Mechanism of adsorption of ferric iron by extracellular polymeric substances (EPS) from a bacterium

*Acidiphilium* sp.

J. M. Tapia, J. A. Muñoz, F. González, M. L. Blázquez and A. Ballester

**ABSTRACT**

The aim of this study was to assess the sorption of Fe(III) by extracellular polymeric substances (EPS) of the *Acidiphilium* 3.2Sup(5) bacterium, which has promising properties for use in microbial fuel cells (MFC). The EPS of *A.* 3.2Sup(5) was extracted using EDTA. The sorption isotherms were determined using aliquots of purified EPS. The exosubstances loaded with metal were characterized by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), X-ray diffraction spectroscopy (XRD) and Fourier transform infrared spectroscopy (FTIR). The sorption uptake approaches to $536.1 \pm 26.6 \text{ mg Fe(III)} (\text{g EPS})^{-1}$ at an initial ferric concentration of $2.0 \text{ g l}^{-1}$. The sorption of Fe(III) by EPS can be fitted to the Freundlich model. The sorption process produces hydrated iron (III) oxalate [$\text{Fe(OH)(C}_2\text{O}_4) \times 2\text{H}_2\text{O}$] by a reversible reaction ($\log K = 1.06 \pm 0.16$), indicating that a shift in the sorption of the cation can be easily achieved. Know the magnitude and form of iron sorption by EPS in MFC can foresee the potential impact on the metabolism of iron-reducing and iron-oxidizing bacteria and, therefore, on the feasibility of the system.

**Key words** | *Acidiphilium*, EPS, ferric iron, iron oxalate, MFC, sorption

**INTRODUCTION**

Biomass is a very attractive and sustainable energy source because of its availability and cost, especially organic wastes can be of great interest in microbial fuel cells (MFC) (Wall *et al.* 2008). Different bacteria of genus *Shewanella* and *Geobacter* are a promising alternative for energy production since they can convert directly chemical energy from organic substances into electric energy using metabolic pathways (Dewan *et al.* 2010).

A characteristic of these systems is that microorganisms involved are strictly anaerobes. This is a serious operational constrain that could condition the feasibility and applicability of fuel cells. However, some microorganisms can perform similar functions but in aerobic environments. Preliminary studies have shown that certain bacteria of genus *Acidiphilium*, isolated from the ecosystem of Rio Tinto (Huelva, Spain), were able to degrade aerobically organic compounds type $(\text{CH}_2\text{O})_n$ into $\text{CO}_2$ and $\text{H}_2\text{O}$, coupled to the reduction of Fe(III) (Malki *et al.* 2006). In fact, that ecosystem contains oxygen-tolerant bacteria *Acidithiobacillus ferroxidans* able to oxidize Fe(II) to Fe(III).

It has been reported that the acidophilic bacterium *Acidiphilium* 3.2Sup(5) is able to transfer electrons, in aerobic conditions, directly to carbon supports (up to $3 \text{ Am}^{-2}$ at a potential of $+0.15 \text{ V SCE}$). Bacteria can perform this in the absence of redox mediators, in saturated air and at very low pH (Malki *et al.* 2008). Thus, implementation of fuel cell could be feasible using both microorganisms and, in addition, would be ecological since combine the generation of power energy with the degradation of residual organic compounds within the iron cycle.

The extracellular polymeric substances (EPS) are metabolic products of biological origin that consist essentially of high molecular weight substances, such as carbohydrates and proteins and are mainly produced by secretion or by cellular lysis (Quintelas *et al.* 2008).

It is expected that the interaction of EPS, through active functional groups, with iron in solution will produce some metal (bio)sorption. For instance, it has been reported that, during the bioleaching of pyrite, EPS of *A. ferroxidans* can retain Fe(III) inside its structure by complexation with exosubstances (Sand & Gehrke 2006). In this way, iron
removal from solution would affect both energy source availability for bacterial growth of A. ferrooxidans and fuel cell viability. Therefore, the interaction of EPS generated by bacteria with Fe in solution needs to be studied.

The EPS-Fe interaction study was carried out with extracellular material of pure cultures of Acidiphilium 3.2Sup(5). It shows 99% genetic similarities with the species A. cryptum, A. multivorans and A. organovorum (Malki et al. 2008). The choice of these EPS was related to the fact that the heterotrophic bacteria A. 3.2Sup(5) has a much higher metabolic activity than the autotrophic bacteria A. ferrooxidans. Thus, the mixed culture was mainly formed by cells of the former bacteria (at least 90%) and EPS released into solution and interacting with Fe, in a bacterial culture formed by both types of microorganisms, would be generated mainly by bacteria A. 3.2Sup(5). Since the culture medium of these bacteria lacks iron, the exosubstances are free of this element and constitute a better approach to the study of the interaction with that metal.

