Adsorption of Cd(II), Zn(II) by extracellular polymeric substances extracted from waste activated sludge

Zheng Lei, Tian Yu, Ding Ai-zhong and Wang Jin-sheng

ABSTRACT

Adsorption of Cd(II) and Zn(II) ions in single solutions using extracellular polymeric substances (EPS) from activated sludge was investigated. Langmuir and Freundlich models were applied to describe metal adsorption. The results showed that EPS was an effective adsorbent for the zinc and cadmium ions from aqueous solution. The equilibrium metal uptake was increased with increasing the initial concentration of metal ion. Constants calculated from isotherms model showed that the maximum uptake capacity of cadmium was estimated to be 45 mg/g of Cd(II) and 80 mg/g of Zn(II). Both Langmuir and Freundlich isotherms were suitable for describing adsorption of Cd(II) by EPS, while the Langmuir isotherm equation fit the date of Zn(II) adsorption better, indicating that EPS adsorb Cd(II) and Zn(II) by different mechanisms.

Analysis of FTIR spectra demonstrated that C-O-C of polysaccharides at 1,150–1,030 cm⁻¹, group of the amide(I), CH₂ group of the lipids, carboxyl and -OH groups of proteins and polysaccharides were involved in cadmium and zinc binding, of which the -OH groups and the C-O-C group of polysaccharides.

Key words | adsorption, cadmium, extracelluar polymeric substances, waste activated sludge, zinc

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INTRODUCTION

Bacterial extracellular polymeric substances (EPS) have shown great promise to act as heavy metal adsorbents (Geesey & Jang 1989; Fukushi *et al.* 2007; Guibaud *et al.* 2003). EPS are mixtures of macromolecules. They comprise a mixture of polysaccharides, mucopolysaccharides and proteins (Urbain *et al.* 1992). EPS contain ionisable functional groups such as carboxyl, phosphoric, amine and hydroxyl groups, which enable EPS to sequester heavy metals (Sutherland 1984). Ion exchange (Chang *et al.* 1995), complexation with functional groups of negatively charged (Brown & Lester 1982), adsorption and precipitation (Oliver & Cosgrove 1974) are the mechanisms involved in metal biosorption onto EPSs.

Previous studies have demonstrated that EPS from waste activated sludge seemed to be an attractive heavy metal adsorbent due to of their low toxicity and cost-effect doi: 10.2166/wst.2008.646 (Rudd *et al.* 1984; Chen *et al.* 1995; Tian *et al.* 2006). Liu *et al.* (2001) reported that the adsorption capacities of EPS for Cd^{2+} , Co^{2+} , Cr^{3+} and Ni^{2+} are higher that those of resin by 83.8, 92.5, 96.3 and 64.8%, respectively. In addition, extraction of EPS from waste activated sludge had the potential to reduce waste sludge volume and mass considering the high water binding properties of EPS (Wingender *et al.* 1999) and the finding that the mass of total EPS represents up to 80% of the mass of activated sludge (Frølund *et al.* 1996). However, there is still very limited information on the sorption of heavy metals by EPS extracted from waste activated sludge. The objective of this study was to investigate cadmium and zinc sorption capacity by EPS produced by waste activated sludge and the metal-binding mechanism.

MATERIALS AND METHODS

EPS preparation

Sludge samples used for EPS extraction were grown in batching bioreactors. The microbial seed was obtained from the primary settling tank effluent from the city of Harbin wastewater treatment plant. The reactors were fed synthetically with the medium, the composition of which is given in Table 1.

The reactors had a working volume of 10 L and were operated at a cycle of 10 h aeration followed by 2 h settling. The temperature of the system was kept at $20-25^{\circ}$ C, pH was 7.0 ± 0.2, and the DO concentration was kept at a level of 2-3 mg/L.

Extraction of EPS was carried out using steaming followed by centrifugation, as suggested by Brown & Lester (1980). Prior to extraction, the sludge was concentrated using a MR 23i (Jouan) type centrifuge at 4,300 g for 10 min. The residues recovered were rinsed twice in double-distilled water. The rinsed activated sludge was steamed at 80°C under 100 Mpa pressure for 10 min and then centrifuged while still hot at 8,000 g for 10 min. The supernatant was filtered through a sterile 0.2 μ m filter into an autoclaved flask and the filtered solutions were concentrated under vacuum at 40°C using a rotary evaporator and were then stored at -20°C before use.

