Adsorption of heavy metals by EPS of activated sludge

Y. Liu, M.C. Lam and H.H.P. Fang

Environmental Engineering Research Centre, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China

e-mail: *hrechef@hkucc.hku.hk*

Water Science and Technology **Vol 43 No6 pp 59–66** © IWA Publishing 2001

Abstract Extracellular polymeric substances (EPSs) were extracted by high-speed centrifugation at 20,000 G for 30 min from an activated sludge treating municipal wastewater. Each gram of sludge, as measured in volatile suspended solids (VSS), contained 7.3 mg of EPS, including 6.5 mg of protein (EPS_p) and 0.8 mg of carbohydrate (EPS_c). The EPS_p had a mean MW of 2.0×105; about 18% of EPS_p had MW over 5×104 and 16% below 5×103. For heavy metal concentrations ranging 10–100 mg/l, EPS on average removed 99% of Zn^{2+}, 98% of Cu^{2+}, 97% of Cr^{3+}, 85% of Cd^{2+}, 69% of Co^{2+}, 37% of Ni^{2+}, and 26% of $CrO4^{2-}$. The relative degrees of metal removals were inconsistent with those reported for the activated sludge process. Each mg of ESP had the capacity to remove up to 1.48 mg of Zn^{2+}, 1.12 mg of Cu^{2+}, 0.83 mg of Cr^{3+}, 0.90 mg of Cd^{2+}, 1.10 mg of Co^{2+}, 0.25 mg each of Ni^{2+} and $CrO4^{2-}$. Results suggest the feasibility of recovering ESP from waste sludge for use as adsorbent. Freundlich isotherm correlated satisfactorily with the adsorption data of Ni^{2+}, Cu^{2+}, Cd^{2+}, and $CrO4^{2-}$ (R2 ranging 0.89–0.97), whereas Langmuir isotherm correlated satisfactorily with those of Zn^{2+}, Cr^{3+} and Ni^{2+} (*R*2 ranging 0.93–0.96). Both correlated poorly for those of Co2+.

Keywords Activated sludge; adsorption; EPS; extracellular polymeric substances; heavy metals

Introduction

The electroplating industry in Hong Kong discharges large quantities of toxic heavy metals, such as cadmium, chromium, cobalt, copper, nickel and zinc, into the environment. In the secondary wastewater treatment plants, these metals are adsorbed by the biomass and removed from wastewater along with the wasted sludge. Studies have been conducted to investigate the adsorption of heavy metals by pure culture of bacteria isolated from activated sludge, including *Zoogloea* (Norberg, 1984; Sag and Kutsal, 1989a, b; Sag *et al*., 1995a, b) and *Klebsiella aerogenes* (Brown and Lester, 1982a). The adsorption of heavy metals is attributed to the extracellular polymeric substances (EPSs) excreted by the microorganisms (Freidman and Dugan, 1968; Dugan and Pickrum, 1972; Forster and Lewin, 1972; Bitton and Freihofer, 1978; Brown and Lester, 1982b). However, little is known about the adsorption of EPS produced by the mixed microorganisms in wastewater treatment systems. This study was thus conducted to investigate the adsorption mechanism of this type of EPS and its adsorption capacities on various heavy metals. Results may lead to a better understanding of the EPS characteristics and its adsorption isotherms.

Materials and methods

EPS characteristics

Activated sludge samples were collected from the Shatin Wastewater Treatment Works using the Bardenpho process. The volatile suspended solids (VSS) content of the sludge was measured according to the Standard Methods (*Standard Methods*, 1989). A 10 ml sludge sample containing 117 mg of VSS was washed twice and then re-suspended in 10 ml of 0.85% NaCl solution. ESP was separated from the sludge by high-speed centrifugation at 20,000 G for 30 min either as-is or after extraction at 80°C. The EPS-rich supernatant was further concentrated by removing low molecular weight (MW) solutes and 30–50% of water through a flow cell that had a membrane with a MW cut-off of 1,000 (Type OMEGA, Pall Filtron). The protein content of EPS (EPS_p) in the supernatant was measured according to the Folin method (Lowry *et al.*, 1951) and the carbohydrate content (EPS_c) by the anthrone-sulfuric method (Dische, 1962). The MW distribution of EPS_p was examined by HPLC (SCL-10A) using a gel permeation column (TSK-Gel G4000 SWxl, Supelco). Heavy metals were analyzed by the atomic absorption spectrophotometer (Perkin Elmer, Model 300).

