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Biosorption properties of extracellular polymeric substances towards Zn(II) and Cu(II)

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ABSTRACT

The aim of this paper was to assess the biosorption properties of extracellular polymeric substances (G-EPS, P-EPS and W-EPS) extracted from three different activated sludges called AS-G, AS-P and AS-W. The compositions of the EPSs were determined. Sludge grown in lab had more EPS than those from a Sewage Treatment Plant, and that sludge fed on glucose had more EPS than if fed on peptone. The biosorption capacities of the EPSs with two metals Cu and Zn were examined successively. The maximal biosorption capacity of EPS is increased in the following order: G-EPS > W-EPS > P-EPS. All EPSs showed stronger binding properties for Zn than Cu, and this adsorption process was described well by Langmuir and Freundlich models, respectively. The excellent fit between pseudo second-order equations and adsorption process indicates that the chemisorption may be the rate limiting step. FTIR analyses revealed that the main chemical groups involved in the interactions between metals were apparently alcohol, carboxyl and amino. These groups were part of the EPS structural polymers, polysaccharides, proteins and hydrocarbon-like products.

Keywords: Activated sludge; Extracellular polymeric substances; Zn; Cu; Equilibrium isotherm; Kinetics; FTIR

1. Introduction

A variety of environmental problems now affect our entire world. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Heavy metals such as copper, cadmium, lead, chromium, zinc, cobalt etc., in wastewaters are hazardous to the environment [1–4]. Due to their toxicity, their presence in aquatic ecosystems poses risks for human health and also causes harmful effects to living organisms in water. Their toxicity also means that any consumers of them are at risk [1]. Thus, the development of clean-up technologies for the treatment of large volumes of water contaminated with heavy metals is of major interest.

Several engineering processes such as chemical precipitation, chemical oxidation or reduction, evaporation, adsorption, ion exchange and membrane technologies have been used to treat the metal contaminants. From a techno-economic perspective, biosorption is one of the economic, practical, and efficient technique technologies for the removal and recovery of heavy metal ions from industrial waste streams.

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Activated sludge produces high amounts of extracellular polymeric substances (EPS). Many studies [5–8] have shown that EPS play a crucial role in biosorption of heavy metals. EPS are metabolic products of bacteria but they also result from organic matter of the effluent and from microbial lysis or hydrolysis [9,10]. EPS are mainly composed of polysaccharides, proteins, humic substances, nucleic acids, and lipids [9], containing ionizable functional groups such as carboxylic, phosphoric, amino, and hydroxylic [11]. The EPS matrix is involved in the mechanisms of surface adhesion, bacterial aggregation in flocs and biofilms, stabilization of the biofilm structure, formation of a protective barrier against biocides, water retention, sorption of exogenous organic compounds [10].

In recent years, there have been a lot of research achievements on EPS, especially on the adsorption of heavy metals [12–14], and consequently, a large amount of information about the structural [15] and functional properties [16] of EPS and their environmental behavior [17] has been obtained. However, little information can be obtained concerning the binding characteristics of metal ions onto EPS of activated sludges cultivated with different organic substrates.

Therefore, the main objectives of our research are (1) to investigate the main compositions of EPSs from three different activated sludges (2) and to identify the adsorption mechanisms, using Fourier transform infrared spectroscopy (FTIR) spectra analysis, biosorption kinetics and equilibrium studies.

2. Biosorption kinetics and equilibrium

2.1. Kinetic modeling

Pre-equilibrium kinetic profiles were characterized in order to determine the rate limiting steps involved in the process of biosorption of zinc and copper ions onto EPS. Lagergren pseudo first-order [Eq. (1)] and pseudo second-order [Eq. (2)] kinetic models were applied [1,18]:

$$\ln(q_e - q_t) = \ln q_e - K_{1,\mathrm{ad}}t \tag{1}$$

$$\frac{t}{q_t} = \frac{1}{K_{2,\mathrm{ad}}q_e^2} + \frac{t}{q_e} \tag{2}$$

where q_e and q_t are the amounts of zinc or copper adsorbed (mg g^{-1}) at equilibrium and at time t, respectively; $K_{1,\mathrm{ad}}$ (min^{-1}) is the Lagergren rate constant of adsorption and $K_{2,\mathrm{ad}}$ (g mg^{-1} min^{-1}) is the equilibrium rate constant of pseudo second-order adsorption.