Sorption test

Fifteen ml of concentrated EPSs-rich solution (containing about 20 mg EPS) was transferred to the dialysis sack with a molecular weight cutoff of 3,000 Da. The dialysis sack was sealed and placed into a 150 mL conic flask containing 85 mL of Cd(II)/Zn(II) bearing solution with metal concentration ranged from 1.5 to 35 mg/L. After 24 h sorption at 25° C, 5 mL of solution was sampled and metal concentration was determined. Cd(II)and Zn(II) were applied in the form of Cd(NO₃)₂ and Zn(NO₃)₂ diluted in deionised water. All the glassware used in this study was dipped in 0.1 M HNO₃ for 48 h, rinsed with deionised water thoroughly and dried for use.

Analysis methods

Protein and sugars were examined as the major constituents of EPS using the following colourimetric methods: Lowry

Constituent	Concentration (mg/L)		
Glucose	935		
Peptone	200		
K ₂ HPO ₄	600		
KH ₂ PO ₄	300		
NH ₄ Cl	225		
$MgSO_4 \cdot 7H_2O$	112.5		
FeSO ₄ ·7H ₂ O	3.75		
$ZnSO_4$ ·7 H_2O	3.75		
$MnSO_4 \cdot 7H_2O$	3.75		
CaCl ₂	15		
NaHCO ₃	180		

Table 1 | Synthetic feed medium composition

et al. (1951) for protein content, bovine albumin serum as standard; Dubois *et al.* (1956) for sugar content, glucose as standard. All the contents of heavy metal were analysed using a furnace atomic absorption spectrometer (type AA anlyst 200, PE Co., USA).

The functional groups of EPS were analysed with a Fourier-transform infrared (FTIR) spectrometer. The samples of EPS solution before and after heavy metal adsorption were dried at -20° C and were then prepared by pressing powered KBr pellets thoroughly mixed with 5% of finely ground powder of the each sample and determined on a Nicolet 740SX FTIR spectrophotometer (USA). The instrument was performed with a MCT-B detector and the spectra were recorded in the region of 4,000–625 cm⁻¹ at a resolution of 4 cm⁻¹ with 32 scans.

RESULTS AND DISCUSSION

EPS characteristics

The initial metal content in the EPS was used for characterisation of EPS while protein and sugars were examined as major constituents of EPS. The characteristics of the EPS used are shown in Table 2.

Approximately 40 mg total EPS was extracted from one gram of VSS, of which 25 mg was protein and 15 mg was sugar. The C/P (ratio of carbohydrates to protein) was 0.6. A review of the literature showed that C/P of active sludge ranges from 0.07 to 14.7 (Jang *et al.* 2001; Liu *et al.* 2001).

 Table 2
 Characteristics of EPS extracted from activated sludge

Parameter	Range		
Initial concentration in metal(mg/gvss)			
Cd	Undetected		
Pb	Undetected		
Zn	0.03 ± 0.002 mg/L		
Biochemical composition (mg/gvss)			
Protein	25.0 ± 5.0		
Sugars (mg/L)	15.0 ± 3.0		

The C/P could be influenced by many factors, including the extraction methods of EPS (Liu *et al.* 2001), heavy metals in solution (Jang *et al.* 2001) and types of active sludge. The background of metals in the EPS are low (as shown in Table 2), which is reasonable for the adsorption experiment.

Cd(II) and Zn(II) adsorption by EPS

Figure 1 illustrates that dissolved EPS was effective in removing cadmium and zinc from solution at an initial concentration ranging from 1.5 to 35 mg/L. The adsorption capacity of EPS increased first with increasing of the initial concentration of metal ions and reached a saturated value. When the initial cadmium (II) concentration was increased from 1.5 to 35 mg/L approximately, the loading capacity increased from 8.5 mg/gl to 42 mg/g. When the initial zinc (II) concentration was increased, approximately from 1.5 to 35 mg/L, the loading capacity increased from 9.3 mg/g to 69 mg/gl.



Figure 1 | Adsorption isotherm of Cd(II)and Zn(II).

The difference in removal of Cd and Zn is believed to be due to their difference in binding strength to EPS. Guibaud *et al.*'s (2003) research showed that the binding site number of EPS solutions toward Cd was higher. However, the stability of the EPS-Cd was lower, which was believed to be related to the electron density of the heavy metal.