The study was focused on the Fe(III) sorption uptake by EPS of A. 3.2Sup(5) through the determination of sorption isotherms. Sorption was corroborated by X-ray microanalysis EDS and complemented with the morphological characterization of EPS by scanning electron microscopy (SEM). A proposal mechanism is also given to count for the interaction between exosubstances and metal, which was derived from the X-ray diffraction analysis (XRD) of such substances.

**MATERIALS AND METHODS**

**Bacterial growth**

The growth of bacterial cultures of A. 3.2Sup(5) was accomplished aerobically in a liquid medium at pH 2.5 and 30 ± 1 °C and stirred at 150 rpm. This strain was provided by Dr. R. Amils of the Molecular Biology Centre (CBM-CSIC, Spain) and its origin was an ecosystem of Rio Tinto (Huelva, Spain). The nutrient medium used was similar to that employed during the isolation and characterization of the strain (per litre): 2.0 g (NH₄)₂SO₄, 0.1 g KCl, 0.25 g MgSO₄ × 7H₂O, 0.25 g K₂HPO₄, 0.01 g Ca(NO₃)₂ × 4H₂O, 0.1 g of yeast extract and 1.0 g of glucose (Tapia et al. 2009). All chemical reagents used were of analytical grade (PA). The cell growth was performed under aseptic conditions to obtain homogeneous bacterial cultures regarding its phenotypic characteristics.

**EPS extraction**

The EPS extraction method used was developed and validated for these bacteria (Tapia et al. 2009). The following procedure was used: 40 ml of pure strain was mixed (without stirring) with a solution of 2% (w/v) EDTA disodium salt (Panreac, PA), considering a ratio of 3.2 g of EDTA per gram of cell dry weight. After 3 h of contact at 4 °C, the solution was centrifuged at 14,000 rpm (20,817 g) for 20 min in an Eppendorf centrifuge model 5804 R V.6. Then, bacterial cells were separated from the supernatant through a 0.22 μm millipore filter. The filtering, containing the raw EPS, was then dialyzed in order to remove metabolites and salts of low molecular weight using a Pierce Slide-A-Lyzer membrane of 3500 MWCO in 1.0 L of deionized water, for 24 h and at 4 °C. The EPS solution was finally stored at 4 °C for chemical analysis.

**EPS-Fe(III) interaction**

**Sorption kinetics.** Sorption tests were performed at different initial metal concentrations (50, 100 and 200 mg l⁻¹) and at different contact times (30, 60 and 180 min). EPS aliquots ranged between 5 and 20 ml. Fe(III) was added from a stock solution of hydrated ferric sulphate, Fe₂(SO₄)₃ × xH₂O (99.9%, Panreac), at 4.0 g l⁻¹ and pH 2.0 adjusted with H₂SO₄ (50% v/v). Then, each solution was dialyzed in order to purify the EPS with the Fe(III) adsorbed. For that, a membrane (Slide-a-Lyzer, Pierce) of 3500 MWCO was used in a volume of deionized water 50 times greater than the total volume of the mixture of exosubstances and iron solution. Dialysis, performed under stirring at 180 rpm and 4 °C for 24 h, led to a purified [EPS + Fe(III)] mixture.

**Sorption isotherms.** Sorption isotherms were obtained from five tests using aliquots of EPS between 5 and 20 ml and the corresponding volume of stock ferric sulphate solution to produce an initial metal concentration of 50, 100, 200, 1,000 and 2,000 mg l⁻¹ Fe(III). The contact time necessary to reach equilibrium was 60 min. Then, the mixture of EPS plus Fe(III) was purified as in the kinetic study.

The results of the sorption tests were fitted to the linear Freundlich model. This model is given by the expression: $q_e = K C_e^{1/n}$; where $q_e$ is the specific amount of metal adsorbed (mg metal g⁻¹ of EPS), $C_e$ is the equilibrium metal concentration (mg l⁻¹) and $K$ and $n$ are Freundlich constants that represent the sorption uptake and performance of the biosorbent, respectively. In all cases, iron was measured in duplicate in both the EPS loaded with metal
and the dialyzation water, by atomic absorption spectroscopy (AAS) using a Perkin-Elmer spectrometer model 1100B. The mass balance for iron showed a good degree of reliability, with an average standard deviation of ±5.3%.

