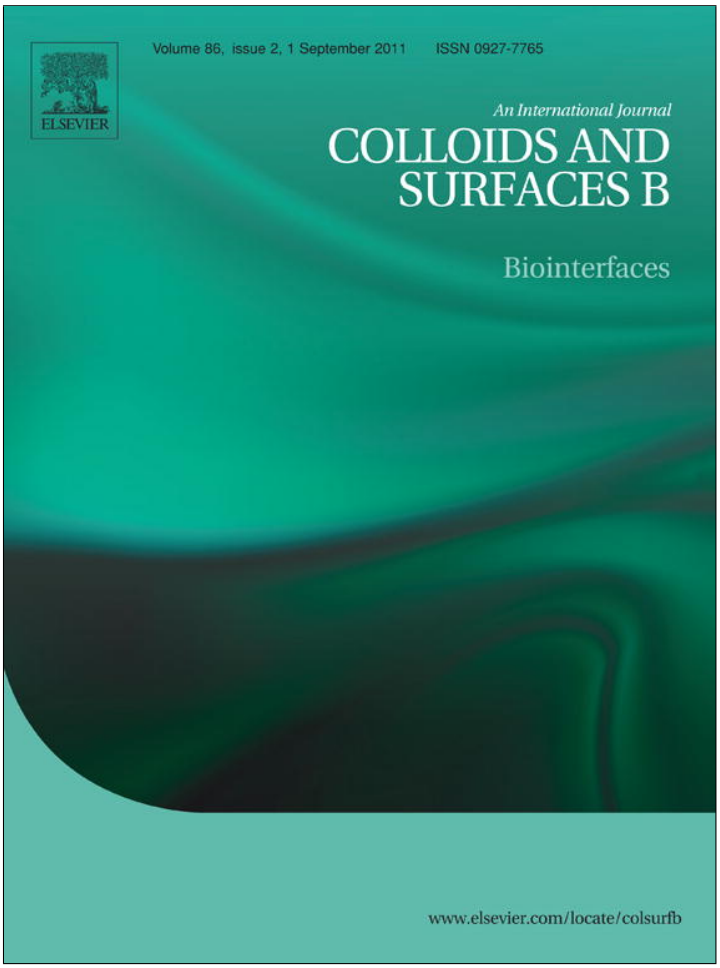

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Effect of saponins on cell surface properties of *Penicillium simplicissimum*: Performance on adsorption of cadmium(II)

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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.

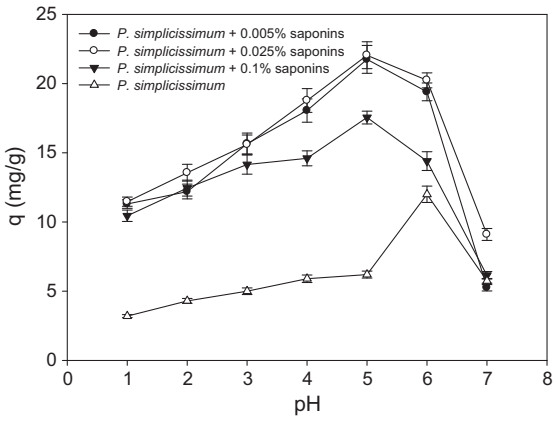

Fig. 1. Effect of pH on cadmium(II) biosorption by *P. simplicissimum*. Results are expressed as mean ± standard deviation ($n = 3$).

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].

Table 1
The optimal pH for cadmium adsorption by various biomasses.

Biosorbent type	Microorganism species	Optimal pH	References
Phanerochaete chrysosporium	Fungus	Around pH 6.0	[25]
P. simplicissimum	Fungus	pH 4.0	[3]
Saccharomyces cerevisiae	Yeast	pH 6.0	[26]
Escherichia coli	Gram-negative bacterium	pH 5.0	[13]
Pseudomonas sp.	Gram-negative bacterium	pH 7.0	[27]
Staphylococcus xylosus	Gram-positive bacterium	pH 6.0	[27]
Intact *P. simplicissimum*	Fungus	pH 6.0	This work
Saponins-pretreated *P. simplicissimum*	Fungus	pH 5.0	This work

Table 1 shows that the optimal pH values were different for cadmium(II) adsorption by various biosorbents. The main reason may be due to the different cell surface characteristics of biosorbents. In this study, the pretreatment of saponins not only changed the optimal pH value for Cd(II) adsorption, but also enhanced the adsorption capacity, indicating the increased amount of adsorption sites. These results suggested that the addition of saponins changed the cell surface properties of *P. simplicissimum*.

