

# Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values

S. Comte, G. Guibaud\*, M. Baudu

*Laboratoire des Sciences de l'Eau et de l'Environnement, Université de Limoges, Faculté des Sciences et Techniques,  
123 Avenue A. Thomas, 87060 Limoges Cedex, France*

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## Abstract

The aim of this study was to assess the influence of pH on the metal biosorption of extracellular polymeric substances (EPS) extracted from two different activated sludges called A and B. The composition and physico-chemical characteristics of EPS were determined. The biosorption capacities of the EPS were examined at pH 4, 6, 7 and 8 successively with three metals Cu, Pb and Cd using differential pulse polarography (DPP) as an investigation tool and Ruzic's model was used to produce polarographic titration curves. Two apparent  $pK_a$  were obtained, the first were 6.6 (EPS A) and 5.7 (EPS B), attributed to carboxylic and phosphoric groups whereas the second was 8.7 for EPS A and 9.4 for B and these were attributed to phenolic and amino functional groups. Whatever the EPS and the metal considered, the conditional binding constant did not show significant differences in the strength of complex formed between the EPS and metals. But for all metals, the number of EPS binding sites was significantly lowered by a decrease in the pH of the medium. At pH 4, the metal biosorption capacity of EPS is very low. At pH 6, the number of EPS binding sites increased in the following order:  $Pb > Cu > Cd$  whereas at pH 7 and 8, this order changed and was:  $Cu > Pb \gg Cd$ . Simulations of the speciation states of Cu, Pb and Cd at the different pH values in ultra-pure water (25 °C, ionic strength 0.045 M) were performed with MINEQL 4.5 software and indicated the presence of hydroxylated forms and sometimes solid forms for Pb and Cu. But the polarographic titration curves revealed precipitation of Cu only at the end of the experiments at pH 8.

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**Keywords:** Activated sludge; Biosorption; EPS; Heavy metals; pH

## 1. Introduction

When wastewater treatment is performed with the activated sludge process, the quality of the effluent is highly dependent on the efficiency of the solid–liquid separation processes. Poor separation of sludge in a secondary clarifier could result in a metal removal problem. Moreover, the activated sludge can be reused in agriculture for land treatment as it is very rich in organic matter and the accumulation of heavy metals in the sludge can counteract metal mobilization in the environment.

The presence of metals in the activated sludge is often attributed to the binding of metals on to the bacterial cell surface [1]. However, bacteria may produce macromolecules outside their own cell wall, commonly called extracellular

polymeric substances (EPS). Studies [2–5] have shown that EPS also play a crucial role in biosorption of heavy metals.

As has already been said, EPS are metabolic products of bacteria but they also result from organic matter of the effluent and from microbial lysis or hydrolysis [6,7] and serve as a protective barrier for cells against the harsh external environment. EPS also have the function of sorption of inorganic ions which constitute metabolic elements for bacteria [8,9]. Their composition is complex but EPS are mainly composed of polysaccharide, protein, humic substances, uronic acid, nucleic acid and lipids [6,7,10], containing ionisable functional groups such as carboxyl, phosphoric, amine, and hydroxyl groups [11]. These functional groups represent potential binding sites for the sequestration of metal ions [2,3,11].

It is assumed that metal biosorption involves a physico-chemical interaction between the metal and functional groups on the cell surface, based on physical adsorption, ion exchange, complexation and precipitation [12]. Moreover, metal biosorp-

\* Corresponding author. Tel.: +33 5 55 74 28; fax: +33 5 55 45 73 67.  
E-mail address: [gilles.guibaud@unilim.fr](mailto:gilles.guibaud@unilim.fr) (G. Guibaud).

tion performance depends on external factors, such as pH, other ions in bulk solutions (which may be in competition), organic material in bulk solution and temperature [12–14]. Unfortunately, few data concerning the effect of pH on EPS metal biosorption properties are available.

A wide variety of techniques can be employed to determine the binding capacity of a macromolecular ligand. Among these techniques, electrochemical techniques such as anodic stripping, voltammetric and differential pulse polarographic (DPP) methods have been the most widely used [15–17]. We have chosen to employ a polarographic method as a means of investigation due to its ease of use. The polarographic titration curves obtained were exploited with Ruzic's modelisation [18].

The major objective of this study is to investigate the effect of pH on the EPS biosorption of heavy metal ions. We have therefore studied the biosorption properties of EPS at different pH values (4, 6, 7 and 8) with three metal ions ( $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ ).

## 2. Materials and methods

### 2.1. Sludge samples and EPS extraction

EPS extraction was carried out on two activated sludges obtained from the aeration tanks of two wastewater treatment plants (WWTP) called A and B in order to obtain two EPS with different compositions.

The WWTPs A and B present different characteristics. The treatment capacity in inhabitant equivalent is 285,000 and 4000 for WWTP A and B, respectively. The organic loads are between 0.24 and 0.30 and between 0.13 and 0.16  $\text{kg BOD}_5 \text{ m}^{-3} \text{ day}^{-1}$  for WWTP A and B, respectively.

Less than 4 h after sampling, the sludges were concentrated at  $4300 \times g$  for 10 min at  $4^\circ\text{C}$ . The residues were recovered and suspended in ultra-pure water. For each sludge (A and B), a 40 W sonication treatment was applied for 2 min followed by two ultra-centrifugations ( $20,000 \times g$  for 20 min at  $4^\circ\text{C}$  then  $10,000 \times g$  for 15 min at  $4^\circ\text{C}$ ) in order to obtain EPS samples A and B [19]. The EPS composition (dry weight, volatile dry weight, total organic carbon, nitrogen, phosphorus, protein, polysaccharide and humic acid contents) was determined according to the protocols described by Guibaud et al. [20]. The main characteristics of both EPS studied are summarized in Table 1.