Scanning electron microscopy (SEM) and energy dispersive X-ray microanalysis (EDS)

Each sample removed from solution (or EPS as appropriate) was coated with a conductive layer of gold and then analyzed using a scanning electron microscope (JEOL JSM-6330 F) at an accelerating voltage of 20 kV, coupled with an energy dispersive X-ray microanalysis (SEM-EDS).

Fourier transform infrared spectroscopy (FTIR)

Two milligrams of freeze-dried EPS (24 h at −56 °C, Labconco SciLab) solution was mixed with 300 mg of KBr and compacted to form a pellet. The IR spectra were then determined using a spectrum Nicolet Magna 750, at 4 cm⁻¹ of resolution.

Titration of EPS

5.0 ml of A. 3.2Sup(5) EPS solution purified by dialysis were titrated with 0.1 M NaOH solution and acidity was recorded.

X-Ray diffraction spectroscopy

Before analysis, EPS were ground and homogenized in an agate mortar. In both cases, a Philips X’pert-MPD diffractometer with copper anode was used. Measurements were recorded at a wavelength of 1.5406 Å (CuKα) and the scan lasted for 1.0 h between 5° and 85°.

RESULTS

Kinetics of bacterial growth

The bacterial population of A. 3.2Sup(5) at the stage of maximum growth (1.14 × 10⁹ cells ml⁻¹), was approximately 10 times higher than for A. ferrooxidans (1.03 × 10⁸ cells ml⁻¹). Therefore, the amount of biomass produced by the former bacteria (0.244 g l⁻¹) was substantially greater than A. ferrooxidans (0.035 g l⁻¹). This suggests that the EPS generated in the mixed culture of A. 3.2Sup(5) and A. ferrooxidans could consist mostly of EPS of the former bacteria.

Sorption of Fe(III) by EPS

Sorption kinetics of Fe(III) by EPS depends on the amount of iron available in the system. At 60 min, metal sorption increased with initial Fe(III) concentration. Furthermore, the amount of Fe(III) adsorbed, at each concentration, reached a constant value for times longer than 60 min, as correspond to the equilibrium between EPS and iron in solution. This equilibrium time was used in tests to determine the Fe(III) sorption isotherms.

Sorption isotherms of Fe(III) are shown in Figure 1(a). An increase of metal concentration improved the iron uptake by the polymeric substances of A. 3.2Sup(5) cells. This trend would be an indication that EPS were unable to reach saturation of their functional groups in the
concentration range studied and that iron uptake by these exopolymers is the result of complex physico-chemical reactions (Zhang et al. 2006).

This performance seems to indicate that the exosubstances adsorbed Fe(III) according to Freundlich model. The fitting of experimental data to the linear expression of such model (Figure 1(b)) was corroborated by the good regression coefficient \( R^2 = 0.99 \). In addition, the sorption parameters obtained \( K = 2.05 \text{ mg}^{-1/1} \text{L}^{1/1} \text{g}^{-1} \) and \( n = 1.13 \) were relatively high compared to those given in the literature (Zhang et al. 2006; Kiran & Kaushik 2008). This is an indicative of the high iron uptake capacity of these substances. Similar results were obtained for the sorption of ferric ion with Chlorella vulgaris (Aksu et al. 1997) and sorption of Fe(III) by EPS of Bacillus licheniformis (McLean et al. 1992).

Characterization of active centres in the biosorption of Fe(III)

The functional groups involved in metal uptake were identified by transformed Fourier infrared spectroscopy (FTIR) of EPS, with and without Fe(III).

Both spectra are shown in Figure 2. In spite that some bands are coincident, some are specific and can be attributed to the interaction of functional groups of EPS with iron (Figure 2(b)). For instance, the bands in the spectrum of pure EPS, at 1,728 and 1,101 cm\(^{-1}\), are shifted to the right when iron is present (at 1,716 and 1,073 cm\(^{-1}\), respectively). These bands correspond to the asymmetric stretching vibration of C=O bond in the carboxylic group (Schmitt & Flemming 1998) and to the stretching vibration of the hydroxyl group (–O–H) (Omoike & Chorover 2004). This would be an indication of the preferential participation of these groups in metal uptake, as previously reported (Ueshima et al. 2008).

