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Contributions of different fractions of extracellular polymeric substances from waste-activated sludge to Cu(II) biosorption

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ABSTRACT

The extracellular polymeric substances (EPS) extracted from waste-activated sludge (WAS) were used as a novel biosorbent. The goal of this study was to quantify the contribution of EPS to copper immobilization by WAS. Through comparison of copper sorption using sludge with tightly bound EPS (TB-EPS) and sludge with minimal EPS, we demonstrated that TB-EPS from WAS contributed significantly to copper sorption, especially at low initial Cu^{2+} concentrations. EPS probably became pseudo-saturated in terms of binding sites for copper sorption at high Cu^{2+} concentrations; thus, cells may play a more dominant role. To quantify the relative contributions of copper sorption by EPS fractions, loosely bound EPS (LB-EPS) and TB-EPS were isolated from WAS and used in batch biosorption experiments. The results showed that TB-EPS exhibited a higher sorption capacity than LB-EPS in any pH solution applied in this study (3–6.5), due to a higher amount of functional groups (carboxylic groups) of TB-EPS. Scanning electron microscopy observation revealed that TB-EPS had a rough surface with long chains, making it more available for copper binding. Fourier transform infrared analysis further confirmed that the functional groups responsible for Cu^{2+} biosorption primarily consisted of hydroxyl (–OH), carboxyl (–COOH), and amide (N–H). The functional groups with weaker affinity in EPS may play a more significant role in copper removal from the aqueous phase at high copper concentrations.

Keywords: Biosorption; Copper; Extracellular polymeric substances; Functional groups; Waste-activated sludge

1. Introduction

Heavy metal contamination of the aquatic environment has aroused intense concerns with the increased awareness of its toxicity and other adverse effects on the environment and human health [1,2]. It has been established that all metals or elements with metallic characteristics can form compounds that are toxic to humans [3]. The traditional methods for heavy metal removal include chemical precipitation, membrane, ion exchange, activated carbon adsorption, and co-precipitation/adsorption which involve high capital and operational costs and may have secondary wastes after processes [4–6].

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In recent years, research attention has been focused on biosorption, a cost-effective biological method which has been demonstrated to possess good potential for replacing conventional methods for the removal of heavy metals using various biosorbents [6]. Generally, the biosorption process can reduce capital costs by 20%, operational costs by 36%, and total treatment costs by 28% compared with conventional systems [7]. Thus, interest in the utilization of cheap alternatives has significantly increased. Various biomaterials have been used as biosorbents such as plant biomass, bacteria, fungi, and algae [8]. Activated sludge has drawn particular interests recently because of its good metal-sequestering property, abundant availability, and low-cost nature [9,10]. Due to the abundant binding sites in extracellular polymeric substances (EPS), activated sludge can decrease heavy metal ion concentrations in aqueous solution from the ppm to ppb level within hours through adsorption [11].

Containing a significant amount of natural organic macromolecule substances, abundant waste-activated sludge (WAS) exists in municipal wastewater treatment plants (WWTP), and it needs to be further treated or reused [12]. Thus, a suitable reuse of WAS is in accordance with sustainable development and the needs for the development of sludge disposal methods. Yu et al. [13] found that WAS can be considered as a good source of EPS, and Zhang et al. [12] have used WAS and extracted EPS for metal removal from water. Recently, there have been some reports about EPS being used as effective biosorbents for the removal of toxic metals [14] and organic pollutants such as dyes, antibiotics, and nanoparticles [15–17]. Because EPS are usually the first barrier of microbial cells that directly contact and interact with metals in the aqueous environment, they are of vital importance not only for protecting the interior microbial cells [18], but also for geochemical cycling and the remediation of metals in the natural environment [8]. Accordingly, EPS in sludge flocs can be stratified into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) [17,18]. However, most of the current studies have not specifically addressed the role of the two types EPS in biosorption in solution [12,14,19]. Thus, little information is available to distinguish between the various compositions of the two types of EPS and their effects on surface properties and biosorption.

Waste biomass adsorbents have been extensively applied to remove heavy metals from industrial wastewater [6]. To better use WAS and the extracted EPS as novel biosorbents, detailed knowledge about biosorption mechanisms is necessary. Cu(II) was selected as a typical candidate of heavy metal pollution. To the best of our knowledge, the relative contributions of LB-EPS, TB-EPS, and EPS-free sludge to copper biosorption have not been investigated. Therefore, the objective of this study was (1) to quantify the contribution of EPS to copper immobilization by WAS through a comparison of copper sorption using sludge with TB-EPS and sludge with minimal EPS (2) and to identify the adsorption mechanisms, determine the relative contributions of EPS fractions (LB-EPS and TB-EPS) to biosorption by scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectroscopy spectra analysis.

2. Materials and methods

2.1. WAS and aerobic digestion

The WAS was collected from a secondary sedimentation tank of a municipal wastewater treatment plant (Nanjing, China). This plant treats approximately 40,000 m^3/d of domestic wastewater using anaerobic–anoxic–oxic processes. Samples were collected right before each experiment and stored in an ice box during the 30-min transport to the laboratory. The pH of the wastewater was 6.9 ± 0.4, and the ionic strength was 21.5 mM. The ratio of volatile suspended solids (VSS) to total suspended solids (TSS) of the sludge was 64.5 ± 6%. The suspended solids and VSS of sludge were determined according to the Standard Methods [20].