### 2.2. $pK_a$ determination

#### 2.2.1. Theoretical considerations

The determination of the  $pK_a$  for the EPS solutions was not possible by simple acid–base titration. According to Guibaud et al. [4], a method previously developed for solid-state chemistry was modified and applied to EPS in order to determine the  $pK_a$  of the EPS. The acid–base surface reactions are described only by the law of mass action [21] based on the protonation of the surface functional groups and determined by analogy with

amphoteric compounds [22].



with:

$$K_{a1} = [\text{H}^+][\text{S-OH}]/[\text{S-OH}_2^+] \quad (3)$$

$$K_{a2} = [\text{H}^+][\text{S-O}^-]/[\text{S-OH}] \quad (4)$$

$$[\text{S}]_{\text{tot}} = [\text{S-OH}_2^+] + [\text{S-OH}] + [\text{S-O}^-] = \text{PEC} \quad (5)$$

with  $[\text{S}]_{\text{tot}}$  as the total number of surface sites and PEC as the protonic exchange capacity.

In this study, the determination of both the EPS surface charge ( $Q$ ) [23,24] and the EPS protonic exchange capacity (PEC) [25] were carried out by potentiometric titration. Finally, the  $Q$  and PEC parameters allowed two apparent  $pK_a$  of EPS solutions to be calculated for the predominant functional groups.

The PEC indicates the total number of sites potentially active for binding fixation [25].

#### 2.2.2. Apparatus

Titrations were carried out with an automatic titrator, Metrohm 716 DMS, coupled to a Metrohm 727 Ti Stand and equipped with a pH electrode (CRISON 5202, pH 0–12/0;  $80^\circ\text{C}$ ; KCl 3 M).

#### 2.2.3. Analytical procedure

Twenty-five millilitre of EPS solution (*i.e.* 12.4 and 13.9 mg of DW, respectively, for EPS A and B) was placed in a  $22 \pm 1^\circ\text{C}$  thermostated cell. The titration was carried out adding NaOH or  $\text{HNO}_3$  ( $10^{-2} \text{ M}$ ) under nitrogen atmosphere. The  $pK_a$  characteristics of both EPS studied are given in Table 1.

Table 1  
Main characteristics of EPS A and B

	EPS A	EPS B
General characterization of EPS solution ( $\text{g L}^{-1}$ )		
Dry weight (DW)	$0.50 \pm 0.1$	$0.56 \pm 0.1$
Volatile dry weight (VDW)	$0.43 \pm 0.1$	$0.49 \pm 0.1$
General characterization of EPS		
Total organic carbon ( $\text{mg C g}^{-1} \text{ VDW}$ )	$228 \pm 11$	$255 \pm 13$
Nitrogen ( $\text{mg N g}^{-1} \text{ EPS VDW}$ )	$77 \pm 8$	$73 \pm 6$
Phosphorus ( $\text{mg P g}^{-1} \text{ EPS DW}$ )	$38 \pm 2$	$26 \pm 2$
Biochemical composition of EPS ( $\text{mg g}^{-1} \text{ EPS VDW}$ )		
Proteins	$350 \pm 14$	$391 \pm 17$
Polysaccharides	$143 \pm 13$	$158 \pm 13$
Humic acids	$62 \pm 4$	$206 \pm 14$
Physico-chemical characterization		
PEC ( $\mu\text{mol g}^{-1} \text{ EPS DW}$ )	$3500 \pm 175$	$3000 \pm 150$
$pK_{a1}$	$6.6 \pm 0.1$	$5.7 \pm 0.1$
$pK_{a2}$	$8.7 \pm 0.1$	$9.4 \pm 0.1$

### 2.3. Biosorption study

#### 2.3.1. Theoretical considerations

The binding capacity of the EPS could be defined as the maximum ability of the polymers to fix a given metal ion. The method commonly used to estimate this capacity consists in the titration (*i.e.* the progressive saturation) of the ligand (EPS) with the metal ion of interest at fixed pH. The theoretical study of the complexation equilibrium associated to the law of mass action can be described as follows, assuming the formation of a 1:1 complex.



$$K = [ML]/[M][L] \quad (7)$$

with  $K$  as the conditional binding constant,  $[M]$  the free metal concentration,  $[L]$  the ligand concentration or number of binding sites, and  $[ML]$  as the complex concentration.

The modelisation of the EPS saturation curves was carried out according to Ruzic's graphic methods and allowed  $[L]$  and  $K$  to be determined [18,26]. During the modelisation, error evaluation on the slopes and the  $y$ -intercepts was carried out.

#### 2.3.2. Theoretical polarographic titration curve

To obtain the saturation curve of EPS by a metal, a polarographic method was used [26]. The polarographic titration allows the concentration of free metal in the solution to be easily measured and, with the use of Ruzic's model, the EPS biosorption ability to be determined [18].

A theoretical polarographic titration curve (Fig. 1) can be divided into three parts: the first called the complexation part corresponding to the biosorption of metal by a ligand, the second called the linear part corresponding to the saturation of the binding sites of the ligand by the metal (*i.e.* the addition of free metal is proportional to the height of polarographic peak recorded) and a third called the precipitation part corresponding to the possible formation of hydroxylated insoluble form or metal solid form (*i.e.* the metal added is free but it can be found soluble or in solid form). Only the complexation and lin-

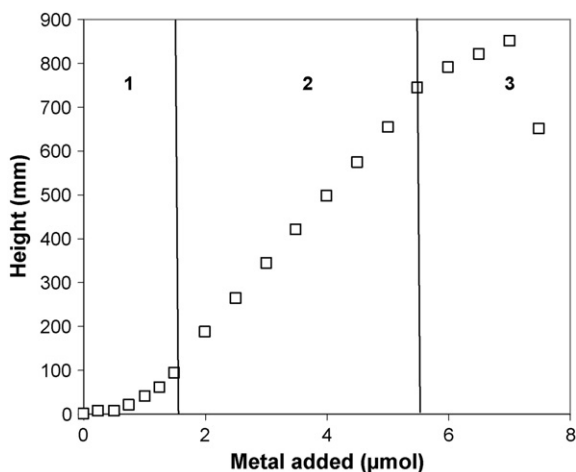


Fig. 1. Three parts of the theoretical polarographic titration curve: (1) complexation part, (2) linear part, (3) precipitation part.