3.2. Adsorption isotherm

In this study, Langmuir and Freundlich isotherms were applied to describe the adsorption equilibrium. The Langmuir isotherm is consistent with strong monolayer sorption whose energy is constant [15]. There is no migration of adsorbed molecules in the surface plane in this adsorption model. The Langmuir isotherm equation is described by the following linearized equation:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{bq_{\max}} \quad (2)$$

where q_e (mg/g) is the adsorption capacity; C_e (mg/l) is the equilibrium concentration in solution; $q_{\max}$ (mg/g) is the maximum adsorption capacity; b (l/mg) is a constant related to adsorption energy of adsorption. The results were shown in Fig. 2A.

The Freundlich isotherm is purely empirical based on sorption on a heterogeneous surface, which is commonly presented as:

$$\ln\ q_e = \ln K_F + a \ln C_e \quad (3)$$

where q_e (mg/g) and C_e (mg/l) are the same as above; K_F is the adsorption coefficient; a is the Freundlich constant. The results were shown in Fig. 2B.

The adsorption constants (R^2) in Table 2 showed that the adsorption of Cd(II) was better described by the Langmuir model than the Freundlich model. Several studies also reported that heavy metal ions adsorption by fungus followed the Langmuir model better than the Freundlich model [3,16,17]. According to the Langmuir isotherm, we found that the monolayer saturation adsorption capacity ($q_{\max}$) increased with saponins concentrations from 0.005% to 0.025%. The 0.025% saponins-pretreated *P. simplicissimum* showed the greatest potential to adsorb Cd(II) ($q_{\max}$, 74.6 mg/l). Then, $q_{\max}$ decreased to 58.1 mg/l with 0.1% saponins, but it was still higher than that of the intact biomass (51.6 mg/l). These results were consistent with those shown in Fig. 1 and indicated that the pretreatment of saponins enhanced Cd(II) adsorption capacity, which also demonstrated the changed cell surface properties of *P. simplicissimum*.

3.3. FTIR analysis

The functional groups, such as carboxyl, amino, and hydroxyl groups, play important roles in the adsorption of heavy metals. The changed characteristics of these functional groups can influence their adsorption ability of heavy metals [2]. The FTIR spectra of the intact and 0.025% saponins-pretreated biomass before and after Cd(II) adsorption were measured to show the possible changes (Fig. 3). The presence of saponins shifted the peaks at 3356 cm^{-1} (indicative of the overlapping of the O–H and N–H stretching [18]) to 3361 cm^{-1}. These functional groups worked on the adsorption of Cd(II), since after adsorption the peaks of the intact and pretreated biomasses increased to 3388 and 3432 cm^{-1}, respectively. The peaks at 1653 cm^{-1} (indicative of the C=O stretching in carboxyl or amine groups), 1545 cm^{-1} (indicative of the N–H bending), 1232 cm^{-1} (indicative of the C=S stretching), and 1035 cm^{-1} (indicative of the C–N stretching vibrations [15]) also shifted in a certain extent after the saponins pretreatment and/or the adsorption of cadmium(II). These results indicated that the addition of saponins changed the chemical structures of the biomass and therefore affected the adsorption of Cd(II).