2.2. Langmuir and Freundlich isotherms

The Langmuir adsorption isotherm assumes that the adsorbed layer is one molecule in thickness and all sites are equal, resulting in equal energies and enthalpies of adsorption, whereas the Freundlich isotherm is derived by assuming a heterogeneous surface with a non-uniform distribution of heat of adsorption over the surface. The Langmuir model [Eq. (3)] and the Freundlich model [Eq. (4)] are express by the following equation [19]:

$$q_e = \frac{q_m K_L C_e}{1 + C_e K_L} \tag{3}$$

$$q_e = K_F C_e^n \tag{4}$$

The constants q_m (mg g^{-1}) is the amount of metal at complete monolayer coverage, and K_L (l mg^{-1}) is a coefficient related to the heat of biosorption. q_e (mg g^{-1}) is the amount of a metal adsorbed; C_e (mg l^{-1}) is the concentration of heavy metal ions in the equilibrium solution. K_F (mg^{1-n} g^{-1} l^n) and n are the indicators of adsorption density and adsorption intensity, respectively.

3. Materials and methods

3.1. Activated sludge reactors

Two 10 l buckets were used as activated sludge reactors, and were stirred with the same type of electric mixers. Each reactor contained a sludge suspension of 5.0 l. The activated sludge reactors and their operation have been previously described [20,21]. In brief, the reactors were seeded with sludge collected from a full-scale municipal wastewater plant, the Fuzhou Municipal Sewage Treatment Plant. The sludge in the reactors was well suspended by the electric mixers at 60 rpm and continuous aeration from the bottom through stone air diffusers. The reactors were fed twice a day with synthetic wastewater that contained a carbon source, dipotassium phosphate, monopotassium phosphate and other inorganic salts for microbial growth, in accordance with the basic recipe that is given in the Environmental Engineering Process Laboratory Manual of AEESP (2001). In one of the activated sludge reactors, glucose was used as the carbon source (AS-G), and for the other reactor peptone was the carbon source (AS-P). The chemical oxygen demand COD: N: P ratio was kept at 100:5:1 for both the glucose-fed and the peptone-fed sludge. The reactors were operated at room temperature (water temperature: 20 ± 5°C), the pH of the sludge suspensions was in the range of 7.0 ± 0.5, and the sludge retention time (SRT) was 15 d. The sludge concentrations in two reactors were maintained at a similar level of around 2000 mg l^{-1} in mixed liquor suspended solids (MLSS) by adjusting the strength of the feeding substrates. The two reactors reached a steady state after 45 d, at which point, the fluctuations in the major daily monitoring parameters, such as MLSS, sludge volume index (SVI) and the

COD in the effluent, were less than 20% for a week or so. Sludge samples were then collected from the reactors before daily feeding for bioflocculation characterization and EPS analysis. In addition, the actual activated sludge from the Fuzhou Municipal Sewage Treatment Plant (AS-W) was sampled and analyzed for comparison. Treatment works was sampled and analyzed for comparison. The treatment works uses a conventional AS system for treating domestic wastewater with an SRT of approximately 15 d.

3.2. EPS extraction and analysis

A heat extraction method was used to extract the EPS from the activated sludge. A sludge suspension was first washed with deionized water for three times in a row. The sludge suspension was heated to 80°C in a water bath for 30 min, followed by centrifugation at 12,000 rpm for 30 min at room temperature.

Finally, all extracted EPS were purified according to Liu and Fang [22]: 100 ml of EPS solution contained in a dialysis membrane (MWCO, 14,000D) were placed in 1000 ml of deionized water for 24 h at 4°C. The EPS were stored at −18°C before use.

Different parameters were used to characterize the EPS extractions: polysaccharides (PSs), proteins (PNs), elemental of carbon, elemental of nitrogen and total EPS. The polysaccharide content was measured with the anthrone method using glucose as a standard [23]. The proteins substances were analyzed by a UV/VIS spectrophotometer (721, Shanghai spectral instrument Co., China) following the modified Lowry method [24] using bovine serum albumin as the standards. CHNSO elemental analyzer (Flash EA1112) was used for the elementals of carbon and nitrogen analyses.