Usually, the carboxylic group (–COOH), or its derivate carboxylate (–COO\(^{-}\)), has two peaks in the IR spectrum: one in the range 1600–1800 cm\(^{-1}\), corresponding to the C=O bond vibration and another around 1400 cm\(^{-1}\), related to the deformation vibration of C–O bond in the carboxylate group. In this case, the peak intensity of the carboxyl group is higher in the spectrum of the pure EPS (Figure 2(a)) than for EPS with iron (Figure 2(b)). This suggests a preferential interaction between carboxyl groups and iron in solution. This interaction has also been reported in literature. Quintelas reported the preferential interaction of the carboxyl group of Escherichia coli with Fe(III) (Quintelas et al. 2009).

Acid-alkali titration of EPS

From titration of A. 3.2 Sup(5) EPS was obtained that pK\(_{a}\) for these EPS was approximate 3.0. This value is usually assigned in the literature to the carboxylic group (Volesky 2003). For instance, Ueshima determined a pK\(_{a}\) for the carboxylic group around 3.2 by titration of EPS obtained by enzymatic extraction of Pseudomonas putida strains (Ueshima et al. 2008), while that Ginn and Fein found a pK\(_{a}\) of 3.1 for the carboxylic group in a sorption study of Cd and Pb with Acidiphilium angustum, bacteria of the same genus that A. 3.2Sup(5) (Ginn & Fein 2008). Then, EPS of A. 3.2Sup(5) mostly consist of the carboxyl group.

Figure 2 | FT-IR spectra of EPS: (a) pure and (b) with Fe(III).
which would be mainly responsible for the interaction with the ferric ion.

**Characterization of EPS loaded with Fe(III)**

Loaded EPS were obtained from tests at an initial ferric ion concentration of 1000 mg l\(^{-1}\) and after 60 min of contact. Then, known volumes of EPS loaded with ferric ion were lyophilized for 48 h at \(56^\circ\text{C}\) and at \(1.2 \times 10^{-6}\) torr. The surface characterization of the lyophilized material involved scanning electron microscopy (SEM) complemented with energy dispersive X-ray microanalysis (EDS) (Figure 3). The exopolymeric substances with a gel-type texture can be seen in Figure 3(a). The energy spectrum confirmed that the exosubstances adsorbed Fe internally up to 13% of the total weight (Figure 3(b)).

**Sorption mechanism of Fe(III) by EPS**

A key aspect of the EPS-Fe(III) interaction is the predominant chemical species of cation in solution during sorption. For the working conditions used, pH between 2.0 and 2.3 and 1.0 g l\(^{-1}\) of Fe(III), the cation, mostly found in hydrolyzed state as Fe(OH)\(^{2+}\), would interact with the carboxyl group (Casas et al. 2005).

In addition, the solution acidity increased after contact between EPS and Fe(III). This would be an indication that protons from biomass were released into solution, probably through deprotonation of carboxylic groups. Moreover, the increase of acidity can provide information on the amount of Fe(III) adsorbed by EPS and on the reaction stoichiometry between the active group and the metal cation. In this case the amount of protons generated and ferric ions consumed molar ratio during interaction was practically 2.0.

A possible source of protons release into solution from biomass could be the dissociation of carboxylic groups, RCOOH (where R is an exopolysaccharide chain). In such case, the carboxylate anion, RCOO\(^-\), would be responsible for the interaction with the ferric ion. That dissociation is very feasible since the carboxyl group tends to release its proton generating the carboxylate anion, more stable, which could interact with Fe(III) through mono, bidentate or bridge-type bonds (Nakamoto 1997).

The interaction mode with the cation can be deduced from the difference between experimental values, in the infrared spectrum of EPS with Fe(III), of the bands corresponding to the stretching vibration of (C = O) and (C–O) bond in the carboxyl group. It has been found that the distance between both bands is related with the relative symmetry of the mentioned group and gives information on the nature of the coordination bond (Atwood & Steed 2004). In this case, those bands (Figure 2), appear at values of 1,716 cm\(^{-1}\) (symmetric stretch of C = O bond in the carboxyl group) and at 1,401 cm\(^{-1}\) (asymmetric stretch of C–O bond in the carboxylate group), respectively. The difference between both values, 315 cm\(^{-1}\), would be typical for the formation of bidentate chelates by this functional group (Fukas et al. 2006).