The aerobic digestion process of WAS was performed at approximately 23℃ with a pH of 6.8 ± 0.3 and dissolved oxygen (DO) level of 2–3 mg/L. According to the study of Zhang et al. [12] and our experimental results on the parameter optimization, the WAS samples were aerobically digested for approximately 4 h and then applied to the biosorption experiment and EPS extraction.

2.2. Sludge preparation and EPS extraction

As shown in Fig. 1, the WAS samples were divided into sludge with TB-EPS (A) and sludge with minimal EPS (C). The EPS were divided into LB-EPS (B) and TB-EPS (D), through different extraction procedures (see details described below). Then, the sludge with TB-EPS and the sludge with minimal EPS were used to perform the sludge biosorption experiments; meanwhile, LB-EPS, and TB-EPS were used for adsorption experiments to study the roles of different EPS fractions in biosorption for heavy metals.

After aerobic digestion, the extraction of EPS fractions from WAS was conducted according to the protocol with negligible contamination from cellular

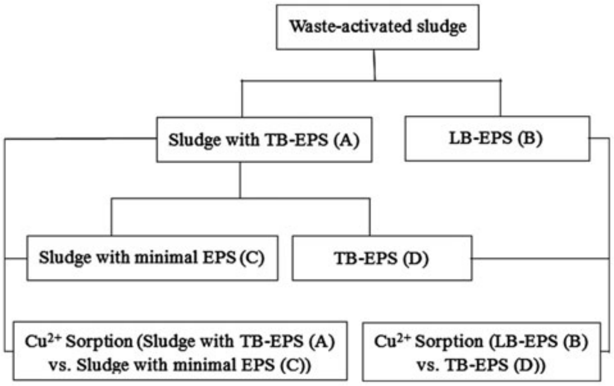

Fig. 1. Schematic illustration of sludge preparation and EPS extraction for comparative analyses of copper sorption: (A) sludge with TB-EPS, (B) LB-EPS, (C) sludge with minimal EPS, and (D) TB-EPS.

materials [21]. Briefly, WAS was firstly washed three times with 0.05% NaCl solution by centrifugation at 2,000*g* for 15 min. The sludge samples in the tube were then resuspended in 0.05% NaCl and centrifuged at 8,000*g* for 20 min, and the supernatants were defined as LB-EPS. As for TB-EPS, the dispersed sludge samples (sludge with TB-EPS) were resuspended in 0.05% NaCl and then heated at 60˚C for 30 min, and they proved able to obtain a relatively high extraction efficiency and low cell lysis [22]. Afterwards, the TB-EPS were harvested by centrifugation at 12,000*g* for 20 min. The sludge samples with minimal EPS were pelleted by centrifugation with 2,000*g* for 15 min and washed three times using 0.05% NaCl. The sludge with TB-EPS and sludge with minimal EPS were kept at 4˚C for further use in biosorption experiments. Finally, both the LB-EPS and TB-EPS were filtered through 0.22 μm cellulose acetate membranes and purified with a dialysis membrane (5,000 MWCO) against deionized water for 24 h at 4˚C at least three times. The harvested solutions were then freeze dried and reserved.

Both the LB-EPS and TB-EPS extractions were analyzed for total organic carbon (TOC), polysaccharide and proteins. TOC was measured by a TOC analyzer (Liqui TOC II, Elementar, Germany) using the combustion-infrared method. The polysaccharides and proteins were analyzed using the anthrone-sulfuric acid method and modified Lowry method, respectively, according to the literature [23].

The morphological changes of WAS before and after TB-EPS removal were observed using a SEM (Hitachi S-4800) following the procedures in our previous study [17]. The viability of sludge communities with TB-EPS and sludge with minimal EPS was evaluated using a confocal laser scanning microscope (CLSM) (Nikon A1, Japan) and a BacLight live/dead staining kit (Invitrogen). The green and red fluorescing cells were counted at five different locations within each sample, and viability was expressed as the percentage of green cells averaged over the five locations.

2.3. Cu^{2+} sorption by sludge with TB-EPS and sludge with minimal EPS

A standard stock solution of Cu^{2+} (20 mM) was prepared by dissolving $Cu(NO_3)_2$ in deionized water separately. The solution was sterilized by filtering with a 0.22 μm-pore-size filter. Before the tests, all glassware was dipped in 0.1 M HNO_3 for 48 h, rinsed with deionized water thoroughly, and then dried. All chemicals were of analytical grade.

Biosorption experiments were carried out in 100-mL Erlenmeyer flasks, and the final concentrations of Cu^{2+} in the suspension (100, 500, and 1,000 μM) were prepared by diluting the standard stock solution at prescribed proportions. After adding 0.1 g (dried weight) sludge samples (sludge with TB-EPS and sludge with minimal EPS), the metal suspensions were agitated in a water bath shaker (125 rpm) at a constant temperature of 23 ± 1˚C [15]. The pH was controlled to 6.5 by adding 0.01-M NaOH or 0.01-M HNO_3 to avoid precipitation of Cu^{2+} [14]. Each test lasted for 6 h to reach equilibrium according to our preliminary experiments. At fixed time points, 1-ml samples were taken out, diluted 5–10 times with pure water, and centrifuged at 4,000*g* for 10 min, and then the supernatant was filtered through a 0.22 μm cellulose acetate membrane [12]. Residual Cu^{2+} in the supernatant was determined using ICP-MS (Agilent 7700X). All the biosorption tests were conducted in triplicate, and the average value was used. The negative controls (without biosorbent) were simultaneously conducted to ensure that sorption was caused only by the biosorbents applied. The biosorption rate *r* (%) was calculated as follows:

$$r = \frac{(C_0 - C_t)}{C_0}$$

where C_0 and C_t (μM) are the concentration of Cu^{2+} initially and at time *t*, respectively.