Table 2

Instrumental parameters used for DPP measurements

Parameters	Value
Drop life time (s)	1
Scan rate (mV/s)	2
Cathodic pulse amplitude (mV)	50
Pulse duration (ms)	60
Potential range scanned (V)	
Cu(II)	+0.10 to -0.20
Pb(II)	-0.20 to -0.50
Cd(II)	-0.45 to -0.75

ear part are used to determine the biosorption capacity of a ligand [18].

#### 2.3.3. Apparatus

DPP measurements were carried out with Metrohm 663 VA polarograph fitted to a three-electrode arrangement. The working and auxiliary electrodes were a stripping mercury dropping electrode (SMDE) and a platinum wire, respectively. The reference electrode, to which all potentials are referred, was a saturated electrode (Ag/AgCl-KCl). The instrumental parameters are listed in Table 2.

The pH of the different samples in the analysis cell was continuously measured using a CRISON pH meter (Basic 20) equipped with a micro-electrode (CRISON 58.02).

#### 2.3.4. Analytical procedure to record the polarographic titration curve

All samples were treated in exactly the same way in order to obtain the polarographic titration curve. The polarographic measurements were performed at  $25 \pm 1$  °C under a nitrogen atmosphere and with a 20 mL total volume of solution containing EPS (*i.e.* 0.45 and 0.51 mg of DW, respectively, for EPS A and B) in the analysis cell.

The solution of EPS for biosorption experiments was prepared with 1 M  $KNO_3$  as a supporting electrolyte (10 mL), ultra-pure water (200 mL) and EPS (10 mL). The pH was adjusted to 4.0, 6.0, 7.0 and  $8.0 \pm 0.1$  by the addition of micro-quantities of a dilute solution of sodium hydroxide or nitric acid. In the polarographic measurement cell, after each metal (metal stock solution  $10^{-2}$  M for Cu and Pb,  $10^{-3}$  M for Cd) micro-addition (10, 20, 50 or 100  $\mu$ L), the pH was readjusted. The response of the system (*i.e.* determination of free metal concentration in EPS solution) was recorded after the de-aeration of the sample by 10 min nitrogen bubbling with stirring. The titration curve (height of peak (in mm) or  $i$  versus total added metal) was then recorded.

The measurement of number of sites initially occupied by the metals studied, on EPS was performed by determination of total metal content of EPS by FAAS [4]. The number of sites initially occupied (considered at a ratio 1:1 (metal:occupied site according to Ruzic, 2003 [18]) by the metals studied, on EPS, was not taken into account for the determination of the total number of sites in EPS as it was negligible (<1% total complexation determined).

## 2.4. Simulation of metal speciation

Speciation states of Cu, Pb and Cd were studied in ultra-pure water with an ionic strength attributed to the presence of  $\text{KNO}_3$  at 0.045 M by simulation with the MINEQL 4.5 computer program. For each pH value (4.0, 6.0, 7.0 and 8.0), different concentrations in metal were analysed corresponding to characteristic part of the polarographic titration curve (Fig. 1):

- Initial condition corresponding to the addition of 10  $\mu\text{L}$  of metal to the polarographic cell (*i.e.* for Cu and Pb,  $4.6 \times 10^{-6}$  M and for Cd  $4.6 \times 10^{-7}$  M).
- Final condition corresponding to the fifth plot on the linear part of polarographic titration curve (Fig. 1, part 2) (saturation of EPS in metal). As a consequence, for each pH value and the metal studied, the final condition was different.
- Finally, we studied the speciation state of the metal under precipitation conditions corresponding to the metal concentration at the point where the titration curve decreased, if such experimental condition were reached (Fig. 1, part 3).

Note as polarographic titration curve are different according to the metal, EPS and pH investigated, the concentration used for metal speciation determination with MINEQL are not constant except for initial condition.

## 3. Results and discussion

### 3.1. Physico-chemical characterization of EPS

The PEC and the  $\text{p}K_a$  values of EPS A and B were determined and are given in Table 1.

The PEC represents an estimation of EPS total binding sites [25]. The PEC values are 3500 and 3000  $\mu\text{mol g}^{-1}$  EPS DW for EPS A and B, respectively. It indicates that EPS A has the better capacity to bind metallic cations when the protonic exchange mechanism is used to explain biosorption properties of EPS.

Two  $\text{p}K_a$  values were found for each EPS.  $\text{p}K_{a1}$  are 6.6 and 5.7 and  $\text{p}K_{a2}$  are 8.7 and 9.4 for EPS A and B, respectively. In

the literature data, Liu and Fang [11] and Guibaud et al. [19,20] have established that in EPS solution,  $\text{p}K_{a1}$  (about 6) is characteristic of carboxylic and phosphoric functional groups and  $\text{p}K_{a2}$  (about 9–10) is attributed to phenolic and amino functional groups. Moreover, EPS A and B are mainly composed of proteins, carbohydrates and humic substances (Table 1). Thus, our  $\text{p}K_a$  values are in accordance with the literature and the EPS composition.

### 3.2. EPS biosorption results

#### 3.2.1. Conditional binding constant

The biosorption capacities of EPS A and B were studied at the four pH values and the conditional binding constants (expressed in  $\log K$ ) obtained are presented in Fig. 2.