Adsorption tests

Seven series of tests were conducted on the adsorption of heavy metals by the ESP extracted from activated sludge as-is without thermal treatment. The six most common heavy metals polluting Hong Kong, i.e. cadmium, cobalt, copper, nickel, zinc and chromium (both tri- and hexa-valent), were selected for the study. The adsorption tests were conducted at ambient temperature in 250 ml glass bottles with cap seals. Each bottle contained 60 ml of aqueous solution containing 36.3–55.6 mg of EPS and an individual heavy metal at 10, 20, 50, 80 or 100 mg/l; one bottle without metal dosage served as a control. After 24 hours of mixing, heavy metals were adsorbed by the EPS. The mixed liquor was then filtered through the OMEGA membrane (Pall Filtron). Contents of the residual metals in the filtrate were compared with those in the original solutions. The difference represented the quantity of the metal adsorbed by the EPS that was retained by the membrane.

Results and discussion

EPS characteristics

The EPS content of biomass varies widely, strictly depending on the extraction method (Fang and Jia, 1996). At present, there are no standard procedures for EPS extraction established by the research community, making it difficult to compare results from one study to another. The EPS_p and EPS_c contents in each gram of activated sludge (as measured by VSS) are summarized in Table 1. It shows that from each gram of activated sludge as-is a total of 7.3 mg EPS was extracted, of which 6.5 mg was EPS_p and only 0.8 mg was EPS_c. The same sludge after extraction at 80°C yielded over 8 fold of total EPS (57.9 mg). As compared to the as-is sludge, extraction at 80°C yielded 18 fold of EPS_c (13.0 mg) and 7 fold of EPS_p (44.9 mg), lowering the EPS_p/EPS_c ratio from 8.6 to 3.5. This indicates that the carbohydrate fraction of EPS was more sensitive to the thermal extraction.

Corresponding results in the literature for EPS extracted from activated sludge are also listed in Table 1 for comparison. Some of the EPS data were originally reported on the basis of SS (suspended solids); they have been converted in Table 1 to the basis of VSS by assuming a VSS/SS of 0.7. Table 1 also lists ESP data in the literature using a variety of extraction

Table 1 Composition of EPS extracted from activated sludge

Extraction method	Protein (mg/g-VSS)	Carbohydrate (mg/g-VSS)	EPSp/EPSc	Reference
20,000 G, 30 min	6.5	0.8	8.6	Present study
80°C, 30 min	44.9	13.0	3.5	Present study
33,000 G, 10 min	3.4	0.6	6.1	Brown & Lester, 1980
Steaming, 10 min	54.0	11.1	4.9	Brown & Lester, 1980
80°C, 10 min	8.0	9.8	0.8	Morgan *et al*., 1990
80°C, 10 min	9.9	16.7	0.6	Morgan *et al*., 1990
Sonication	127	9.5	13.40	Dignac *et al*., 1998
Ion-exchange	88	21.0	4.2	Rudd *et al*., 1984
Sonication / Ion-exchange	242	11.4	21.20	Dignac *et al*., 1998

methods, including thermal (Brown and Lester, 1980), ion-exchange (Rudd *et al*., 1984; Dignac *et al*., 1998), and sonication (Dignac *et al*., 1998). Results show that there are large discrepancies in reported data, mainly due to the variations in the extraction methods. However, data of the as-is sludge are in general comparable between this study and the literature (Brown and Lester, 1980): EPS_p 6.5 mg/l vs 3.4 mg/l; EPS_c 0.8 mg/l vs 0.6 mg/l; and EPS_p/EPS_c 8.6 vs 6.1.

In pure cultures, most EPSs were composed of carbohydrates, and the EPSs were often referred as extracellular polysaccharides or exopolysaccharides (Sutherland, 1977; van Geel-Schutten *et al*., 1998; Wingender *et al*., 1999; Guillouet *et al*., 1999). In some cases, the EPS_p/EPS_c ratio was as low as 0.04 (Ford *et al*., 1991). But as shown in Table 1, the EPSs in activated sludge were mostly composed of EPS_p with an EPS_p/EPS_c ratio ranging from 3.5 to 21.2. The high EPS_p/EPS_c ratio is probably due to the presence of large quantities of proteinaceous exoenzymes entrapped in the ESP matrix of the microorganisms in the activated sludge (Frolund *et al*., 1995; Dignac *et al*., 1998).