3.3. Preparation of zinc and copper solution for biosorption

Stock solutions of Zn(II) and Cu(II) were prepared by dissolving $Zn(NO_3)_2 \cdot 6H_2O$ and $Cu(NO_3)_2 \cdot 3H_2O$ in deionized water separately. All working solutions were prepared by diluting each stock solution with appropriate volume of deionized water to the desired concentration. The initial pH of each solution was adjusted to a desired value in the range of 3–8 with HNO_3 and $NH_3 \cdot H_2O$ solutions.

3.4. Biosorption kinetic studies

Biosorption kinetic experiments were performed in 1000 ml glass beakers filled with solutions containing zinc or copper alone in equimolar binary systems at the concentration of 20 mg l^{-1}. The concentration of EPS (dry weight) in the biosorption media was 200 mg l^{-1}. Mixing was ensured by a magnetic stirrer with gentle agitation at a constant temperature of 25°C. The pH was adjusted to 6 ± 0.2. Samples (5 ml) were taken at given intervals and dialyzed in a dialysis membrane to remove the residual zinc and copper in 50 ml of deionized water for 24 h at 4°C. The dialysate was used to analysis the residual zinc and copper concentration.

3.5. Biosorption equilibrium studies

The experiments were conducted in duplicate using 250 ml flasks containing 20 ml of zinc or copper solution and amount of EPS (200 mg l^{-1}). The zinc or copper ion concentration was varied from 10 to 140 mg l^{-1}. The pH was adjusted to 6.0 ± 0.2 and the temperature was set at 25°C. Flasks were agitated on a shaker at 100 rpm for 2.0 h to ensure equilibrium was reached. Then samples were dialyzed in a dialysis membrane to remove the residual zinc and copper in 50 ml of deionized water for 24 h at 4°C. The dialysate was analyzed for the remaining zinc and copper concentration. Residual, unsorbed zinc or copper in the dialysate was determined by an atomic absorption spectrophotometer (TAS-990; Beijing geological instrument plant, China). All experiments were performed in triplicate. The pH of suspension was monitored with a pH meter (PB-10 pH meter, BSISL, China).

3.6. FTIR spectroscopy

FTIR spectroscopy of the G-EPS, P-EPS and W-EPS before and after metal adsorption tests were recorded using FTIR (Aratar 380, Thermo-Nico-Let, USA) connected with a personal computer. Before the analysis, the wet samples were freeze-dried. The freeze-dried sample was mixed with KBr in the ratio of 1:100 and pellets of 13 mm diameter were prepared at 25.35×10^3 kg cm^2 pressure.

4. Results and discussion

4.1. The quantity and components of EPSs

Table 1 presents PSs and PNs are the major constituents of EPS, which is in accordance with literatures [25,26]. For both the G-EPS and P-EPS, PSs were the predominant component at quantities of 41–48%. PNs accounted for a small proportion of the EPS (2–9%). The results of the EPS analysis for the sludge grown in the laboratory were comparable to those for the actual AS collected from the full-scale treatment plant. The sludge that was fed on glucose contained more EPS than the sludge that was fed on peptone, which suggests that EPS production depends considerably on the carbon source. Similar observations have been reported by others [26,27]. The underlying cause for this phenomenon is the different metabolic pathways of glucose and

Table 1
The main compositions of G-EPS, P-EPS and W-EPS from AS-G, AS-P, and AS-W

Type	PSs (mg g^{-1} EPS)	PNs (mg g^{-1} EPS)	C (mg g^{-1} EPS)	N (mg g^{-1} EPS)	Total EPS[a] (mg g^{-1} MLSS)	PSs/PNs	C/N
G-EPS	480	21	392	46	13.4	22.9	8.5
P-EPS	411	85	428	78	12.8	4.8	5.5
W-EPS	421	63	402	66	12.2	6.7	6.1

[a] Expressed as the lyophilized of EPS solution; PSs: polysaccharides; PNs: proteins.