2.4. Cu^{2+} sorption by LB-EPS and TB-EPS

In the biosorption experiments of EPSs, 10 mg of powdered EPS (LB-EPS and TB-EPS) samples were dissolved with deionized water and placed in dialysis sacks (5,000 MWCO). Then, the suspensions were suspended in a clean 100-mL Erlenmeyer flask containing Cu^{2+} solution with three different initial concentrations (100, 500, and 1,000 μM) [24]. The pH was controlled to 6.5 by adding 0.01-M NaOH or 0.01-M HNO_3 to avoid precipitation of Cu^{2+} [14]. The metal solutions with the sacks were shaken at 125 rpm (23 ± 1℃) for 2 h to reach equilibrium according to our preliminary experiments. At fixed time points, 2-ml samples were taken out and filtered through a 0.22-μm cellulose acetate membrane. Then, residual Cu^{2+} in the supernatant was determined [24]. Each of the sets was prepared in triplicate, and the average value was used. The negative controls (without biosorbent) were simultaneously carried out to ensure that sorption was caused only by the biosorbents applied. In addition, the effect of pH on the biosorption was also investigated, in which the Cu^{2+} solution (fixed as 1,000 μM) was adjusted to the desired pH (3–6.5) with 0.01 M NaOH or 0.01 M HNO_3.

2.5. Analytical methods

The surface structure and morphology of LB-EPS and TB-EPS before and after adsorption of copper were observed by SEM (Hitachi S-4800). All EPS were freeze dried. Before SEM analysis, all the samples were coated with gold (Au).

FTIR spectra of the LB-EPS and TB-EPS before and after copper adsorption tests were recorded using FTIR (Tensor 27, Bruker) connected with a personal computer. Before the analysis, the wet samples were lyophilized. All infrared spectra were recorded over the range of 4,000–400 cm^{-1}, and the averaged spectra were obtained at a resolution of 4 cm^{-1}. FTIR spectra were measured on KBr pellets prepared by pressing mixtures of 1-mg dry powdered sample and 100-mg spectrometry-grade KBr under a vacuum to avoid moisture uptake. Spectra were baseline corrected to observe the differences in the functional groups in EPSs after the biosorption of copper with three different concentrations.

2.6. Statistical analysis

All tests were performed in triplicate, and the results are expressed as the mean ± standard deviation. For statistical analysis, the experimental values were compared to their corresponding control values. Statistical analysis (*t*-test) was performed using SPSS 16.0. A difference was judged as being significant at $p < 0.05$.

3. Results and discussion

3.1. Cu^{2+} immobilization by sludge with TB-EPS and sludge with minimal EPS

The preparation of sludge flocs and the isolation of EPS fractions for Cu^{2+} sorption studies are shown schematically in Fig. 1. Representative SEM images of waste activated sludge before and after removal of EPS are shown in Fig. 2. As can be seen from Fig. 2(A), most of the bacterial cells were embedded by secreted EPS. As the LB-EPS bound with cell loosely, centrifugation was chosen to extract the LB-EPS from sludge [21], in order to avoid the inclusion of the TB-EPS [18]. After the removal of LB-EPS, there was little difference in the surface morphology of the sludge flocs compared to the original sludge; in contrast, after TB-EPS removal individual bacterial cells became more apparent (Fig. 2). The removal of TB-EPS did not significantly affect cell viability (81 ± 5% for untreated sludge vs. 75 ± 6% for TB-EPS-removed sludge; $p = 0.256$) as shown in Fig. 3.

Biosorption experiments of the sludge with TB-EPS and the sludge with minimal EPS were conducted under three initial Cu^{2+} concentrations (100, 500, and 1,000 μM). The results are shown in Fig. 4. The sorption of Cu^{2+} onto both sludge with TB-EPS and sludge with minimal EPS were rapid for each initial Cu^{2+} concentration in the first 1 h, and then the sorption rate decreased with time due to the continuous decrease in the driving force of Cu^{2+} concentration [12]. The abiotic controls with the three different initial concentrations showed Cu^{2+} concentrations remaining constant over time. However, there were significant differences ($p < 0.01$) between the copper removal efficiency of sludge with TB-EPS and sludge with minimal EPS, suggesting that TB-EPS account for the differences in copper sorption.

As seen in Fig. 4(D), a higher copper sorption capacity was observed for sludge with TB-EPS at three different initial Cu^{2+} concentrations. These results showed that EPS removal significantly decreases the ability of sludge flocs to immobilize copper. Furthermore, the difference in copper sorption between sludge with TB-EPS and sludge with minimal EPS was proportionally more significant at low Cu^{2+} concentrations (17.6, 8.2, and 5.6% at initial Cu^{2+} concentrations of 100, 500, and 1,000 μM, respectively), indicating that EPS play more important roles at lower Cu^{2+} concentrations. Metal sorption by EPS is believed to be an important self-protection strategy of microbial cells against toxic substances [18]. EPS are usually the first barrier of microbial cells that directly contact and interact with metals in aqueous environments [8]. The biosorption mechanism is not only related to the

