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Cadmium sorption by EPSs produced by anaerobic sludge under sulfate-reducing conditions

Daoyong Zhang [a,b], Jianlong Wang [b], Xiangliang Pan [a,b,*]

[a] *Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, PR China*
[b] *Institute of Nuclear Energy Technology, Tsinghua University, Beijing 100084, PR China*

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Abstract

This study showed that EPS produced by anaerobic sludge under sulfate-reducing bacteria was effective in removing Cd^{2+} from solution. The sorption data could be described by the Freundlich isotherm model. The q_m derived from the Langmuir isotherm model was up to 2720 mg/g EPSs. The presence of copper and nickel ions had significantly reduced the cadmium sorption by EPS and the presence of zinc had little effect. Analysis of FTIR spectra demonstrated that C–O–C of polysaccharides at 1150–1030 cm^{-1}, group of the amide(I), CH_2 group of the lipids, carboxyl and –OH groups of proteins and polysaccharides were involved in cadmium binding, of which the –OH groups and the C–O–C group of polysaccharides played a major role in cadmium sorption by EPSs.

Keywords: Anaerobic sludge; Extracellular polymeric substances; Cadmium; Biosorption

1. Introduction

Most bacteria produce extracellular polymeric substances (EPSs) of biological origin. EPSs comprise a mixture of polysaccharides, mucopolysaccharides and proteins, which depends on the strain and its culture conditions [1]. EPSs plays an important role in aggregation of bacterial cells in flocs [2] and biofilms [3], stabilization of the biofilm structure, retention of water, formation of a protective barrier that buffers harmful effects, but also plays a very important role in biosorption of heavy metals [4–8]. EPSs contain ionizable functional groups such as carboxyl, phosphoric, amine, and hydroxyl groups, which enable EPSs to sequester heavy metals [9]. Quite a few studies demonstrated that EPSs exhibited high affinity for selected metal ions [10–15]. Fukushi et al. [6] have shown that the quantity of metals bound to cells is proportional to the quantity of polymers in each one. Ion exchange [16], complexation with functional groups of negatively charged [17], adsorption [17] and precipitation [18] are the mechanisms involved in metal biosorption onto EPSs. Some kinds of wastewater containing heavy metals (e.g. acid mining drainage) is typical of being under sulfate-reducing conditions. One common treatment method to remove heavy metals from these kinds of wastewater is to form metal sulphide precipitates microbiologically. However, there is still very limited information on the sorption of heavy metals by EPSs produced by sludge under sulfate-reducing condition. The objective of this study was to investigate cadmium sorption capacity by EPSs produced by anaerobic sludge under sulfate-reducing conditions and the cadmium-binding mechanism.

2. Materials and methods

2.1. Preparation of sludge under sulfate-reducing conditions

The activated sludge collected from the anaerobic digester at the BeiXiaoHe sewage treatment plant, Beijing. Five grams of anhydrous sodium sulfate were added into a sterile 1 l Schott bottle containing 1 l of anaerobic sludge. The bottle was incubated at 35 °C for 7 days. Then 50 ml of sludge were transferred to another sterile 1 l Schott bottle containing 950 ml of autoclaved Postgate's B Medium [19]. After 8 weeks' incubation, sludge under sulfate-reducing conditions containing 2 g VSS (volatile suspended solid, dry weight) per liter was obtained.

* Corresponding author. Tel.: +86 1 851 5890459; fax: +86 1 851 5890609.
E-mail address: xiangliangpan@163.com (X. Pan).

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Test showed that this sludge had good sulfate-reducing capacity (data not shown).

2.2. Extraction of EPSs

The dissolved EPSs were separated by centrifugation at 20,000 × *g*, 4 °C for 20 min [20]. This method for EPSs extraction would not cause cell lysis [21]. The supernatant was filtered through a sterile 0.2 μm filter into an autoclaved flask and the filtered solutions were concentrated to 100 ml under vacuum at 40 °C using a rotary evaporator.

2.3. Sorption test

Transfer 2 ml of concentrated EPSs-rich solution (containing about 3 mg EPSs) to dialysis sack with a molecular weight cut-off 3000 Da. The dialysis sack was sealed and placed into a 150 ml conic flask containing 98 ml of Cd^{2+}-bearing solution with Cd^{2+} concentration ranged from 10 to 500 mg/l. After 24 h sorption at 25 °C, 5 ml of solution was sampled and cadmium concentration was determined. Cd(II) was applied in the forms $CdCl_2{\cdot}6H_2O$ diluted in deionized water. All the glassware used in this study was dipped in 0.1 M HNO_3 for 48 h, rinsed with deionized water thoroughly and dried for use.