For Cu, the results reveal no trend with the pH, the conditional binding constant varies from 3.2 to 4.5. The same observation can be made for lead, the conditional binding constant varying from 3.9 to 5.7. For cadmium, at pH 4 the biosorption of the metal by EPS was not detectable. Concerning the other pH values, for cadmium,  $\log K$  varied from 3.7 to 5.0 and did not show a trend when the pH decreased.

Finally, whatever the EPS (A or B) and the metal considered (Cu, Pb, Cd), the conditional binding constant did not show significant differences in the strength of the complex formed between the EPS and the metals for the pH range studied.

#### 3.2.2. Number of binding sites

The biosorption capacity of EPS towards the three metals at different pH values has also been expressed in number of EPS binding sites and this is summarized in Table 3.

For Cu, the number of binding sites varied from 99 to 5304  $\mu\text{mol g}^{-1}$  of VDW EPS and from 29 to 3183  $\mu\text{mol g}^{-1}$  of VDW EPS for EPS A and B, respectively, according to the increase in pH. For lead, this number varied from 69 to 2509  $\mu\text{mol g}^{-1}$  of VDW EPS for EPS A and from 26 to 1789  $\mu\text{mol g}^{-1}$  of VDW EPS for EPS B on increasing the pH. For cadmium, in the pH range 6–8, the number of binding sites of EPS A varied from 11 to 84  $\mu\text{mol g}^{-1}$  of VDW EPS and the

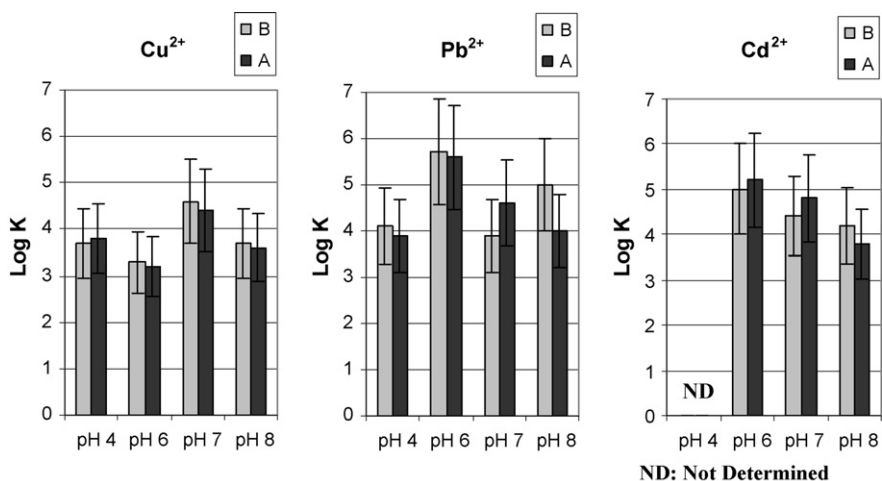


Fig. 2. Conditional binding constants of EPS (A and B) at different pHs for the metals studied.

Table 3  
Number of fixation sites of EPS A and B in  $\mu\text{mol g}^{-1}$  of VDW EPS for different pHs and metals studied

	pH 4	pH 6	pH 7	pH 8
<b>Cu<sup>2+</sup></b>				
A	99 ± 7	125 ± 8	4228 ± 166	5304 ± 1350
B	29 ± 6	135 ± 14	2630 ± 87	3183 ± 460
<b>Pb<sup>2+</sup></b>				
A	69 ± 9	1841 ± 41	2205 ± 89	2509 ± 89
B	26 ± 8	1272 ± 24	1468 ± 79	1789 ± 35
<b>Cd<sup>2+</sup></b>				
A	ND	11 ± 1	23 ± 3	84 ± 10
B	ND	8 ± 1	21 ± 2	85 ± 4

number of binding sites of EPS B varied from 8 to 85 in  $\mu\text{mol g}^{-1}$  of VDW EPS. At pH 4, no complexation was determined with Cd and either EPS.

Finally, for all metals studied, the number of EPS binding sites was significantly lowered by a decrease in the pH of the medium. For most cases, the number of binding sites of EPS A is higher than that of EPS B. At pH 4, the metal biosorption capacity of both EPS is very low. At pH 6, the number of EPS binding sites increased in the following order, depending on the metal ion: Pb > Cu > Cd. But at pH 7 and 8, this order changed and was: Cu > Pb ≫ Cd.

### 3.2.3. Discussion: metal affinity order for EPS

Wang et al. [27] investigated the uptake of several heavy metal ions by sludge particulates as a function of pH (range 3–9). They obtained the following metal-sludge affinity order: Pb > Cu > Cd, this order was not affected by the pH in the range studied. Pardo et al. [28] studied the biosorption of different metals by inactive biomass of *Pseudomonas putida* and found different metal order affinities depending on the pH. At pH 4.5, the sequence was: Pb > Cu > Cd ≈ Zn and at the optimum pH value (different from 4.5), the order becomes Pb > Cu ≈ Zn < Cd. Several authors predict different stability trends for organo-metal complexes depending on the pH and the complexing studied. According to Savvaidis et al. [17] who worked on biosorption of *Pseudomonas cepacia*, the order in which the

metals were removed with the biomass was Cu > Ni > Cd > Zn. Moreover, it has been established that the preference of several hydrous solids (such as lignite) for metals has been related to the metals' electronegativity and the reported effect is a stronger attraction for the higher electronegativity [29]. Thus, the metal order can vary according to the metals' electronegativity (*i.e.* metal speciation) and the biosorbent charge produced by the pH medium changes.

### 3.3. Metal speciation

#### 3.3.1. MINEQL simulations

The speciation state of the different metals (Cu, Pb and Cd) at pH 4, 6, 7 and 8 were carried out in ultra-pure water (at 25 °C) with an ionic strength of 0.045 M using the MINEQL 4.5 software and Table 4 summarizes the results obtained.