Table 2 summarizes the MW distribution of the EPS_p extracted from the activated sludge as-is. Limited MW information is available in the literature for comparison. The EPS_p had a mean MW of 2.0×10^5, considerably higher than the 15,000 reported for EPS_p in activated sludge (Higgins *et al*., 1997). About 18% of EPS_p had MW over 5×10^4 and 15.7% below 5×10^3 (8.7% below 1×10^3). The distribution is comparable to the reported range of 1.2×10^5–2.0×10^6 for EPS_c extracted from activated sludge (Horan and Eccles, 1986).

Heavy metal removal

Figure 1 illustrates the removals of heavy metal at initial concentrations ranging 10–100 mg/l for Cu^{2+}, Cr^{3+} and Co^{2+}. Figure 2 illustrates those for Zn^{2+}, Cd^{2+}, Ni^{2+} and CrO_4^{2-}. They show that Zn^{2+} and Cu^{2+} were consistently removed (over 98%) at all concentrations. Removals of most other species decreased with the increase of metal concentration, as expected. For Cd^{2+}, the removal efficiency reduced from 93% at 10 mg/l to 85% at 100 mg/l. The corresponding efficiencies were 55% and 21% for Ni^{2+}, 100% to 89% for Cr^{3+}, and 39% to 13% for CrO_4^{2-}. However, the removal of Co^{2+} increased from 42% at 10 mg/l to 82% at 100 mg/l.

Table 3 summarizes the average removal efficiencies for the seven tested species by EPS in this study and the corresponding data for activated sludge (Brown and Lester, 1979; Chen and Hao, 1998). It shows that more metals were removed by the EPS than by the activated sludge process, despite the metal concentrations tested in this study (10–100 mg/l) being considerably higher than those in wastewater treated by the activated sludge process. However, the relative degrees of removal were consistent. Zn^{2+}, Cu^{2+} and Cr^{3+} were the three metals most effectively removed by both EPS (97–99%) and activated sludge (66–73%), whereas CrO_4^{2-} was the least effective (26% vs 29%). The poor removal of

Table 2 Molecular weight distribution of EPS

Molecular Weight (×103)	Fraction (%)
< 1	8.7
1 – 5	7.0
5 – 15	15.3
15 – 30	30.8
30 – 50	10.2
50 – 100	8.3
100 – 500	11.5
500 – 1,000	1.1
> 1,000	7.1