peptone in microbes. The citric acid cycle plays a major role in metabolism of organic compounds and biosynthesis of microbial materials. It is a series of chemical reactions which is used by all aerobic living organisms to generate energy through the oxidization of acetate derived from carbohydrates, fats and proteins into carbon dioxide and water. In addition, the cycle provides precursors for the biosynthesis of compounds including certain amino acids as well as the reducing agent NADH that is used in numerous biochemical reactions. Glucose can enter the citric cycle directly, but for peptone, first of all, it has to be degraded to amino acids under the function of exopeptidase and endopeptidase and then oxidised to form acetyl-CoA before it can enter the cycle. In comparison to glucose degradation, peptone metabolism is more complex and likely involves more enzymes including extracellular enzymes. Thus, a higher EPS abundance is expected in the biomass fed with glucose than that grown on peptone. Different PSs/PNs and C/N values of EPSs were obtained. The underlying cause for this phenomenon is also the different metabolic pathways of glucose and peptone.

The composition of EPS presented in Table 1 have a few differences with some of these reported by related literatures [28,16]. There are many possible explanations, such as the varieties of EPS extraction methods, the origin of activated sludge or cultural conditions, etc.

4.2. Biosorption rates and equilibrium adsorption of zinc and copper

Fig. 1 shows the extent of solely zinc and solely copper biosorption onto G-EPS, P-EPS and W-EPS as a function of reaction time at the initial metal concentration of 20 mg l^{-1}. The data demonstrate that the biosoption of Zn(II) and Cu(II) ions by EPSs is fast in the first 10 min and equilibrium is established in 20–30 min for all biosorption. It can be seen that metals biosorption consisted of two phases: a very rapid initial sorption, followed by a long period of much slower uptake. The two-stage kinetics of biosorption was also reported for immobilized biomass as well as some conventional sorbents [29,30]. During the initial stage of sorption, a large number of vacant surface sites are available for biosorption. After a lapse of some time, the remaining vacant surface sites are difficult to be occupied due to repulsive forces between the solute molecules adsorbed on the solid surface and the bulk phase. Besides, the metal ions are adsorbed into the meso-pores that get almost saturated with metal ions during the initial stage of biosorption. Thereafter, the metal ions have to traverse farther and deeper into the pores encountering much larger resistance. This results in the slowing down of the biosorption during the later period of biosorption. The time required to reach the equilibrium is in accordance with the result of Sun et al [31], who investigated zinc and cobalt biosorption by aerobic granules. In another study, the copper

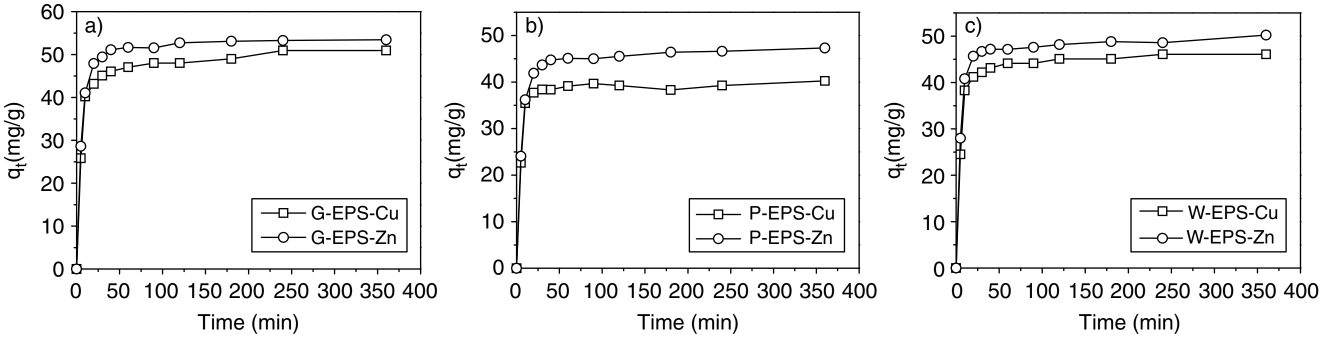

Fig. 1. Respective effect of the contact time on biosorption of zinc (20 mg l^{-1}) and copper (20 mg $^{-1}$) onto G-EPS (a), zinc (20 mg l^{-1}) and copper (20 mg l^{-1}) onto P-EPS (b), zinc (20 mg l^{-1}) and copper (20 mg l^{-1}) onto W-EPS (c).

sorption processes by bentonite was relatively fast in the first few minutes and then became slow after reaching 80% of the total sorption [32]. Rapid (or instantaneous) biosorption phenomenon has significant practical importance as it will facilitate the scale-up of the process to small reactor volumes ensuring efficiency and economy.