Studying the mechanism for metal ion binding to the biomass is complicated by the fact that metal cations are hydrolyzed in aqueous solutions at the pH of the sorption system studied. Partitioning of the metal species depends on the pH of the solution, the temperature, the total metal concentration in solution, the ionic strength and the presence of ligands. EPS are very complex so the simulations with MINEQL 4.5 were carried out in ultra-pure water for initial, final and sometimes precipitation conditions of metal without the influence of the presence of ligands (*i.e.* EPS).

According to the simulations, for the experiments at pH 4, all metallic species existed only in the divalent cation form. For cadmium, the simulations predicted only the presence of the divalent cation form whatever the pH. At pH 6, for lead and copper, the majority of metal is in the divalent cation form but soluble hydroxylated forms can appear from the beginning of the experiments. The higher the metal concentration and the pH the more the divalent cation forms disappear, and the percentage of the more soluble or insoluble hydroxylated forms increases. For lead and copper, the solid form may also be found. At pH 8, for the same metals, simulations indicated the presence of only the solid form whatever the metal concentration studied.

#### 3.3.2. Experimental observations

Polarographic titration curves of lead with EPS A and B at pH 4, 6, 7 and 8 are presented on Fig. 3.

Table 4  
Main forms of Cu, Pb and Cd calculated with MINEQL 4.5 in ultra-pure water (at 25 °C, ionic strength 0.045 M)

Metal	pH 4	pH 6	pH 7	pH 8
<b>Cu</b>				
Initial condition	100% Cu <sup>2+</sup>	98.1% Cu <sup>2+</sup> , 1.8% CuOH <sup>+</sup>	19.5% Cu <sup>2+</sup> , 3.5% CuOH <sup>+</sup> , 76.9% Tenorite	99.4% Tenorite
Final condition	100% Cu <sup>2+</sup>	98.1% Cu <sup>2+</sup> , 1.7% Cus	99.5% Tenorite	100% Tenorite
Precipitation condition	–	–	100% Tenorite	100% Tenorite
<b>Pb</b>				
Initial condition	100% Pb <sup>2+</sup>	98.6% Pb <sup>2+</sup> , 1.4% PbOH <sup>+</sup>	62.5% Pb <sup>2+</sup> , 8.8% PbOH <sup>+</sup> , 28.6% Pb(OH) <sub>2</sub> s	98.5% Pb(OH) <sub>2</sub> s
Final condition	100% Pb <sup>2+</sup>	98.6% Pb <sup>2+</sup> , 1.4% Pb(OH) <sub>2</sub> s	2.6% Pb <sup>2+</sup> , 97.1% Pb(OH) <sub>2</sub> s	99.9% Pb(OH) <sub>2</sub> s
Precipitation condition	–	–	98% Pb(OH) <sub>2</sub> s	100% Pb(OH) <sub>2</sub> s
<b>Cd</b>				
Initial condition	100% Cd <sup>2+</sup>	100% Cd <sup>2+</sup>	100% Cd <sup>2+</sup>	100% Cd <sup>2+</sup>
Final condition	100% Cd <sup>2+</sup>	100% Cd <sup>2+</sup>	100% Cd <sup>2+</sup>	100% Cd <sup>2+</sup>

NB: s, soluble; Tenorite is a solid (Cu=O).



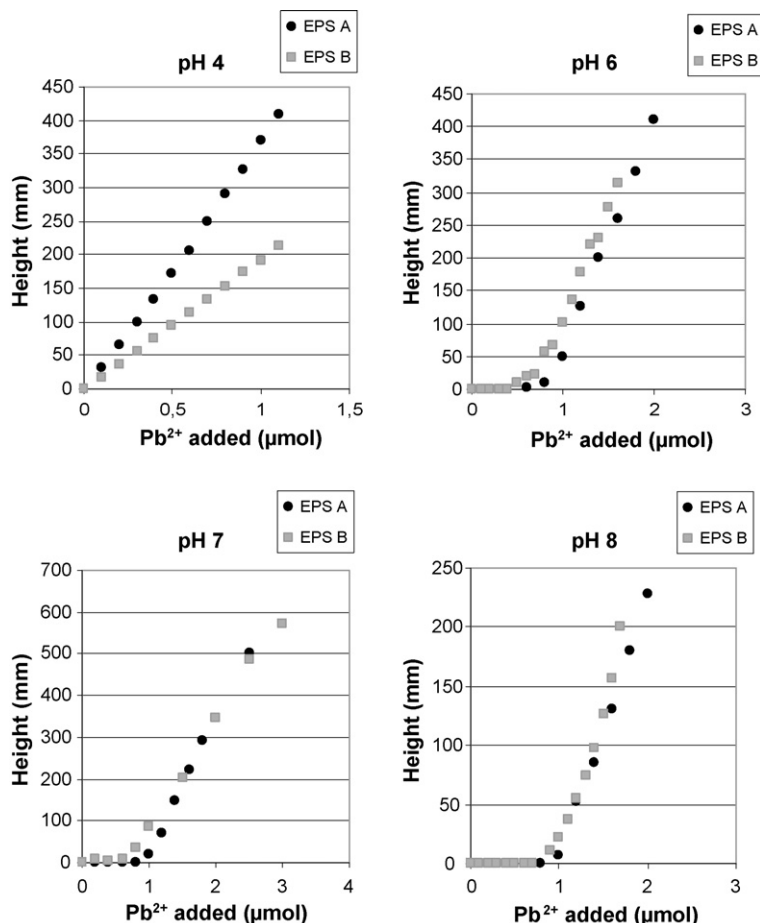


Fig. 3. Polarographic titration curves of  $Pb^{2+}$  with EPS (A and B) at pH 4, 6, 7 and 8.