3.4. EDAX and SEM analysis

EDAX analysis is one of the useful tools to evaluate the elemental characteristics of biomass [19]. The element analysis of the intact

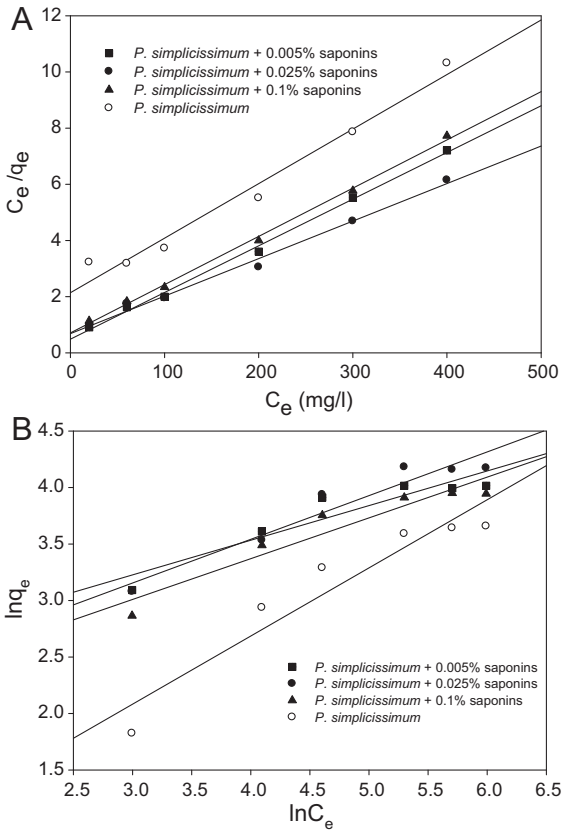

Fig. 2. Langmuir isotherms (A) and Freundlich isotherms (B) for Cd(II) biosorption by the intact and saponins-pretreated biomasses. Results are expressed as means of 3 independent measurements and less than 5% standard deviation.

Table 2
Langmuir and Freundlich constants and correlation coefficients (R^2) for Cd(II) adsorption. Results are expressed as means of 3 independent measurements and less than 5% standard deviation.

Biosorbent type	Langmuir			Freundlich		
	q_{max} (mg/l)	b (l/mg)	R^2	K_F	a	R^2
P. simplicissimum	51.6	0.0091	0.9748	1.313	1.658	0.9075
P. simplicissimum + 0.005% saponins	60.2	0.0341	0.9967	10.046	3.261	0.8748
P. simplicissimum + 0.025% saponins	74.6	0.0197	0.9907	7.349	2.585	0.9278
P. simplicissimum + 0.1% saponins	58.1	0.0243	0.9977	6.865	2.769	0.9088

Table 3
The element percents of the intact and 0.025% saponins-pretreated *P. simplicissimum*. Results are expressed as means of 3 independent measurements and less than 5% standard deviation.

Biosorbent species	Element weight percents (%)						
	C	N	O	P	S	Cl	K
P. simplicissimum	76.31	2.3	16.07	2.01	0.44	1.19	1.68
P. simplicissimum + 0.025% saponins	67.60	7.49	17.67	1.27	0.85	3.11	2.01

and 0.025% saponins-pretreated biomass was shown in Table 3. The results indicated that the pretreatment of saponins changed the chemical component of the biomasses. Previous investigations found that there are certain relationships between the electrical properties and the element (such as phosphate, nitrogen and carbon) concentrations on cell surface, which is dependent on the microorganism species [20,21]. For certain microorganisms, the origin of the negative surface potential seems to be mainly phosphate (or phosphodiester), amines and carboxylic groups present at the cell surface [21]. In this study, the oxygen concentration only changed a little. The pretreatment of saponins decreased the carbon and phosphate concentrations to 89% and 63%, respectively, which may reduce the negative charge on the cell surface [20,21]. However, it markedly increased the nitrogen concentration (3.3 fold), probably indicating the increased amount of the negative charges on the cell surface [20,21]. We postulated that the increased concentrations of S (1.9 fold) and Cl (2.6 fold) may also increase the negative charge on the cell surface. The K concentration on cell surface also increased after the pretreatment of saponins, indicating the increased adsorption ability of heavy metals. Kapoor and Viraraghavan [22] found that *Aspergillus niger* released potassium, calcium and magnesium ions when it adsorbed metal ions. Tunali et al. [19] also found that the peaks indicative of Mg^{2+} and K^+ disappeared after Cu(II) sorption by *Bacillus* sp. through EDAX analysis. In this study, the K^+ peaks between 3 and 4 keV disappeared after Cd(II) adsorption by both intact and saponins-pretreated biomasses through the EDAX analysis (data not shown), suggesting that the ion-exchange mechanism was also involved in the biosorption process.