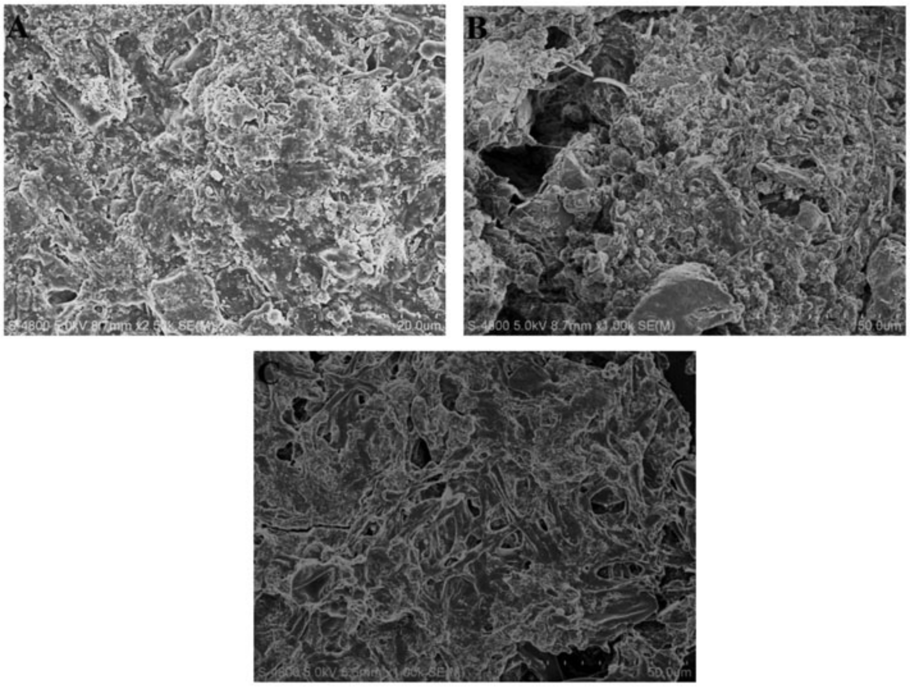

Fig. 2. Representative SEM images of waste activated sludge before and after EPS extraction: (A) original sludge, (B) sludge with TB-EPS, and (C) sludge with minimal EPS.

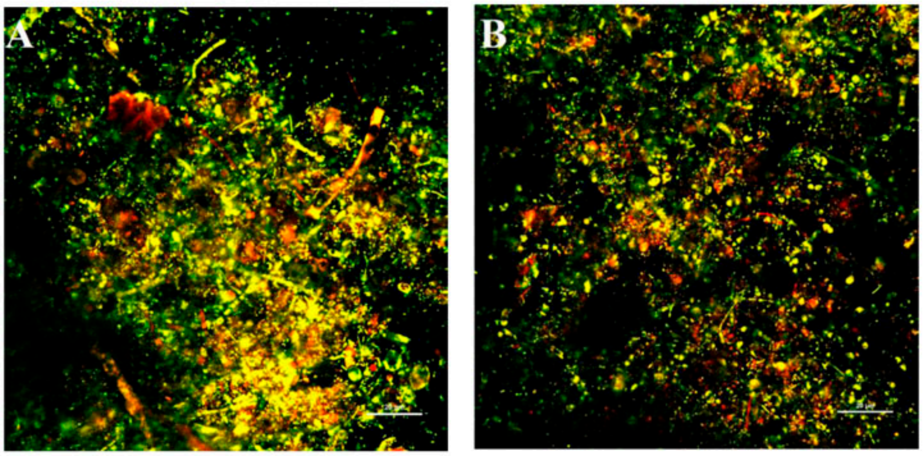

Fig. 3. Representative CLSM images of waste activated sludge after EPS extraction: (A) sludge with TB-EPS and (B) sludge with minimal EPS. Live cells in the sludge fluoresce green while the dead ones fluoresce red.

binding sites of a system as a whole, but to the availability of binding sites in a location that surrounds the bacteria [8,24]. Thus, EPS likely contribute more significantly to copper sorption at low Cu^{2+} concentrations; at high Cu^{2+} concentrations, EPS probably becomes pseudo-saturated for Cu^{2+} sorption due to the obstruction of the spatial structure of sludge [24], so cellular protective mechanisms may play a more dominant role. Previous studies have demonstrated that EPS have important roles in metal removal by sludge [9,25], and similar saturated biosorption of EPS has been reported by Li and Yu [8] and More et al. [26].