2.4. Binary metals sorption test

Transfer 2 ml of concentrated EPSs-rich solution (containing about 30 mg EPSs) to dialysis sack. The dialysis sack was sealed and placed into a 150 ml conic flask containing 98 ml of binary metal-bearing solution. The cadmium concentration was 20 mg/l and the other metal concentration was 10 mg/l. After 24 h sorption at 25 °C, 5 ml of solution was sampled and cadmium concentration was determined. Cu(II), Cd(II), Ni(II), Zn(II)were applied in the forms of $CdCl_2{\cdot}6H_2O$, $CuSO_4{\cdot}5H_2O$, $NiCl_2{\cdot}6H_2O$, $ZnSO_4$ diluted in deionized water.

2.5. Metal sorption by sludge before and after extraction of EPSs

A 10 ml of sludge were centrifuged at 20,000 × *g*, 4 °C for 20 min. The supernatant was discarded and sludge was used as biomass after EPSs extraction. Another 10 ml of sludge was transferred into a 10 ml centrifuge tube and centrifuged at 10,000 × *g* for 1 min and the supernatant was discarded. The precipitated sludge was used as biomass without EPS extraction. A 10 ml of cadmium-bearing solution with a concentration of 20 mg/l were added into the tubes and the tubes were shaken at 800 rpm to make biomass resuspended. After 2 h of sorption at 25 °C, the suspension was centrifuged at 10,000 × *g* for 1 min. The cadmium concentration in the supernatant was measured. The experiment was triplicated and the mean value was used.

2.6. Analytical methods

Polysaccharide content was determined by the phenol–sulfuric acid method, with glucose as the standard [22] and protein was quantified according to the Folin method, BSA as the standard [23].

The content of EPSs were determined with a TOC determiner (Model high TOC V2.6, ELEMENTAR, Germany). The EPSs containing solution was lyophilized, mixed with solid KBr in the ratio of 1:100 and pellets of 13 mm diameter of the mixture were prepared at 8×10^{13} kg cm^{-2} pressure [24]. The functional groups of EPSs was analyzed with Fourier-transform infrared (FT-IR) spectrometer (Nexus 470 FT-IR).

Cadmium(II) concentrations in the filtrate were determined by AAS (AAS6, Vaio).

3. Results and discussion

3.1. Cadmium sorption by EPSs

31.78 mg EPSs were extracted from each gram VSS, of which 15.62 mg was carbohydrates and 13.71 mg was protein. The C/P (ratio of carbohydrates to protein) was 1.14. A review of literatures showed that C/P of active sludge ranges from 0.07 to 14.7 [14,15,25]. The C/P could be influenced by many factors including extraction methods of EPSs [15], heavy metals in solution [14] and types of active sludge.

Fig. 1 illustrated that dissolved EPSs was effective in removing cadmium from solution at an initial concentration ranging from 10 to 500 mg/l. More than 99% of the cadmium was removed at initial concentration ranging from 10 to 100 mg/l. The removal efficiency dropped to 78.5% when the initial concentrations were greater than 100 mg/l. However, as the initial increased, cadmium removal efficiency increased again. 90.2% of the cadmium was adsorbed at an initial concentration of 500 mg/l. The cadmium sorption capacity increased from 33.06 mg/g EPSs at an initial concentration of 10 mg/l to 1437 mg/g EPSs at an initial concentration of 500 mg/l (Fig. 2). White floccules were observed in the dialysis sacks when the initial cadmium concentration was greater than 100 mg/l. The increasing adsorption efficiency when initial cadmium concentration was greater than 100 mg/l might be ascribed to these