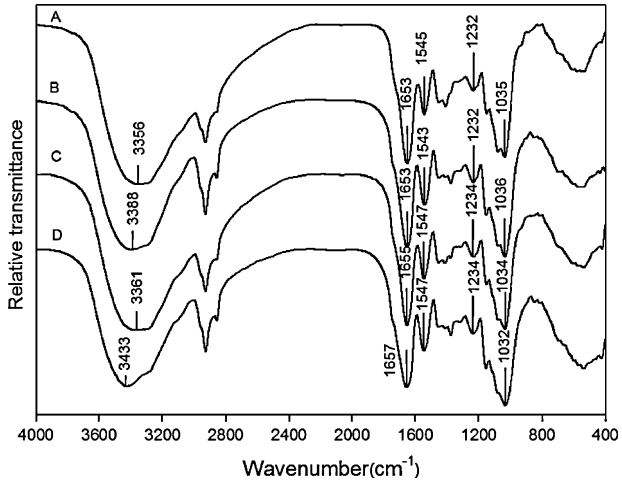

Fig. 3. FTIR spectra of: (A) the intact biomass; (B) the intact biomass with cadmium; (C) the 0.025% saponins-pretreated biomass; (D) the 0.025% saponins-pretreated biomass with cadmium. The adsorption was carried out at pH 5.0 with 20 mg/l Cd(II).

Tunali et al. [19] found that the morphology of *Bacillus* sp. upon adsorption of Pb(II) and Cu(II) was different, indicating that the morphology of the biomasses is dependent on the characteristics of biosorbents and heavy metals. To examine morphologic changes at the ultrastructural level, the intact and 0.025% saponins-pretreated biomasses before and after adsorption of Cd(II) were observed using SEM. Before adsorption of Cd(II), both biosorbents have large spaces (Fig. 4A and B). However, both the intact and saponins-pretreated biomasses were densely packed and individual mycelia pieces were not found after cadmium uptake (Fig. 4C and D). The results indicated that the adsorption of Cd(II) exactly occurred during the experiments. The morphology upon Cd(II) adsorption between the intact and saponins-pretreated biomasses was different. The intact biosorbents after uptake of Cd(II) looked like aggregated spawn (Fig. 4C), while the saponins-pretreated biosorbents after adsorption were like aggregated tharm (Fig. 4D). The changed morphology upon Cd(II) adsorption was also probably caused by the change of cell surface characteristics.

3.5. General mechanisms

Previous studies have reported some general mechanisms for the influence of biosurfactants on cell surface charges. First, biosurfactants can adsorb to the cellular envelope. Saponins can built into the bio-membrane lipophilic part with their hydrophilic groups towards the external environments [6]. Some negatively charged groups, such as hydroxyl and carboxyl, are present on the hydrophilic moiety of saponins and can interact with cations such as cadmium(II), lead(II) and copper(II) [23]. Thus, the adsorption of saponins on the cellular envelope could increase the amount of adsorption sites. Second, biosurfactants can increase biomembrane permeability. Kaczorek et al. [6] showed that saponins may increase membrane permeability of *Aeromonas hydrophila*. Sotirova et al. [4] also reported that the biosurfactant PS probably forms molecular aggregates in surface bacterial membranes, leading to the formation of transmembrane pores that serve as channels to the periplasm. Some components (such as proteins, polysaccharides, glycoproteins, and lipopolysaccharides) able to