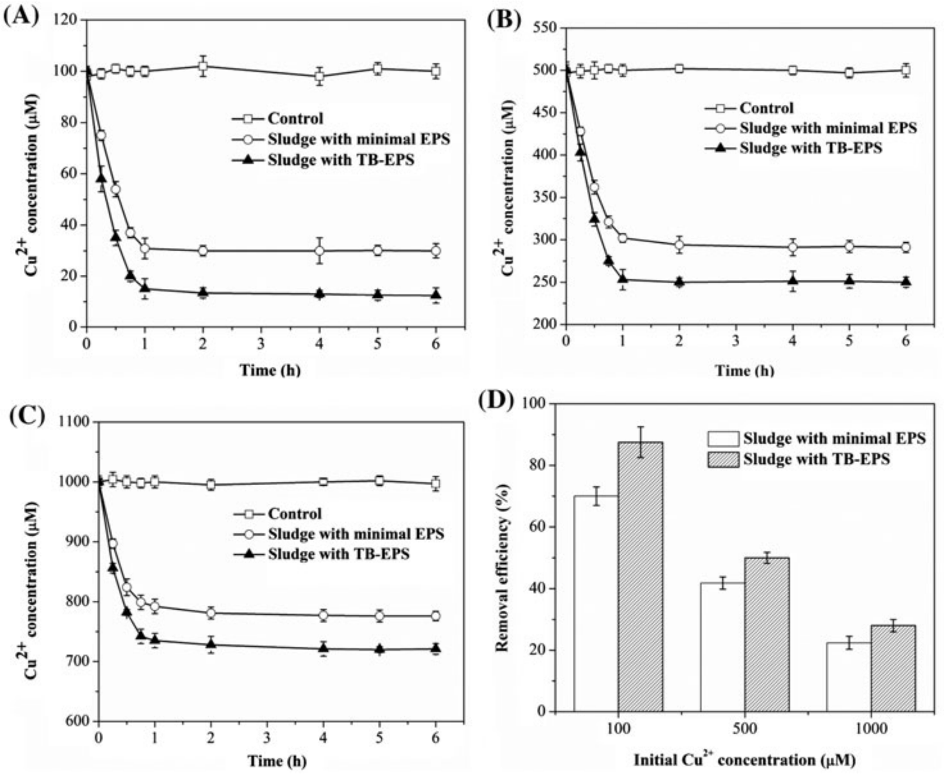

Fig. 4. Sorption of Cu^{2+} at different initial concentrations by sludge with TB-EPS and sludge with minimal EPS: (A) 100 μM, (B) 500 μM, and (C) 1,000 μM. Abiotic controls at the different initial Cu^{2+} concentrations are provided; (D) Copper removal efficiency (expressed as % of initial Cu^{2+} removed from aqueous phase) of sludge with TB-EPS and sludge with minimal EPS at different initial Cu^{2+} concentrations. Error bars represent standard deviation (SD) (*n* = 3).

3.2. Biosorption tests on LB-EPS and TB-EPS

3.2.1. Characteristics of EPS and effect of pH on biosorption

To further quantify the relative contributions of sorption in copper immobilization by EPS, we directly conducted the biosorption experiments of the isolated LB-EPS and TB-EPS for Cu^{2+}. The main contents of LB-EPS and TB-EPS measured by standard quantification are listed in Table 1. Proteins and polysaccharides are the major constituents of EPS, which is in accordance with the previous literature [18,27]. The protein/polysaccharide ratios were 0.7 and 3.8 in LB-EPS and TB-EPS, respectively. The contents of protein and polysaccharide in both EPS showed the same of order of magnitude (at mg/g SS) with the results reported by Sassi et al. [5], who extracted EPS from activated sludge using the heating method.

The comparisons of Cu^{2+} biosorption on LB-EPS and TB-EPS are shown in Fig. 5. It is noticeable that the rate of Cu^{2+} immobilization was very rapid in the first 20 min and reached equilibrium within 40 min. This rapid initial biosorption was consistent with the results reported by Zhang et al. [12] in which the time required for equilibrium was 30 min at an initial Cu^{2+} concentration of 50 mg/L. The copper removal efficiencies by LB-EPS and TB-EPS at different initial Cu^{2+} concentrations were calculated and are shown in

Table 1
The main compositions of EPS[a] (LB-EPS and TB-EPS) extracted from WAS after short-term aerobic digestion

	Proteins (mg g^{-1} SS)	Polysaccharides (mg g^{-1} SS)	TOC (mgC g^{-1} SS)	Proteins/polysaccharides
LB-EPS	17.6 ± 3.2	25.2 ± 1.4	7.5 ± 2.1	0.7
TB-EPS	114.6 ± 6.7	29.8 ± 3.2	28.4 ± 3.7	3.8

[a] The data reported are the averages and their SDs in triplicate measurements.