Cd(II) and Zn(II) adsorption isotherm

The well known Langmuir and the Freundlich adsorption isotherms were used to evaluate the adsorption data. Langmuir and Freundlich isotherm equations were used to describe the equilibrium state for single-ion adsorption experiments. The theoretical basis of the Langmuir equation relies on the assumption that there is a finite number of binding sites which are homogeneously distributed over the adsorbent surface of the absorbent, having the same affinity for adsorption of a single molecular layer, and there is no interaction between adsorbed molecules.

The mathematical description of the Langmuir isotherm equation is as follow:

$$q = q_{\rm m} b C_{\rm e} / (1 + b C_{\rm e})$$

where q is the amount of metal adsorbed, mg/g; Q_m is the maximum metal uptake value corresponding to sites saturation, mg/g; C_e is the equilibrium metal concentration in solution, mg/L; and b is the ratio of adsorption/desorption rates, representing the biomass-metal binding affinity.

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. This empirical equation has the form:

$$q = kC_e^{1/n}$$

where k and n are constants indicating adsorption capacity and adsorption intensity, respectively (Bayramoğlou *et al.* 2005)

The metal initial concentration provides an important driving force for overcoming the mass transfer limitations of metal ion between the EPS and water. Figure 1 shows that the EPS capacity *Q* increases as the initial metal concentration increases. The Langmuir and Freundlich adsorption constants Table 3 | Related coefficients for isotherm equations of Cd(II) and Zn(II) by EPS

	Freundlich isotherm equation		Langmuir isotherm equation			
Metal	k	n	R ²	q _m	b	R ²
Cd	3.5823	1.5649	0.9967	46.17	0.05944	0.9913
Zn	5.7546	1.6637	0.9864	81.97	0.05902	0.998

calculated from linearisation of the corresponding isotherms with the correlation coefficients are presented in Table 3.

The estimation of the correlation coefficients shows that the experimental points of Cd sorption fits better to the Freundlich model, while those of Zn sorption fit the Langmuir model better. This suggests that Cd(II) ions are adsorbed onto the surface in a multilayer pattern and Zn (II) ions are adsorbed in a monolayer pattern. Comparing $q_{\rm m}$ values, with those reported for peat with $q_{\rm m}$ of 22 mg Cd(II)/g and 10 mg Zn(II)/g (Wase et al. 1997), EPS seem to be a better biosorbent. However, their capacity is much less than that of the results reported by Liu et al. (2001) and Zhang et al. (2006). This is believed to be due to the fact that the EPS used in Liu and Zhang's report had a higher molecule weight than the present study and former was extracted at a higher separate force (Brown & Lester 1980; Frølund et al. 1996; Zhang et al. 2006). The lower initial metal concentration in the present study may also contribute to the difference in the adsorbed capacity (Liu et al. 2001).

The constants of the Freundlich isotherm equation and Langmuir isotherm equation are shown in Table 3. The results showed that the data of Cd(II) sorption could be satisfactorily represented by both the Freundlich isotherm Equation ($R^2 = 0.9967$) and the Langmuir isotherm Equation ($R^2 = 0.9913$), which indicated that cadmium sorption by EPS was a physicochemical process and certain reactions were involved between cadmium and functional groups of EPSs, while the Langmuir isotherm equation fits the date of Zn(II) adsorption better than the Freundlich equation, indicating a different adsorption mechanism compared with Cd(II) adsorption.

Mechanisms of Cd(II) and Zn(II) adsorption by EPS

IR spectra $(400-4,000 \text{ cm}^{-1})$ of blank EPS and those exposed to Cd^{2+} and Zn^{2+} are shown in Figure 2. The IR-spectra of the three samples studied were quite similar.

For the native EPS, there were several distinct, characteristic, sharp stretching frequencies of chemical groups contained in the EPS. The different functional groups observed in the EPS samples agree with the results of Guibaud *et al.* (2003). The analysis of the IR-spectra shows the presence of numerous functional groups. Several intense characteristic bands can be attributed to functional groups present in proteins and in polysaccharides. Some less intense bands show carboxylic groups under acid or basic salt form and they suggest, when they are combined with the other bands observed, the presence of uronic acids (notably with the bands characteristic of sugars) and of humic substances.

Some groups of molecules present in lower proportions in the solutions of EPS, such as lipids or nucleic acids, are more difficult to detect with IR-spectra. Some bands observed in the "fingerprint" region could be attributed to the phosphate group, which is one of the functional groups of which nucleic acids are composed. The presence of CH_2 and of carboxylic groups should indicate the presence of lipids. The analysis of the IR-spectra allowed the previously determined composition of the EPS to be confirmed.