At the four pH values, the titration curves do not show precipitation parts for either EPS at the metal concentrations studied. So no presence of the solid form was detected during our experiments. MINEQL 4.5 simulations (Table 4) predicted the presence of solid forms of lead at pH 7 and 8 throughout the experiments.

Fig. 4 presents polarographic titration curves of Cu with EPS A and B at the four pH values.

At pH 4, 6 and 7, we noticed that the titration curves only presented complexation and linear parts for all the metal concentration ranges considered. But at pH 8, at the end of the experiments, for both EPS, some plots (corresponding to free metal in solution) greatly decreased which could indicate precipitation of Cu. Thus, these curves show that if there was formation of solid copper, our analyses only detected this phenomenon at the end of the experiments at pH 8.

In conclusion, MINEQL simulations of metallic speciation were not in accordance with the experimental results observed with metal biosorption of EPS. These simulations only underlined the possible presence of different speciation forms for the metals studied. They thus indicate that several biosorption mechanisms are also possible according to the speciation states of the metal studied and the EPS forms produced by the pH of the medium.

### 3.4. Possible mechanisms for metal biosorption by EPS as function of pH

#### 3.4.1. Implication of electrostatic nature of EPS and metal

To explain the influence of the pH, it should be remembered that it is now widely recognized that the deprotonated form of the reactive sites (*i.e.* carboxylate groups) is primarily responsible of the binding of metal ions on to polymers of a carboxylic nature [16,30]. The carboxylic and phosphoric groups identified with  $pK_{a1}$  values (6.6 and 5.7) could be mainly implicated in the binding of metal ions by EPS at pH 6, 7 and 8 [11,31]. The medium's pH affects the ionization state of the functional groups like carboxylate, phosphate and amino groups of EPS. The carboxylate and phosphate groups carry negative charges that allow the EPS components to be potent scavengers of cations [32]. Rudd et al. [33] assumed that the complexation of Cu and Cd with EPS from activated sludge was controlled by two distinct types of binding site.

In fact, different mechanisms have been proposed to explain the biosorption of metals. One is based on ionic exchange equilibrium. According to Lopez et al. [34], as the pH increases, metal biosorption increases since ion exchange is more effective when less protons are available to compete with the metal for negatively charged metal-binding sites. Moreover, PEC val-

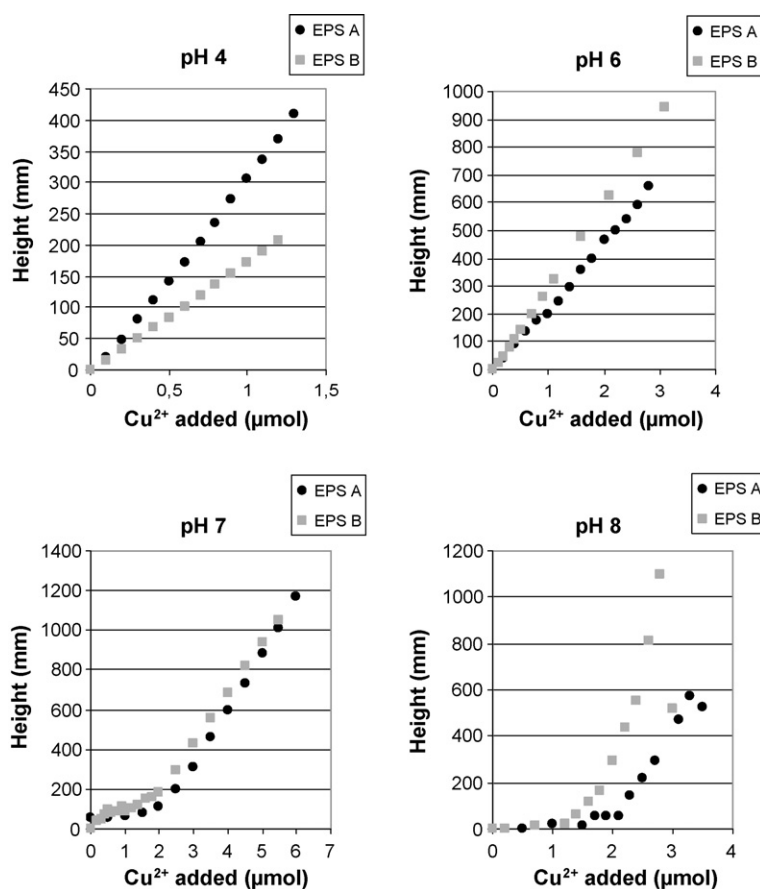


Fig. 4. Polarographic titration curves of  $\text{Cu}^{2+}$  with EPS (A and B) at pH 4, 6, 7 and 8.

ues showed that the PEC of EPS A is higher than the PEC of EPS B (Table 2) and the number of binding sites of EPS A was higher than that of EPS B in the most cases (Table 3). Thus, the PEC results and the number of binding sites are in accordance.

In the case of ionic exchange equilibrium, the charge density of the metal ions will be the governing factor [28]. For the three metal ions studied, Pb(II) has the smallest radius (450 pm), followed by Cd(II) (500 pm) and Cu(II) (600 pm). Our results showed that the metal order at pH 6 was  $\text{Pb} > \text{Cu} > \text{Cd}$  and at pH 7 and 8 was  $\text{Cu} > \text{Pb} \gg \text{Cd}$ . Therefore, ionic attraction and exchange do not fully explain the biosorption ability of EPS as function of pH.

According to Loaec et al. [35] who have studied the biosorption properties of a bacterial exopolysaccharide (*Alteromonas macleodii* subsp. *fijiensis*), the formation of a metal–ligand coordination bond is based on the theory of hard and soft acids (*i.e.* electron acceptor) and bases (*i.e.* electron donor). The main electron donor atoms of exopolymers are nitrogen present as amino-sugars, oxygen as hydroxyl and carboxyl and sulphate ester. According to Gadd [36], Pb has a strong preference for ligands such as O and N, while Cd ions prefer soft ligands such as sulphide. Table 1 shows that EPS are composed mainly of proteins and carbohydrates containing ligands such as O and N. Taking into consideration the composition of EPS, the order of the biosorption of the metal should be Pb and then to a lesser extent Cd, as is the case in our study (Table 3).