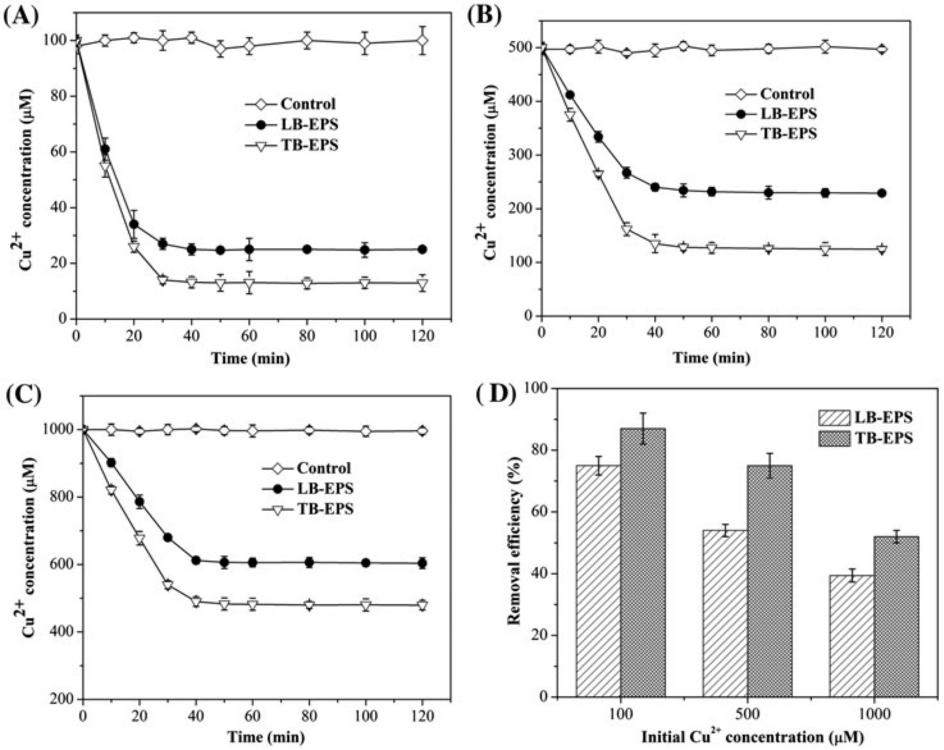

Fig. 5. Sorption of Cu^{2+} at different initial concentrations by LB-EPS and TB-EPS: (A) 100 μM, (B) 500 μM, and (C) 1,000 μM. Abiotic controls at the different initial Cu^{2+} concentrations are provided; (D) Copper removal efficiency (expressed as % of initial Cu^{2+} removed from aqueous phase) of sludge with TB-EPS and sludge with minimal EPS at different initial Cu^{2+} concentrations. Error bars represent SD (*n* = 3). Error bars represent SD (*n* = 3).

Fig. 5(D). A relative higher removal capacity was obtained from biosorption on TB-EPS at any initially applied Cu^{2+} concentration, indicating that TB-EPS gave a higher contribution to copper biosorption. In addition, the raising Cu^{2+} concentration resulted in increased sorption capacity of the TB-EPS from 65.4 (100 μM Cu^{2+}) to 330.2 mg/g (1,000 μM Cu^{2+}). According to the literature, average Cu adsorption capacity of bioadsorbents is observed in the following order as activated carbon (AC) > algal > bacterial > agriculture and forest > fungal > yeast biomass [6,8]. The increased surface area and porosity of activated carbon are the main factors responsible for higher copper ion removal, with Cu sorption capacity about 50–100 mg/g [6]. Values obtained at high initial Cu^{2+} concentrations in this study are significantly greater than the sorption capacity of AC, indicating that the EPS could be probable to widely apply for wastewater treatment containing heavy metals through further study.

The effect of pH on Cu^{2+} biosorption from aqueous solution by EPS was studied at various pH (ranging from 3 to 6.5) and results are shown in Fig. 6(A). At lower pH (3–4), the H_3O^+ and Cu^{2+} protons compete for binding sites on the EPS, and result in lower Cu^{2+} sorption [8]. The adsorption capacity of both the EPS for Cu^{2+} significantly increased with increasing pH value as it was below 5, and then with rising pH, the adsorption capacity exhibited slightly increase. These observed trends for both EPS were consistent with those reported in the literature [12,18]. TB-EPS showed a higher sorption capacity than LB-EPS in any pH solution applied in this study, due to a higher amount of functional groups (carboxylic groups) of TB-EPS, as confirmed by the acidity test of both the EPS in Fig. 6(B). In this study, experiments were not performed beyond pH 6.5, in order to avoid Cu^{2+} precipitation in the form of hydroxide [28].

The excellent metal-binding capacity of EPS could be due to their potency to interact with positively charged metals [19]. The adsorption capacity of extracted EPS was 2.34 times as much as that of pristine disintegrated aerobic granules, and ion exchange was the most important biosorption mechanism [28]. Comte et al. [9] determined the numbers of total potential binding sites in soluble and bound EPS extracted from activated sludge and suggested that different mechanisms could be implicated in the metal

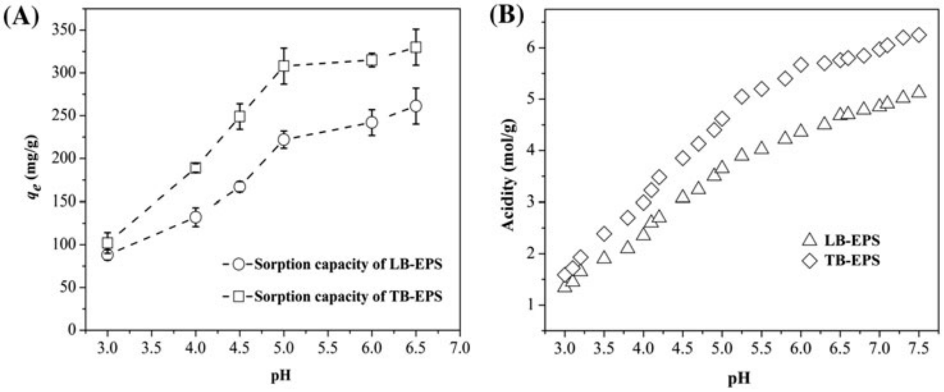

Fig. 6. (A) Effect of pH on the Cu^{2+} biosorption by EPS with an initial Cu^{2+} concentration of 1,000 μM and (B) the acidity of EPS as a function of pH and concentration of EPS is 100 mg/L.

biosorption of EPS, principally in the case of Cu^{2+}. Some studies have demonstrated that the composition of EPS is an important factor affecting the metal adsorption performance of microbial EPS [8] and that proteins were strongly associated with copper removal due to their abundant metal-binding functional groups [29]. In this study, the higher adsorption amounts of TB-EPS for copper in this study implied that the protein/polysaccharide ratios of the EPS greatly influence sorption ability, and the proteins may play major role in copper biosorption. In addition, the biosorption capacity for heavy metals may also differ from the forms (LB-EPS or TB-EPS) and the functional groups in EPS [24], which will be investigated next.

