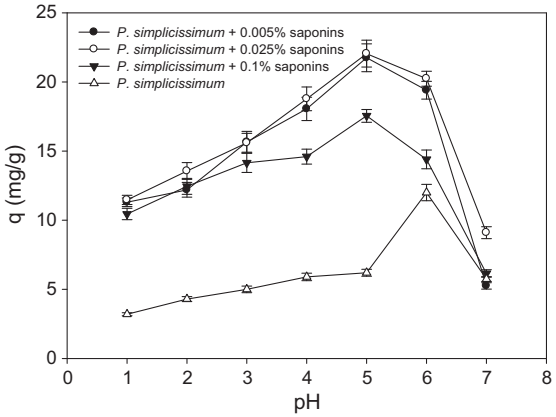

Fig. 1. Effect of pH on cadmium(II) biosorption by *P. simplicissimum*. Results are expressed as mean ± standard deviation ($n = 3$).

2.4. Biosorption characterization

The chemical characteristics of the samples were analyzed by Fourier transform infrared spectrometer (FTIR, WQF-410). The spectra were recorded in FTIR spectrometer with the samples prepared as KBr discs. All spectra were plotted using the same scale on the transmittance axis. The surface structure of biosorbents was analyzed by scanning electron microscope (SEM, Qutanta 200) coupled with energy dispersive X-ray analysis (EDAX, Qutanta 200). The biosorbent samples before and after adsorption were coated with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs. Then the samples were mounted on a stainless steel stab with a double-stick tape.

3. Results and discussion

3.1. Effect of pH

The medium pH can significantly influence adsorption of heavy metal ions. Therefore, the effect of the initial pH on Cd(II) adsorption by both the intact and pretreated biomasses of *P. simplicissimum* was studied first. As shown in Fig. 1, the amount of the adsorbed Cd(II) increased markedly with pH at relatively low pH values. The maximum adsorption of Cd(II) was observed at pH 6.0 for the intact biomass and at pH 5.0 for the saponins-pretreated biomasses. Then the adsorption amount of Cd(II) decreased with the increasing pH values. Saponins at concentrations of 0.005% and 0.025% had similar enhancement on Cd(II) adsorption. While the effect of saponins on Cd(II) adsorption was weakened with saponins concentration up to 0.1%, but the adsorption amount of Cd(II) was still higher than that of the intact biosorbents.

It is well known that the external pH influences the activity of functional groups (such as carboxylate, phosphate, and amino groups) and the availability of metal in solution [13]. At low pH values, the biomasses obtained low adsorption capacity, probably due to the protonation of the functional groups on the cell surface. Moreover, low pH environments may lead to high concentration of H_3O^+, thereby intensifying the competition between H_3O^+ and Cd(II) for negatively charged adsorption sites [14]. As pH increased, there is an increase in ligands with negative charges on cell surface, which results in increased binding of cations [2]. In addition, the competition between H_3O^+ and Cd(II) decreased, leading to enhanced metal uptake. On the other hand, the decrease in adsorption capacity at higher pH was probably due to the slowly increase of the OH^- concentration which leaded to the increase of hydroxyl complexes in the solution [14].