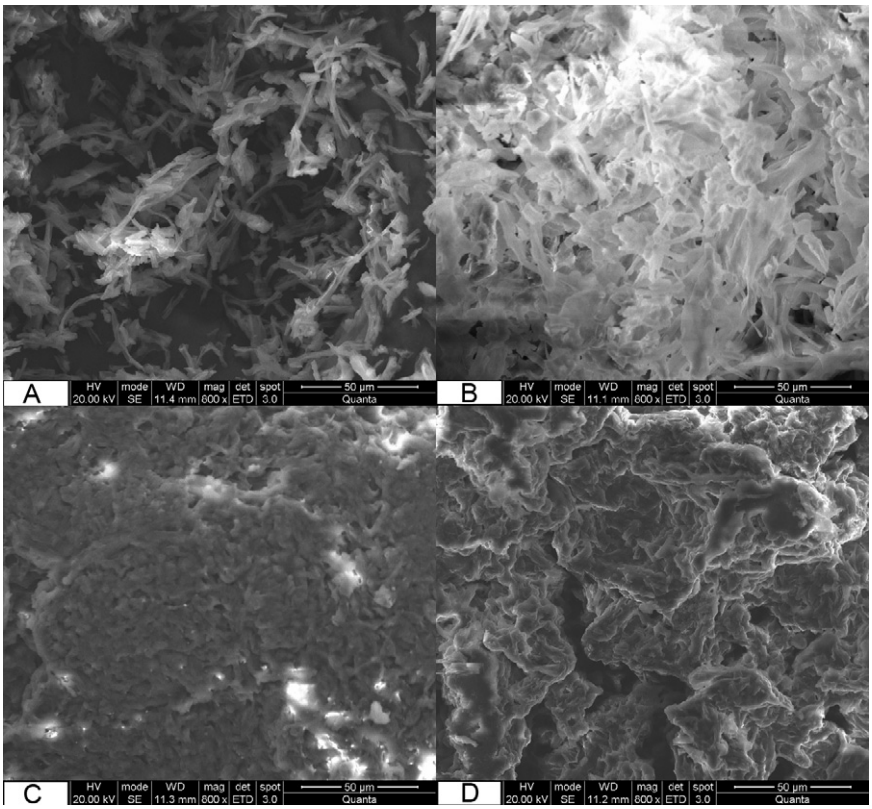

Fig. 4. SEM micrographs of *P. simplicissimum*: (A) the intact biomass; (B) the 0.025% saponins-pretreated biomass; (C) the intact biomass after cadmium uptake; (D) the 0.025% saponins-pretreated biomass after cadmium uptake. The adsorption was carried out at pH 5.0 with 20 mg/l Cd(II).

adsorb heavy metals may release from the inside of cells and remain on cell surface, which probably influence the cell surface charge [24]. Third, biosurfactants can cause the release of chemical components from the cell surface. Sotirova et al. [4] reported that the interaction of biosurfactant PS with surface proteins may cause the direct removal of proteins by solubilisation. Al-Tahhan et al. [8] also found that biosurfactant rhamnolipids can induce the release of lipopolysaccharide from the cell membrane of *Pseudomonas aeruginosa*. Because these components, such as proteins and lipopolysaccharide, confer a considerable negative charge upon the cell surface and favour electrostatic interactions with cations, removal of these components may reduce the negativity of the cell surface charge, thus reducing the interactions with cations, such as cadmium(II) [11]. In this study, the pretreatment of saponins enhanced the adsorption capacity. We postulated that the first and second mechanisms played the main roles in the change of cell surface charges of *P. simplicissimum*.

4. Conclusions

In this study, the addition of saponins enhanced the adsorption capacity of Cd(II) and changed the optimal pH for the adsorption by *P. simplicissimum*. All the biosorption followed the Langmuir isotherm better than the Freundlich isotherm. The FTIR spectra indicated that the pretreatment of saponins changed the cell surface properties and therefore influenced the adsorption kinetics of Cd(II). The EDAX analysis showed that the element percent of the biomass was changed by the saponins pretreatment. The SEM analysis also showed the differences between the intact and saponins-pretreated biomasses before and after cadmium(II) adsorption, which also demonstrated that the addition of saponins changed the cell surface characteristics of *P. simplicissimum*.

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