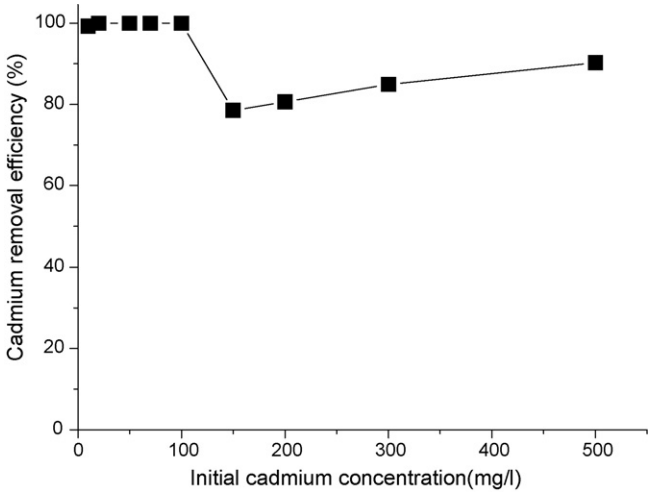

Fig. 1. Cd removal efficiency by EPSs. (The relative standard deviations of the measurement were below 1%.)

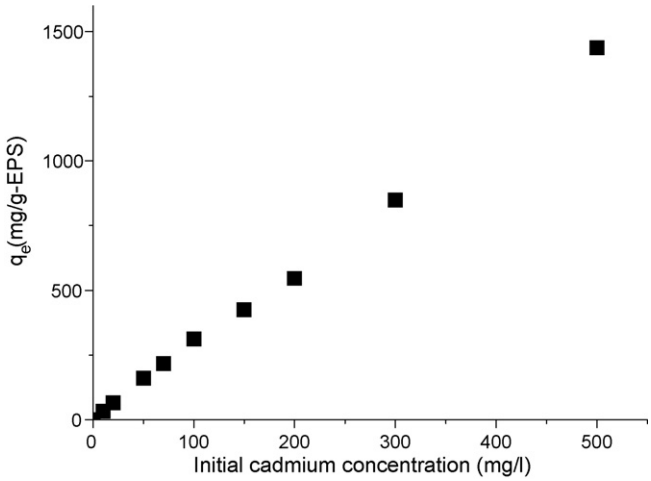

Fig. 2. Equilibrium sorption capacity of Cd by EPSs.

floccules, which might have some adsorptive capacity. However, the mechanisms involved need further research.

3.2. Sorption isotherm

The well known Langmuir and the Freundlich adsorption isotherms were used to evaluate the adsorption data.

The Langmuir isotherm model is a theoretical model for monolayer adsorption:

$$q = \frac{q_m b C_e}{1 + bC_e} \quad (1)$$

where q is the amount of metal adsorbed, mg/g (dry mass); q_m the maximum metal uptake value corresponding to sites saturation, mg/g (dry mass); C_e the equilibrium metal concentration in solution, mg/l; and b is the ratio of adsorption/desorption rates.

Freundlich isotherm model is an experimental model and is usually expressed as follows:

$$q = kC_e^{1/n} \quad (2)$$

where q is the amount of metal adsorbed in mg/g, k and $1/n$ are the Freundlich constants.

The constants of Freundlich isotherm equation and Langmuir isotherm equation are shown in Table 1. The results showed that the sorption data could be satisfactorily represented by Freundlich isotherm equation ($R^2 = 0.9880$) (Fig. 3) but poorly by the Langmuir isotherm equation ($R^2 = 0.8638$), which indicated that cadmium sorption by EPSs was a physicochemical process and certain reactions were involved between cadmium and functional groups of EPSs. The value of q_m derived from Langmuir isotherm equation was up to 2720.9 mg/g EPSs and this value

Table 1
Constants of Freundlich equation and Langmuir equation

Freudlich isotherm equation			Langmuir isotherm equation		
k	$1/n$	R^2	q_m (mg/g EPSs)	b (1 mg^{-1})	R^2
0.1027	0.5349	0.9638	2720.9	1.6493	0.8638

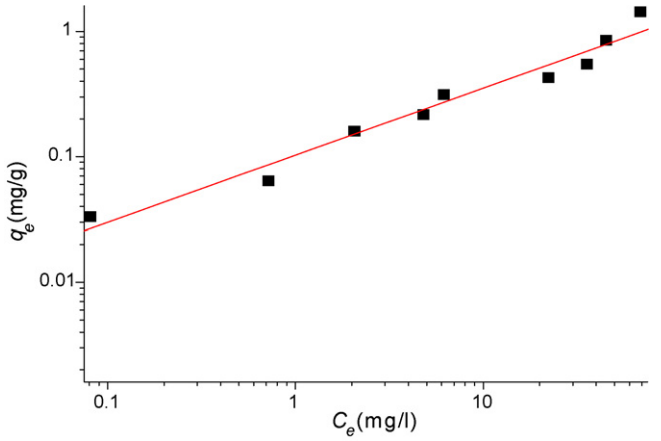

Fig. 3. Freundlich isotherm of cadmium sorption by EPSs.