4.3. Biosorption kinetics

In order to clarify the biosorption kinetics of Zn(II) and Cu(II) onto EPSs, two kinetic models, Lagergren's first-order and pseudo second-order model were applied to the experimental data. The pseudo first-order model assumes that a metal ion only sorbs on one sorption site of the sorbent surface and is represented by the following scheme[33]:

$$S_{(solid)} + Me^{2+}{}_{(aqueous)} \rightarrow SMe^{2+}{}_{(adsorbed \cdot phase)} \qquad (5)$$

where S is the biosorption site.

On the other hand, the essential assumption of the pseudo second-order model is that one metal ion is adsorbed onto two surface sites, as indicated by the following equation [33]:

$$2S_{(solid)} + Me^{2+}{}_{(aqueous)} \rightarrow S_2Me^{2+}{}_{(adsorbed \cdot phase)} \qquad (6)$$

Table 2 present the results of rate constant studies for Zn(II) and Cu(II) in single as well as in binary combinations by the pseudo first-order and pseudo second-order models. The value of correlation coefficient R^2 for the pseudo second-order adsorption model is obviously high, and the adsorption capacities calculated by the model are close to those determined by experiments. However, the values of R^2 for the pseudo first-order model are not satisfactory. Therefore, it has been concluded that the pseudo second-order adsorption model is more suitable to describe the adsorption kinetics of Zn(II) and Cu(II) over EPSs. This suggests that the rate limiting step in this sorption process may be chemisorption involving valent forces through the sharing or exchange of electrons between sorbent and sorbate, as also reported by Gulnaz et al. [34].

The reaction order is directly related to the reaction mechanisms. Metal biosorption by EPS would be through either a specific ion exchange mechanism on the surface of the biosorbent or surface precipitation of metal hydroxide/sulfide/carbonate species. It is certain that the overall reaction order of biosorption is dependant upon the characteristics of the heavy metals as well as the nature of biosorbent studied. The second-order kinetic equation has been reported to effectively describe the sorption of heavy metals by biomass [35].

4.4. Equilibrium isotherms

To understand the adsorption process, it is necessary to correlate the equilibrium adsorption data to different isotherm models such as Langmuir and Freundlich equations. The model parameters are given in Table 3.

The better agreement by Langmuir equation for Zn(II) suggests that Zn(II) sorbed from a monolayer coverage on the adsorbent surface. The equilibrium data of Cu(II) well described by the real heterogeneous nature of the surface sites involved in the metal uptake.

Between the two metal ions, the highest q_m of G-EPS is observed for zinc and copper, whereas P-EPS shows the lowest q_m value towards the two metal ions. G-EPS exhibited the greatest capacity to bind metal ions among G-EPS, W-EPS and P-EPS in all investigated concentrations.

According to the results of Acar and Malkoc [36], the adsorption of an adsorbate is favorable when $0.1 < n < 1.0$. Our findings indicate that the EPSs can be used effectively for the removal of Zn(II) and Cu(II) ions from aqueous solutions. Moreover, the K_F values obtained from the Freundlich model suggest that the metal binding affinity was in the order Zn(II) > Cu(II).

Table 2
First-order kinetic and second-order kinetic constants of zinc and copper absorbed by G-EPS, P-EPS and W-EPS

	First-order kinetic		Second-order kinetic		
	$K_{1,ad}$	R^2	q_e	$K_{2,ad}$	R^2
G-EPS-Cu	0.0114	0.7256	51.5464	0.0038	0.9996
G-EPS-Zn	0.0181	0.8778	54.0541	0.0060	0.9998
P-EPS-Cu	0.0065	0.3216	40.1606	0.0111	0.9994
P-EPS-Zn	0.0116	0.7333	47.6190	0.0056	0.9999
W-EPS-Cu	0.0146	0.7328	46.5116	0.0064	0.9999
W-EPS-Zn	0.0080	0.5781	49.0196	0.0091	0.9997