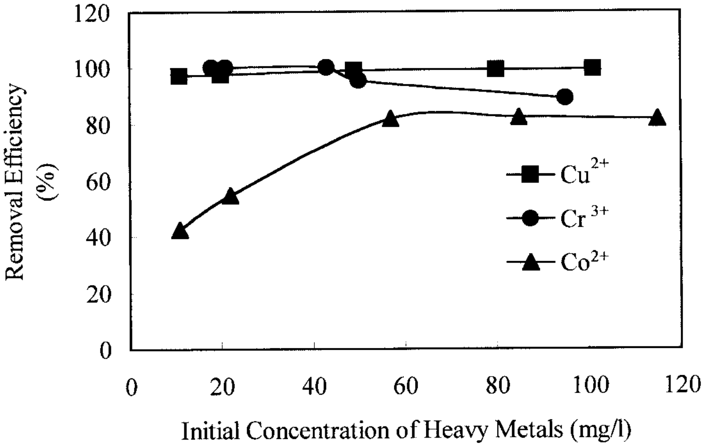

Figure 1 Removal efficiencies of Cu^{2+}, Cr^{3+} and Co^{2+} by EPS

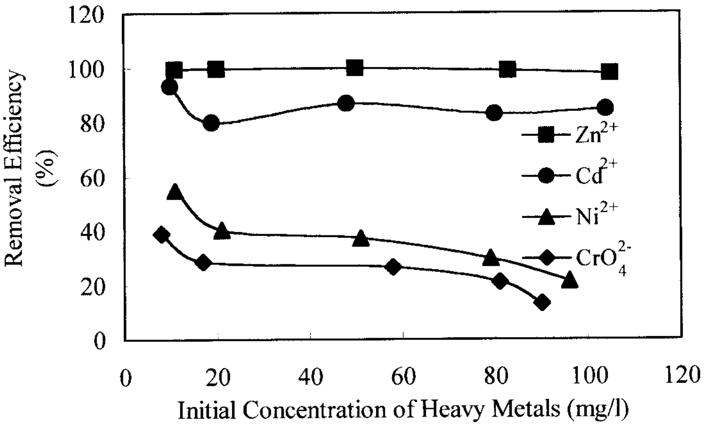

Figure 2 Removal efficiencies of Zn^{2+}, Cd^{2+}, Ni^{2+} and CrO_4^{2-} by EPS

CrO_4^{2-} is likely due to its negative charge, due to the anionic nature of the ESP (Jia *et al.*, 1996).

Adsorption isotherms

The adsorption capacity of an adsorbent is normally dependent on the concentration the adsorbed species at equilibrium. An adsorption isotherm is commonly used to correlate these two parameters. The two most common adsorption isotherms are:

Freundlich isotherm $Q_e = k\,C_e^{1/n}$
Langmuir isotherm $Q_e = Q_m bC_e/(1+bC_e)$

where Q_e is the adsorption capacity (expressed in mg/g-adsorbent) and C_e is equilibrium concentration of the adsorbed species. The Freundlich isotherm is an empirical equation, whereas Langmuir isotherm can be derived by assuming a mono-layer of physical adsorption. Both isotherms have two parameters that are specific to the adsorbent and adsorbed species. The Langmuir parameter Q_m represents the maximum adsorption capacity of the adsorbent.

Table 3 Comparison of average heavy metal removal efficiencies

Heavy metal	Average removal efficiency	
	EPS in this study (%)	Activated sludge (%)
Cd^{2+}	85	46a
Co^{2+}	69	n/a
Cr^{3+}	97	73a
CrO4^{2-}	26	29b
Cu^{2+}	98	66a
Ni^{2+}	37	33a
Zn^{2+}	99	69a

[a]Brown and Lester (1979)
[b]Chen and Hao (1998

Table 4 summarizes the best-fitted Freundlich parameters, k and $1/n$, and the best-fitted Langmuir parameters, Q_m and b, for the adsorption of seven metal species by EPS. Results show that the Freundlich isotherm correlated satisfactorily with the adsorption data of Ni^{2+}, Cu^{2+}, Cd^{2+}, and CrO_4^{2-} (R^2 ranging 0.89–0.97), but poorly for Co^{2+} (R^2 of 0.83) and Cr^{3+} (R^2 of 0.55). On the other hand, the Langmuir isotherm correlated satisfactorily with those of Zn^{2+}, Cr^{3+} and Ni^{2+} (R^2 ranging 0.93–0.96), but poorly for CrO_4^{2-} (R^2 of 0.79), Co^{2+} (R^2 of 0.53) and Cd^{2+} (R^2 of 0.17).

Table 5 summarizes the maximum observed capacities of heavy metals by EPS and the Q_m values, as compared with the corresponding values by other adsorbents in the literature. Results show that each mg of ESP was capable of removing up to 1.48 mg of Zn^{2+}, 1.12 mg of Cu^{2+}, 0.83 mg of Cr^{3+}, 0.90 mg of Cd^{2+}, 1.10 mg of Co^{2+}, and 0.25 mg each of Ni^{2+} and CrO_4^{2-}. Such adsorption capacities are considerably higher than the observed capacities, or the estimated Q_m values by other natural adsorbents, such as peat (Wase *et al*., 1997), algae (Yu *et al*., 1999), rice bran (Wase *et al*., 1997), etc., and synthetic adsorbents, such as activated carbon (Wase *et al*., 1997) and ion-exchange resins (Liu and Tang, 1999). This is likely due to the strong anionic nature of the ESP (Jia *et al*., 1996).

Results in Table 5 also imply that it is highly feasible to recover ESP from the waste sludge of a wastewater treatment plant for use as adsorbent.