3.2.2. SEM and FTIR analysis

The SEM observations were performed to elucidate the surface morphology of LB-EPS and TB-EPS. As can be seen from Fig. 7(A), LB-EPS had a smooth surface with crystal structure, while, TB-EPS had a relatively rough and bumpy surface with long chains (Fig. 7(B)). The surface area of TB-EPS was greater than that of LB-EPS, improving the adsorption by this EPS type. Zhang et al. [12] found that the EPS extracted from WAS exhibited a meshy structure with long chains by SEM observation, and the EPS had an excellent adsorption performance for copper ions. In this study, the surface structures of both LB-EPS and TB-EPS were measured, and the experimental results were consistent with the finding that the TB-EPS had a higher adsorption capacity. Therefore, the surface morphology of EPS may play an important role in Cu^{2+} removal from the solution.

With an initial Cu^{2+} concentration of 1,000 μM, the aqueous Cu^{2+} concentration decreased to 605.8 ± 11.5 and 481.4 ± 13.8 μM after 120 min of biosorption in the presence of LB-EPS and TB-EPS, respectively (Fig. 5). Similarly, with an initial Cu^{2+} concentration of 500 μM, the Cu^{2+} concentration decreased to 231.6 ± 9.4 and 125.3 ± 9.0 μM. It was expected that all the Cu^{2+} would be immobilized by the EPS when the initial concentration of Cu^{2+} was 100 μM. Interestingly, the final aqueous Cu^{2+} concentration remained at

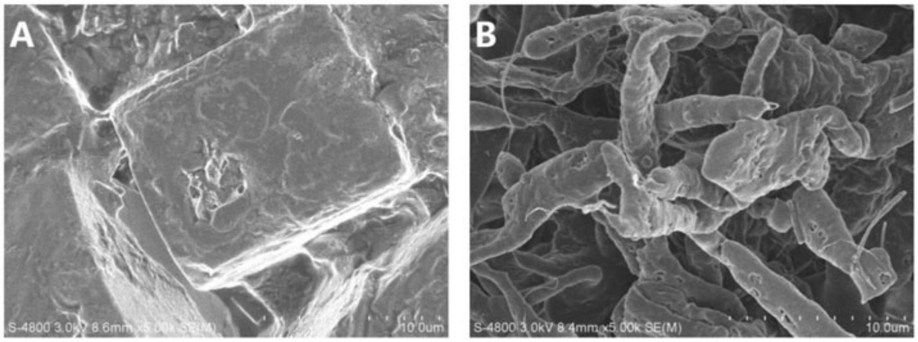

Fig. 7. SEM images of LB-EPS (A) and TB-EPS and (B) extracted from the WAS.

25.2 ± 2.8 and 13.4 ± 3.1 μM. EPS differs in affinity to metal cations, depending mainly on the compositions of functional groups. Hou et al. [24] suggested that carboxylate, carboxylic, hydroxyl, and amino groups are involved in the EPS removal of heavy metals. Guibaud et al. [30] reported that carboxyl groups typically exhibited a stronger binding ability than many other functional groups in EPS, such as hydroxyl groups of neutral polysaccharides, the amide groups of proteins, and some nucleic acids, which are also responsible for metal ion biosorption by EPS [31]. Thus, in this study, there may be a wide range of sorption groups with different binding affinities in the EPS contributing to copper removal from the aqueous phase. The groups with weaker affinity, which play a more significant role at high Cu^{2+} concentrations, may result in a decrease in efficiency at low Cu^{2+} concentrations, which should be further studied.

To confirm the hypothesis above, the pristine and Cu-loaded EPS samples were freeze dried, and FTIR analysis was then performed to identify the major functional groups of EPS and to confirm their possible participation in biosorption. The FT-IR spectrum depicted a number of bands indicating the complex nature of the EPS (Fig. 8). Bands in the FT-IR spectrum were assigned to various groups according to wave numbers (Table 2).

As illustrated in Fig. 8, the functional groups of LB-EPS and TB-EPS observed in this study were in accordance with the results of Guibaud et al. [25] and Zhang et al. [12]. The band approximately 3,400 cm^{-1} was the stretching vibration of –OH in the EPS. The intensive band at 1,640 cm^{-1} was assigned to the –CONH– group of Amide I in proteins. Bands at 1,410 and 1,240 cm^{-1} were attributed to the vibration of the C=O group of carboxylates and carboxylic acids. At 1,000–1,200 cm^{-1}, a wide and intensive band was found. The peaks at 1,120 and 1,080 cm^{-1} were assigned to the stretching vibration of the C–O–C group in the carbohydrate cyclic structures and of –OH, respectively. Some bands at the fingerprint zone (<1,000 cm^{-1}) might be assigned to the phosphate group, which is one of the functional groups composing nucleic acids.

Compared with the pristine EPS, the absorption peaks of the EPS-Cu complex changed greatly. Some peaks shifted and became weak. The intensity of the peaks at 3,400 and 1,640 cm^{-1} declined gradually as the concentration of Cu^{2+} absorbed by EPS increased. Similar results were found in both types of EPS at the bands of 1,410 and 1,080 cm^{-1}. These changes in wave numbers provided evidence for the involvement of different functional groups in EPS in the reaction [14]. Interestingly, as the concentration of applied Cu^{2+} increased, the band at 1,240 cm^{-1} in TB-EPS-Cu complex declined and then disappeared at an initial Cu^{2+} concentration of 1,000 μM. These differences indicated that at high Cu^{2+} concentrations, the groups with weaker affinity may contribute more to copper removal from the aqueous phase, and these potential mechanisms need to be further investigated.