Table 3
Simulated parameters of Langmuir and Freundlich adsorption isotherms

	Freundlich			Langmuir		
	n	K_F	R^2	K_L	q_m	R^2
G-EPS-Cu	0.1923	31.0375	0.9923	0.4	69.4444	0.8806
G-EPS-Zn	0.1909	33.7371	0.9358	0.3394	76.9231	0.9666
P-EPS-Cu	0.2352	23.3500	0.9863	0.2516	64.1026	0.8503
P-EPS-Zn	0.2408	24.7865	0.9376	0.1861	72.9927	0.9730
W-EPS-Cu	0.2203	26.2246	0.9906	0.2792	67.5676	0.8921
W-EPS-Zn	0.2302	26.9720	0.9301	0.2059	75.1880	0.9786

4.5. Fourier transform infrared analysis

FTIR analysis was conducted with raw and metal-loaded EPS in order to identify groups involved in the biosorption. The results are given in Fig. 2. For the pristine G-EPS, P-EPS and W-EPS (samples ai, bi and ci, respectively), the broad adsorption peak around 3432, 3388 and 3437 cm^{-1} is indicative of the existence of the O-H groups of glucose and the N–H groups of proteins. According to Liu and Fang [11], EPS are composed of a variety of organic substances: carbohydrates and proteins being major constituents with humic substances, uronic acids and nucleic acids in smaller quantities. Due to this complex nature of the EPS composition, it is not possible to determine the exact group responsible for this stretching vibration. The band at approximately the 1653 (sample ai) and 1649 cm^{-1} (sample bi and ci) region corresponds to the amine I, which are characteristic of functional groups C=O stretching vibrations of proteins. The peak at 1406 (sample ai), 1442 (sample bi) and 1400 cm^{-1} (sample ci) corresponding to bending of CH_3 and CH_2 groups from proteins may also have a contribution from an amine III band. The peak at ≈1400 cm^{-1} arises from to the stretching C–O of carboxylic groups which overlap with the amide III band making it difficult to distinguish. The spectra bands between 1150 and 1030 cm^{-1} correspond to polysaccharide groups for EPSs. Some bands at “fingerprint zone” (<1000 cm^{-1}) might be assigned to phosphate group, which is one of the functional groups of which nucleic acids are composed. Furthermore, the other bands around 3000–2900 cm^{-1} are the characteristics of alkyl chains (2935 cm^{-1} for G-EPS and W-EPS, 2929 cm^{-1} for P-EPS), and the amide I band is a combination of C=O stretching, being centred near 1653 cm^{-1} for G-EPS, 1649 cm^{-1} for P-EPS and W-EPS. The spectral data thus confirm the presence of amine, hydroxyl and carboxyl groups in the EPS.