### 3.4.2. Implication of global electric field

According to Morlay et al. [37], the binding of cations with polyacids cannot be reduced to pure ion exchange. This interaction proved to be specific of the counterion and thus was not only of an electrostatic nature. It has also suggested that the global electric field surrounding the partially neutralized polyacid molecule has a role to play in the resulting metal–polymer complex stability. In our experiments, we did not observe any great changes in the binding constant stability with the variation in the pH (Fig. 2). However, the pH of the medium influences the binding of metal ions via the change in the degree of dissociation of the polyacid [16]. In our study, the numbers of binding sites (Table 3) obtained at pH 4 were low, particularly for Cd (the biosorption of the metal by EPS was not significant). The first apparent  $\text{pK}_a$  of EPS were at 6.6 and 5.7 so at low pH, EPS had a very low degree of dissociation. The higher the pH the greater the degree of dissociation of EPS is.

Some conformational changes in the polymer structure may also be considered [16]. For example, we can assume that at pH 4, owing to the low degree of dissociation of the polymer and consequently, to the low electrostatic repulsion forces existing in its structure, the macromolecules are in rather compact form. Thus, steric effects would probably lower the accessibility of some inner potentially complexing sites.

The inconsistency in the literature regarding the influence of pH on biosorption seems to indicate that the way in which pH

would alter the adsorption of metal ions to biomass varies with the type of adsorbent (biomass or EPS) and also the type of adsorbates (metal ions) [32].

### 3.4.3. Others possibilities

The pH of the medium also affects the solubility of metals. Fig. 4 shows that there is precipitation of Cu only at the end of the experiments at pH 8. But, micro-precipitation of metals due to the possibility of small changes in the pH in the vicinity of the adsorbent particles could be possible [38].

Finally, the formation of hydroxylated complexes of the metal would also compete with the active sites and as a consequence, the retention would decrease again [28]. In our study, the presence of hydroxylated complexes did not decrease the biosorption capacities of EPS. The kinetics of EPS biosorption could explain this result. The fixation of metallic cations would be more effective than the change in the metal speciation form whatever the pH.

## 4. Conclusion

The EPS A and B extracted from activated sludge had two apparent  $pK_a$ , the first attributed to carboxylic and phosphoric groups and the second attributed to amino and phenolic groups. The study of the biosorption of EPS (A and B) at a pH range 4–8 with Cu, Pb and Cd was carried out. The results show that the number of EPS binding sites increased with an increase in pH for Cu, Pb and Cd, whereas the binding constant did not indicate a variation according to the pH value for all metals. Simulations carried out with metals in ultra-pure water with MINEQL 4.5 software demonstrated the presence of different speciation forms for Cu and Pb divalent cations but also hydroxylated forms and solid forms. The polarographic titration curves indicated that for our experimental conditions (presence of EPS) precipitation of Cu at pH 8 was observed only at the end of the experiment. The speciation state of the metals studied was influenced by the EPS which are complex solutions.

The metal affinity order for EPS has changed with the pH. So to explain the influence of the pH on the metal biosorption, different theories have been developed in the literature. We have summarized that the influence of pH varies depending on:

- (a) the nature of EPS (implicated in the degree of dissociation of EPS, in the conformational changes in the polymer),
- (b) the nature of the metal studied (implicated in metal ionic attraction mechanisms, in metallic speciation with the presence of hydroxylated forms and possible micro-precipitation of the metal in the vicinity of the EPS) or,
- (c) on both the nature of EPS and the nature of the metal (implicated in the protonic exchange mechanisms, in the global electric field concerning the stability of the metal–EPS complex, in the theory of hard and soft acids and bases and in the kinetics of the reaction between metal and EPS).