3.3. Implications of this work

Biosorption is one of the available efficient processes for heavy metal removal, and inexpensive material, fast metal recovery, and regeneration of biosorbents are some the major features of this process

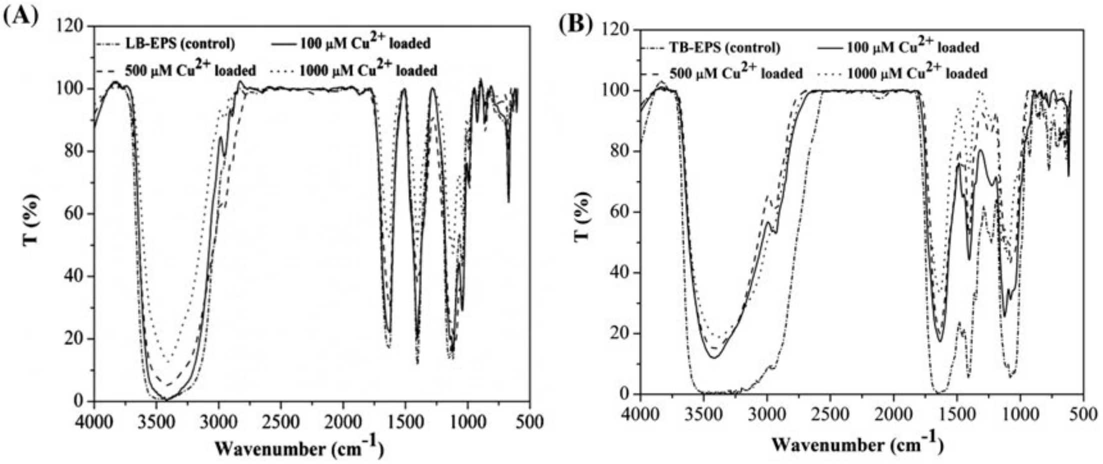

Fig. 8. FTIR spectra of the LB-EPS (A) and TB-EPS and (B) before and after biosorption of Cu^{2+} at different initial concentrations.

Table 2
Main functional groups of EPS observed in FT-IR

	Wave number (cm^{-1})							
Functional group	Pristine LB-EPS	100 μM Cu^{2+}	500 μM Cu^{2+}	1,000 μM Cu^{2+}	Pristine TB-EPS	100 μM Cu^{2+}	500 μM Cu^{2+}	1,000 μM Cu^{2+}
–OH and –NH$_2$ stretching	3,420.36	3,420.58	3,416.82	3,408.89	3,436.56	3,420.24	3,416.65	3,404.77
C–H asymmetric stretching	–	2,956.82	2,962.73	2,956.24	2,939.75	2,927.26	2,933.49	–
C=O and C–N (amide I) stretching	1,633.65	1,626.34	1,626.28	1,626.45	1,653.28	1,636.55	1,633.32	1,635.64
C–H bending	1,403.39	1,404.18	1,404.19	1,404.78	1,409.13	1,404.09	1,405.36	1,406.98
C–O deformation					1,239.85	1,236.28	1,225.94	–
C–O–C stretching	1,149.76	–	–	–				
	1,117.28	1,118.62	1,096.34	1,118.24	1,103.24	1,114.55	1,125.08	1,145.17
C–OH stretching	1,044.85	1,043.28	1,044.85	1,043.42	1,080.87	1,078.29	1,076.84	1,095.39
Fingerprint zone	<1,000	<1,000	<1,000	<1,000	<1,000	<1,000	<1,000	<1,000

[6,32]. EPS are believed to play an important role in metal biosorption by sludge because of their high binding capability [14]. The binding of heavy metals to sludge EPS would affect the bioavailability and mobility of metals, and the high binding strength benefits the further disposal of WAS. Previous studies have demonstrated that EPS extracted from WAS can be applied as a novel biosorbent for the removal of heavy metals from wastewater systems [8,12]. The results from this study facilitate a better understanding about the roles of EPS fractions in the biosorption of heavy metals in wastewater systems. These differences suggest that the influential factors for copper biosorption by LB-EPS and TB-EPS in sludge systems may include the composition of EPS, the functional groups with different binding affinities in EPS, and the physical morphology of EPS. However, further studies should be conducted to investigate exactly how EPS properties and solution chemistry affect the biosorption dynamics and desorption of heavy metals by sludge EPS.

4. Conclusions

In this study, we have determined the contribution of EPS from activated sludge to copper biosorption via (1) a comparison of copper sorption by sludge with TB-EPS and sludge with minimal EPS and (2) direct testing of the interaction between LB-EPS and TB-EPS with Cu^{2+}. Through a comparison of copper sorption using sludge with TB-EPS and sludge with minimal EPS, we showed that TB-EPS from WAS contribute significantly to copper sorption, especially at low initial Cu^{2+} concentrations. EPS probably becomes pseudo-saturated in terms of binding sites for copper sorption at high Cu^{2+} concentrations, so cells may play a more dominant role. By directly testing the biosorption of copper by EPS fractions isolated from WAS, we found that TB-EPS made greater contributions than LB-EPS for copper ion removal in any pH solution applied in this study. SEM observation revealed that TB-EPS had a rough surface with long chains, making it more available for copper binding. FTIR confirmed that the groups with weaker affinity may contribute more to copper removal from the aqueous phase at high Cu^{2+} concentrations. These differences suggest that the chemical composition, the physical morphology of EPS and the functional groups in EPS could be three important, influential factors for copper biosorption by LB-EPS and TB-EPS in sludge systems.

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