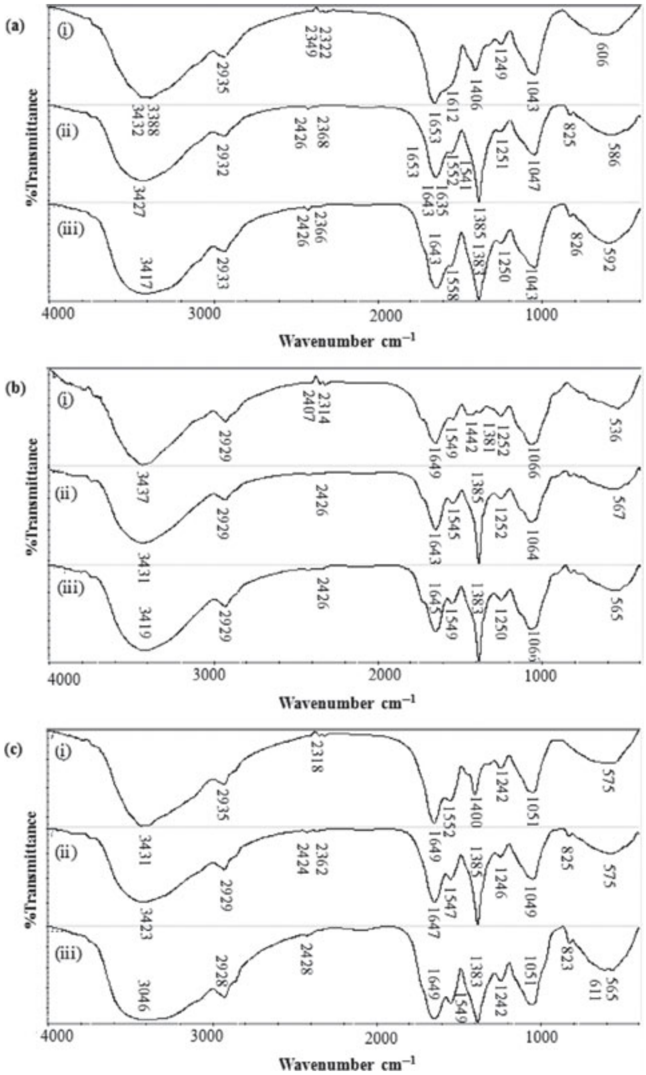

Fig. 2. FTIR spectra of (a) G-EPS, (b) P-EPS and (c) W-EPS. (ai, bi and ci) Fresh EPS; (aii, bii and cii) Zn(II)-loaded; (aiii, biii and ciii) Cu(II)-loaded.

When the G-EPS were loaded with the metal ions, the spectrum exhibits some changes (Fig. 2a), A strong C=O stretching vibration conjugated to a N–H deformation initially present at 1653cm^{-1} was split into 1636 and 1643 cm^{-1} for Zn(II)-loaded G-EPS, and was shifted to 1643 cm^{-1} for Cu(II)-loaded G-EPS, which indicated the complexation of metal ions with the functional groups from protein. The broad overlapping region for N–H and O–H stretching in the range of 3200–3600 cm^{-1} also presents some changes, but it is difficult to distinguish which group causes the shift. The peaks at 1043 cm^{-1} could be attributed to the stretching vibration of C–O that occurs in alcoholic groups or the stretching vibration of C–O–C that occurs in polysaccharides was shifted to 1041 cm^{-1} for Zn(II)-loaded G-EPS, so it led to the conclusion that the formation of complex binding between the Zn and the C–O–C group of polysaccharides. A medium strength absorption peak at 1406 cm^{-1}can be assigned to the symmetrical stretching of the carboxylate anion. A sharp increase in the band intensity and slight shifting of peaks from 1406 to 1385 cm^{-1}(for Zn(II)-loaded G-EPS) and 1383 cm^{-1}(Cu(II)-loaded G-EPS) reveals the chelating character of metal ions complexation to the carboxyl group in the G-EPS.

The spectra of G-EPS, P-EPS and W-EPS are similar with functional groups observed in the results of Guibaud et al. [7]. Several intense characteristic bands can be attributed to protein and polysaccharide functional groups. Similarly to the P-EPS and W-EPS, significant carboxyl and amino group participation was seen for Zn(II) and Cu(II) binding to P-EPS and W-EPS. However, in contrast to G-EPS, it is observed that the peak at 1649 cm^{-1} of P-EPS and W-EPS representing the stretching vibration of the functional groups from protein is much sharper than that at 1653 cm^{-1} of G-EPS, indicating that less coordinated complexes of metal ions and protein were formed in P-EPS and W-EPS.

5. Summary

This study examined biosorption of zinc (II) and copper (II) in single systems by EPSs. The sorption processes were well described by the Langmuir and Freundlich isotherm equations, respectively. The maximal adsorption capacity of G-EPS, P-EPS and W-EPS has been found to be 54.0541 mg g^{-1} Zn and 51.5464 mg g^{-1} Cu, 47.6190 mg g^{-1} Zn and 40.1606 mg g^{-1} Cu, 49.0196 mg g^{-1} Zn and 46.5116 mg g^{-1} Cu, respectively. The Lagergren plot of the kinetic isotherm suggests the applicability of the second-order rate representation, which outlines that the rate limiting step in this sorption process may be chemisorption involving valent forces through the sharing or exchange of electrons between sorbent and sorbate. The analyses by FTIR showed that functional groups on EPSs, such as alcoholic and carboxylate, would be the main binding sites for biosorption of the studied heavy metals by EPSs.

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