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## References

- [1] P.S. Lawson, R.M. Sterritt, J.N. Lester, Factors affecting the removal of metals during activated sludge wastewater treatment. II: The role of mixed liquor biomass, Arch. Environ. Contam. Toxicol. 13 (1984) 391–402.
- [2] J. Brown, J.N. Lester, Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge-I, Water Res. 16 (1982) 1539–1548.
- [3] M.J. Brown, J.N. Lester, Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge-II, Water Res. 16 (1982) 1549–1560.
- [4] G. Guibaud, N. Tixier, A. Bouju, M. Baudu, Relation between extracellular polymers composition and its ability to complex Cd, Cu and Pb, Chemosphere 50 (2003) 1701–1710.
- [5] Y. Liu, M.C. Lam, H.H.P. Fang, Adsorption of heavy metals by EPS of activated sludge, Water Sci. Technol. 43 (2001) 59–66.
- [6] J. Wingender, T.R. Neu, H.C. Flemming, Microbial extracellular polymeric substances: characterisation, structure and function, Springer, Berlin, 1999, 123 pp.
- [7] Y. Liu, H.P. Fang, Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge, Crit. Rev. Env. Sci. Technol. 33 (2003) 237–273.
- [8] S.C. Andrews, A.K. Robinson, F. Rodriguez-Quinones, Bacterial iron homeostasis, FEMS Microbiol. Rev. 27 (2003) 215–237.
- [9] S.F. Aquino, D.C. Stuckey, Soluble microbial products formation in anaerobic chemostats in the presence of toxic compounds, Water Res. 38 (2004) 255–266.
- [10] D.T. Sponza, Investigation of extracellular polymer substances (EPS) and physicochemical properties of different activated sludge flocs under steady-state conditions, Enzyme Microb. Technol. 32 (2003) 375–385.
- [11] H. Liu, H.H.P. Fang, Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data, Biotechnol. Bioeng. 80 (2002) 806–811.
- [12] M. Ledin, Accumulation of metals by microorganisms—processes and importance for soil systems, Earth-Sci. Rev. 51 (2000) 1–31.
- [13] M. Esparza-Soto, P. Westerhoff, Biosorption of humic and fulvic acids to live activated sludge biomass, Water Res. 37 (2003) 2301–2310.
- [14] T. Lebeau, D. Bagot, K. Jézéquel, B. Fabre, Utilization of an exopolysaccharide produced by *Chryseomonas luteola* TEM05 in alginate beads for adsorption of cadmium and cobalt ions, Sci. Total Environ. 291 (2002) 73–83.
- [15] C. Morlay, M. Cromer, Y. Mougnot, O. Vittori, Potentiometric study of Cd(II) and Pb(II) complexation with two high molecular weight poly(acrylic acids); comparison with Cu(II) and Ni(II), Talanta 48 (1999) 1159–1166.
- [16] C. Morlay, M. Cromer, O. Vittori, The removal of Cu(II) and nickel(II) from dilute aqueous solution by a synthetic flocculant: a polarographic study of the complexation with a high molecular weight poly(acrylic acid) for different pH values, Water Res. 34 (2000) 455–462.
- [17] I. Savvaidis, M. Hugues, R. Poole, Differential pulse polarography: a method for the direct study of biosorption of metal ions by live bacteria from mixed metal solutions, Anton. Leeuw. Int. J. G. 84 (2003) 99–107.
- [18] I. Ruzic, Trace metal complexation at heterogeneous binding sites in aquatic systems, Mar. Chem. 53 (1996) 1–15.
- [19] G. Guibaud, S. Comte, F. Bordas, S. Dupuy, M. Baudu, Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel, Chemosphere 59 (2005) 629–638.
- [20] G. Guibaud, S. Comte, F. Bordas, M. Baudu, Metal removal from single and multimetallic equimolar systems by extracellular polymers extracted from activated sludges as evaluated by SMDE polarography, Process Biochem. 40 (2005) 661–668.



- [21] A.M.L. Kraepiel, K. Keller, F.M.M. Morel, On the acid–base chemistry of permanently charged minerals, *Environ. Sci. Technol.* 32 (1998) 2829–2838.
- [22] W. Stumm, *Chemistry of Solid–Water Interface*, Wiley, New York, 1992.
- [23] R. Kummert, W. Stumm, The surface complexation of organic acids on hydrous  $\alpha$ - $\text{Al}_2\text{O}_3$ , *J. Colloid Interface Sci.* 75 (1980) 1170–1182.
- [24] L. Sigg, W. Stumm, The interaction of anion and weak acids with the hydrous goethite ( $\alpha$ -FeOOH) surface, *Colloids Surf.* 2 (1981) 101–107.
- [25] Nowack, B., 1996. Behaviour of EDTA in groundwater—a study of the surface reactions of metal-EDTA complexes, Dissertation ETH Zurich, No. 11392.
- [26] G. Guibaud, N. Tixier, A. Bouju, M. Baudu, Use of a polarographic method to determine copper, nickel and zinc constants of complexation by extracellular polymers extracted from activated sludge, *Process Biochem.* 39 (2004) 833–839.
- [27] J. Wang, C.P. Huang, H.E. Allen, Modeling heavy metal uptake by sludge particulates in the presence of dissolved organic matter, *Water Res.* 37 (2003) 4835–4842.
- [28] R. Pardo, M. Herguedas, E. Barrado, M. Vega, Biosorption of cadmium, copper, lead and zinc by inactive biomass of *Pseudomonas putida*, *Anal. Bioanal. Chem.* 376 (2003) 26–32.
- [29] S. Allen, P. Brown, Isotherm analyses for single component and multi-component metal sorption onto lignite, *J. Chem. Technol. Biotechnol.* 62 (1995) 17–24.
- [30] M. Van den Hoop, F. Leus, H. Van Leeuwen, Stripping voltammetry of zinc/polyacrylate complexes: influence of molar mass, *Collect. Czech. Chem. C* 56 (1991) 96–103.
- [31] S. Singh, S. Pradhan, L.C. Rai, Metal removal from single and multimetallic systems by different biosorbent materials as evaluated by differential pulse anodic stripping voltammetry, *Process Biochem.* 36 (2000) 175–182.
- [32] G. Ozdemir, T. Ozturk, N. Ceyhan, R. Isler, T. Cosar, Heavy metal biosorption by biomass of *Ochrobactrum anthropi* producing exopolysaccharide in activated sludge, *Bioresource Technol.* 90 (2003) 71–74.
- [33] T. Rudd, R.M. Sterritt, J.N. Lester, Complexation of heavy metals by extracellular polymers in the activated sludge process, *J. WPCF* 56 (1984) 1260–1268.
- [34] A. Lopez, N. Lazaro, J.M. Priego, A.M. Marques, Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39, *J. Ind. Microbiol. Biot.* 24 (2000) 146–151.
- [35] M. Loaec, R. Olier, J. Guezennec, Uptake of lead, cadmium and zinc by a novel bacterial exopolysaccharide, *Water Res.* 31 (1997) 1171–1179.
- [36] G.M. Gadd, Metals and microorganisms: a problem of definition, *FEMS Microbiol. Lett.* 100 (1992) 197–204.
- [37] C. Morlay, M. Cromer, Y. Mougnot, O. Vittori, Potentiometric study of Cu(II) and Ni(II) complexation with two high molecular weight poly(acrylic acids), *Talanta* 45 (1998) 1177–1188.
- [38] R.-S. Juang, H.-J. Shao, Effect of pH on competitive adsorption of Cu(II), Ni(II) and Zn(II) from water onto chitosan beads, *Adsorption* 8 (2002) 71–78.