Conclusion

Each gram of VSS of activated sludge contained 7.3 mg of EPS, including 6.5 mg of protein (EPS_p) and 0.8 mg of carbohydrate (EPS_c). For heavy metal concentrations ranging 10–100 mg/l, EPS on average removed 99% of Zn^{2+}, 98% of Cu^{2+}, 97% of Cr^{3+}, 85% of Cd^{2+}, 69% of Co^{2+}, 37% of Ni^{2+}, and 26% of CrO_4^{2-}. Each mg of ESP had the capacity to remove up to 1.48 mg of Zn^{2+}, 1.12 mg of Cu^{2+}, 0.83 mg of Cr^{3+}, 0.90 mg of Cd^{2+}, 1.10 mg of Co^{2+},

Table 4 Freundlich and Langmuir isotherm constants

Metal	Freundlich isotherm			Langmuir isotherm		
	k	$1/n$	R^2	Q_m (mg/mg-EPS)	b (1/mg)	R^2
Cd^{2+}	0.0960	0.721	0.902	2.364	-0.029	0.166
Co^{2+}	0.001	2.560	0.827	–0.148	–0.047	0.534
Cr^{3+}	0.5700	0.066	0.553	0.878	-1.076	0.962
CrO_4^{2-}	0.0180	0.596	0.893	0.265	-0.044	0.787
Cu^{2+}	3.2050	3.010	0.923	–0.153	–1.336	0.883
Ni^{2+}	0.0230	0.605	0.971	0.301	-0.048	0.931
Zn^{2+}	1.0630	0.546	0.801	1.779	-1.958	0.963

Table 5 Maximum adsorption capacities and Q_m for heavy metals of several materials

Metal	Maximum capacity Range (mg/g)	Maximum capacity Average (mg/g)	Q_m Range (mg/g)	Q_m Average (mg/g)	Absorbent	Reference
Cd^{2+}		900			EPS	Present study
Cd^{2+}			93–135	121	Algae	Yu et al., 1999
Cd^{2+}			21–23	22	Peat	Wase et al., 1997
Cd^{2+}	107–217	146			Resin	Liu and Tang, 1999
Co^{2+}		1100			EPS	Present study
Co^{2+}				9	Rice bran	Wase et al., 1997
Co^{2+}	60–142	83			Resin	Liu and Tang, 1999
Cr^{3+}		830		878	EPS	Present study
Cr^{3+}				33	Rice bran	Wase et al., 1997
Cr^{3+}	15–50	31			Resin	Liu and Tang, 1999
$CrO4^{2-}$		250			EPS	Present study
$CrO4^{2-}$				119	Peat	Wase et al., 1997
$CrO4^{2-}$			20–145	76	Activated carbon	Wase et al., 1997
Cu^{2+}		1120			EPS	Present study
Cu^{2+}			69–83	76	Algae	Yu et al., 1999
Cu^{2+}	14–43	30	2–65	23	Fungi	Wase et al., 1997
Cu^{2+}			6–20	14	Peat	Wase et al., 1997
Cu^{2+}				9	Activated carbon	Wase et al., 1997
Cu^{2+}	63–126	87			Resin	Liu and Tang, 1999
Ni^{2+}		250		301	EPS	Present study
Ni^{2+}	5–12	9			Fungi	Wase et al., 1997
Ni^{2+}	1–23	9			Peat	Wase et al., 1997
Ni^{2+}	46–168	88			Resin	Liu and Tang, 1999
Zn^{2+}		1480		1779	EPS	Present study
Zn^{2+}			6–13	10	Peat	Wase et al., 1997
Zn^{2+}	7–24	16			Rice bran	Wase et al., 1997
Zn^{2+}	57–136	85			Resin	Liu and Tang, 1999

0.25 mg each of Ni^{2+} and CrO_4^{2-}. Results suggest the feasibility of recovering ESP from waste sludge of wastewater treatment plants for use as adsorbent. The Freundlich isotherm correlated satisfactorily with the adsorption data of Ni^{2+}, Cu^{2+}, Cd^{2+}, and CrO_4^{2-} (R^2 ranging 0.89–0.97), whereas the Langmuir isotherm correlated satisfactorily with those of Zn^{2+}, Cr^{3+} and Ni^{2+} (R^2 ranging 0.93–0.96). Both correlated poorly for those of Co^{2+}.

Acknowledgement

The authors would like to thank the Hong Kong Research Grants Council for the financial support of this study, and the University of Hong Kong for the Postdoctoral Fellowship for YL.

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