Table 2
Cadmium sorption capacity of some bioadsorbent in literature

q_m (mg/g)	Bioadsorbent	Reference
98–120	*Sargassum* sp.	[26]
17.98–83.18	Alga	[27]
2.6–8.0	*Pseudomonas putida*	[28]
127.01	*Durvillaea potatorum*	[29]
566	Aerobic granular sludge	[30]
15.35	Chitin	[31]
29 ± 3	Moss	[32]
2364	EPSs of anaerobic sludge	[15]
2720.9	EPSs of anaerobic sludge	This study

was much higher than the observed capacities or the estimated q_m values by other natural adsorbents reported in the literature (Table 2).

3.3. Effect of competing cations

Fig. 4 indicated that cadmium sorption capacity was influenced by competing cations. The cadmium removal efficiency of biomass before EPSs extraction was 99.9% at an initial concentration of 20 mg/l. The presence of copper had an appreciable

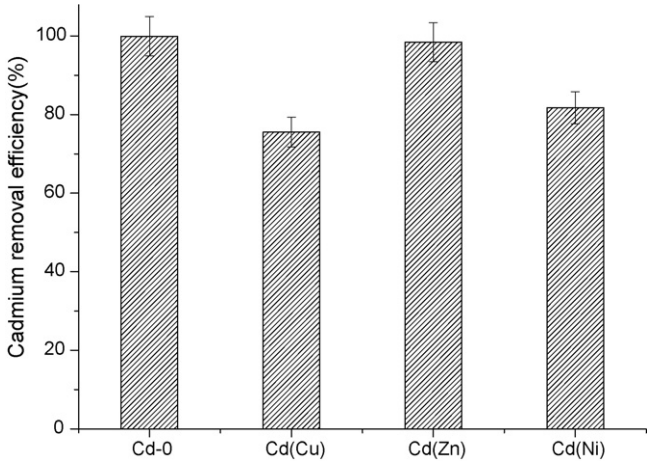

Fig. 4. Effect of competing cations on removal of cadmium by EPSs.

Table 3
Main functional groups observed with IR spectra

Wave number (cm^{-1})	Vibration type	Groups	References
3750–3000	Stretching vibration of OH	–OH of polysaccharides and proteins	[24,34,35,36]
2926 ± 10	Asymmetric stretching, vibration of CH_2		[24,34]
2853 ± 10	Symmetric stretching, vibration of CH_2		[24,34]
1680–1630	Stretching vibration of C=O and C–N (amide I)	Amide I (protein peptidic bond)	[34]
1630–1580	Stretching vibration of C–N and deformation vibration of N–H	Amide II (protein peptidic bond)	[34]
1455	Deformation vibration of CH_2		[24,34]
1240	Deformation vibration of C=O and stretching vibration of OH	Carboxylic acids and phenols	[24,34]
1120–950	Deformation vibration of CH	Benzene ring	[35]
1150–1030	C–O–C stretching	Polysaccharides	[24]
<1000	"Fingerprint" zone	Phosphate or sulphur functional groups	[24]

effect on cadmium sorption capacity of dissolved EPSs and cadmium removal efficiency dropped to 75.5%. The presence of nickel reduced the cadmium sorption capacity of EPSs from 99.96% to 81.74%. It can be concluded that nickel and copper compete the same binding sites of EPSs and cadmium could be preferably complexed by EPS in the binary solutions. However, the presence of zinc had little effect in cadmium sorption capacity, indicating that cadmium and zinc do not compete the same type binding site, which is contrast to the result that Loaec and Olier [33] reported. Loaec and Olier [33] demonstrated the competition for the same binding site of EPSs of *Alteromonas macleodii* with zinc and cadmium.