The IR spectra of the blank EPS displays a shoulder peak at $1,080 \text{ cm}^{-1}$ corresponding to the coupled vibrations band of the hydroxyl group of saccharides.

A comparison of the spectra for native EPS (Figure 2(a)) with that of Cd-loaded (Figure 2(b)) revealed a characteristic "blue shift" (negative shift) in carbonyl, hydroxyl and ammonia groups, indicating the interaction of Cd with these groups of EPS. The disappearance of peaks at $1,550-1,680 \text{ cm}^{-1}$ indicates C–N and N–H in proteins (peptidic bond) involved in Cd adsorption and the disappearance of peaks at $1,190-1,000 \text{ cm}^{-1}$ for the Cd-loaded EPS indicates C = C stretching and C–O–C asymmetric stretching as a consequence of Cd sorption. The above changes suggest that hydroxyl, carboxyl and carbonyl groups of saccarides and amido, peptidic goups of protein in EPS are the mainly active groups for Cd adsorption.

It must be noticed that one new adsorption band at $1,763 \text{ cm}^{-1}$ corresponding to the C = O stretching band of the carboxyl group (COOH) occurred in Figure 2(b). There are two possibilities that account for the occurrence of the new band of the carboxyl group. One is the hydrolysis of some peptide chains of the peptidoglycan layer, since a



Figure 2 | IR spectra of EPS: (a) EPS; (b) EPS-Cd; (c) EPS-Zn.

break in peptide chains of the peptidoglycan layer to shorter peptides or amino acids should produce the carboxyl group that resulted in the occurrence of the corresponding carbonyl groups stretching band in IR. The other is the likelihood of the free aldehyde group from reducing sugars, i.e. the hydrolysates of the polysaccharides, being oxidised to the carboxyl group (Lin *et al.* 2005*b*).

The spectra of EPS loaded with Zn are similar to that of the Cd loaded sample. After Zn and Cd adsorption, a new group occurred at $2,424 \text{ cm}^{-1}$ and $3,114 \text{ cm}^{-1}$ result from C-H, N-H, O-H and NH³⁺ stretching band of EPS adsorbing heavy metal.

After Cd or Zn adsorption, a new peak occurred at 834 cm^{-1} , indicating Cd or Zn adsorption visualises oxidised C, such as carboxyl, carbonyl groups. At the wave number lower than 834 cm^{-1} , there is little change in the spectra before and after metal adsorption, indicating that S, P containing groups make little function in EPS adsorbing Cd or Zn. A peak at $1,090 \text{ cm}^{-1}$ representing -OH stretching band in primary alcohols and carboxyl groups disappeared after Cd adsorption and remains unchanged after Zn adsorption, indicating -OH in primary alcohols and carboxyl groups works in Cd adsorption but does not function in Zn adsorption.

After Cd or Zn adsorption, carboxyl groups at $1,556 \text{ cm}^{-1}$ and $1,405 \text{ cm}^{-1}$ disappears indicating asymmetric and symmetric stretching of C(= O)₂ in (COO-). This is typical of the complexation of the carboxylate anion functional group by coordination with metal cation (Lin *et al.* 2005*a*). In this case, most of the carboxylate anion must have bound, complexed or chelated with metal ions

because the asymmetrical stretching band at $1,338 \text{ cm}^{-1}$ moved to a higher frequency (Lin *et al.* 2005*a*), which may be overlaid by other higher frequency bands, resulting in deepening the peak valley between 1,275 to $1,511 \text{ cm}^{-1}$.

CONCLUSIONS

EPS was an effective adsorbent for the zinc and cadmium ions from aqueous solution. The Cd(II) and Zn(II) sorption was found to be dependent on the initial concentration of metal ion. The equilibrium metal uptake was increased with increasing the initial concentration of metal ion. Both Langmuir and Freundlich isotherms were suitable for describing adsorption of Cd(II) by EPS, indicating that cadmium sorption by EPSs was a complex physicochemical process, while the Langmuir isotherm equation fits the date of Zn(II) adsorption better. Analysis of FT-IR spectra confirmed that cadmium and zinc sorption was attributed to complexation of cadmium and zinc with functional groups of proteins, polysaccharides, lipids and humic acids of EPS. Complexation of cadmium with -OH and C-O-C of polysaccharides played a major role in cadmium and zinc sorption by EPS.

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