3.4. Mechanism of cadmium sorption by EPSs

Fig. 5 shows the FT-IR spectra of EPSs before and after cadmium sorption. Analysis of the FT-IR spectrum of EPSs before cadmium sorption demonstrated there were intensive bands corresponding to proteins (at 1680–1630 and 1630–1580 cm^{-1}), polysaccharides (at 1150–1030 cm^{-1}) and other functional groups (Fig. 5, upper). Some relatively weaker bands indicated the presence of carboxyl group (at 1240 cm^{-1}) that existed in the form of acidic or basic salts, which combined with other bands indicated the presence of uronic acid and humic acid (CH_2 at 2926 ± 10 and 2853 ± 10 cm^{-1} and phenols at 1240 cm^{-1}). The presence of CH_2 and carboxylic groups indicated the presence of lipid [24,34], whose content is usually too low to be detected with IR spectra. Some bands at "fingerprint zone" might be assigned to phosphate group, which is one of the functional groups of which nucleic acids are composed. The main functional groups observed with the FT-IR spectra are shown in Table 3. The different functional groups observed in the EPSs agreed well with the results of other authors [24,34,36,37]. Comparative analysis of the FT-IR spectrum before (Fig. 5, upper) and after (Fig. 5, lower) cadmium sorption showed that the C–O–C group of polysaccharides at 1150–1030 cm^{-1}, the CH_2 group of the lipids, the carboxyl group and the –OH group of proteins and polysaccharides were involved in cadmium binding. The bands at 1455–1000 cm^{-1} almost disappeared, indicating strong binding between cadmium and the C–O–C group of polysaccharides, the CH_2 group of lipids and carboxyl group of the protein. As for whether the groups of polysaccharides or the groups of proteins played a major role in complexation of metal ions, different

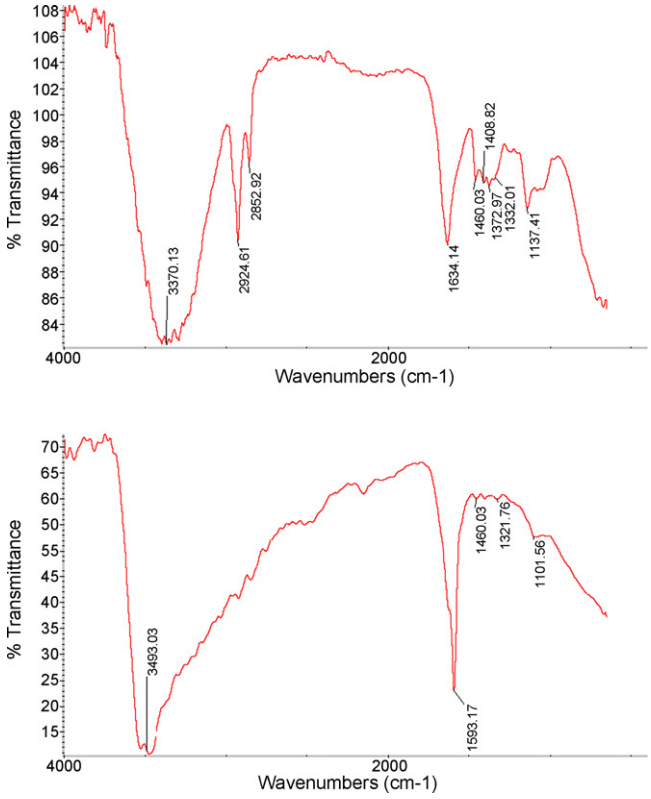

Fig. 5. FT-IR spectra of EPSs before and after sorption of cadmium (upper, before sorption; lower, after sorption).

results were reported. Some authors reported that the polysaccharide part was mainly responsible for metal removal [5,38] while other authors reported that the proteinaceous part of EPSs plays a major role in complexation of metal ions [24]. Guibaud et al. [24] demonstrated that the number of binding sites and the complexation constant were strongly linked to proteins, polysaccharides and humic substances content.

4. Conclusion

EPSs produced by anaerobic sludge under sulfate-reducing conditions played key role in cadmium sorption by sludge and the value of q_m was up to 2720 mg/g EPSs. The sorption data could be represented by Freundlich isotherm model, indicating that cadmium sorption by EPSs was a complex

physicochemical process. Analysis of FT-IR spectra confirmed that cadmium sorption was attributed to complexation of cadmium with functional groups of proteins, polysaccharides, lipids and humic acids of EPSs. Complexation of cadmium with –OH and C–O–C of polysaccharides played a major role in cadmium sorption by EPSs.

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