Hence, a possible mechanism of interaction between EPS of A. 3.2 Sup 5 and Fe(III) ions could be the formation of a bidentate chelate after reaction of two moles of carboxylate groups, COO\(^-\), resulting from deprotonation of carboxylic groups of the biomass, RCOOH, with one mol of ferric ion partially hydrolyzed, Fe(OH)\(^{2+}\), according to reaction (1):

\[
2 \text{RCOOH} + \text{Fe(OH)}^{2+} \rightleftharpoons \text{RCOO}^{-}\text{Fe(OH)} + 2 \text{H}^+ \quad (1)
\]

The equilibrium constant for this reaction is given by the expression:

\[
K = \frac{[\text{H}^+]^2}{[\text{Fe}^{3+}]} \quad (2)
\]
where $[H^+]$ and $[Fe^{3+}]$ represent concentrations of protons release and ferric iron involved in reaction, respectively. The calculated values of $\log K$ are shown in Table 1.

The average value of the equilibrium constant, $\log K = 1.06 \pm 0.16$, suggests that reaction (1) is reversible. In such case, the sorption of Fe(III) by EPS of A. 3.2$\text{Sup}(5)$ would not be irreversible and ferric could be recovered from those substances if needed.

According to reaction (1), the interaction mechanism assumes that each iron atom is associated to two carboxylate groups, COO$^-$, which combined forming an oxalate group, $\text{C}_2\text{O}_4^{2-}$, therefore it is likely the presence of iron oxalates in the EPS loaded with Fe(III) as shown by the XRD analysis of these substances (Figure 3(c)).

One of the compounds detected in the XRD spectrum was an hydroxide-hydrated Fe(III) oxalate, $[\text{Fe(OH)}(\text{C}_2\text{O}_4)\times2\text{H}_2\text{O}]$. Its presence would confirm the mechanism previously mentioned since its stoichiometry agrees with reaction (1); but otherwise this substance could be formed during the dehydration process associated to lyophilization of EPS solutions containing Fe(III). In addition, the spectrum shows the presence of iron-free organic compounds, such as uricite, $[\text{C}_4\text{(NH}_3\text{)}_2\text{O}_2(\text{NH}_2\text{O})]$, formed from proteins contained in EPS. Finally, different iron compounds were also detected: ferric sulphate, $[\text{Fe}_2(\text{SO}_4)_3\times\text{xH}_2\text{O}]$, and sabieite, $[\text{NH}_4\text{Fe(SO}_4)_2]$, formed during lyophilization process.

The formation of iron chelates has also been reported in literature. For instance, Corzo found that EPS, especially of *Bradyrhizobium Chamaecytisus* and to a lesser extent of *Bradyrhizobium japonicum*, were able to uptake Fe$^{3+}$ by bidentate chelation of the complex Fe(OH)$^{2+}$ (Corzo et al. 1994). Similarly, the carboxyl group of *Sargassum fluitans* biomass was able to uptake Fe$^{3+}$ by bidentate chelation (Figueira et al. 1999). Likewise Morillo suggested that, apparently, the ferric cations could interact with two carboxylate groups of the polysaccharide, producing a mesh of polymer large enough to precipitate (Morillo et al. 2008). Finally, Magyarosi reported the removal of nickel from a contaminated soil through the formation of a nickel oxalate dehydrate (Magyarosy et al. 2002).

### Table 1 | Equilibrium constant of the EPS-Fe(III)

<table>
<thead>
<tr>
<th>Initial concentration Fe (III) (mg l$^{-1}$)</th>
<th>Log $K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.88</td>
</tr>
<tr>
<td>1,000</td>
<td>1.12</td>
</tr>
<tr>
<td>2,000</td>
<td>1.19</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The sorption of Fe(III) by A. 3.2$\text{Sup}(5)$ EPS can be fitted to the Freundlich model. The sorption uptake approaches to $536.1 \pm 26.6$ mg Fe (g EPS)$^{-1}$ at an initial ferric concentration of 2.0 g l$^{-1}$. The sorption by exosubstances took place presumably by preferential interaction of ferric ion, partially hydrolyzed as Fe(OH)$^{2+}$, with the carboxyl functional group, RCOOH. This interaction led to the formation of hydrated iron oxalate, Fe(OH)(C$_2$O$_4$)$\times2$ (H$_2$O), by a reversible reaction ($\log K = 1.06 \pm 0.16$).

ACKNOWLEDGEMENTS

J. M. Tapia wants to thank the Universidad Arturo Prat (Iquique, Chile) for funding the grant that made this work possible.

REFERENCES

Aksu, Z., Acikel, U. & Kutsal, T. 1997 Application of multicomponent adsorption isotherms to simultaneous biosorption of iron(III) and chromium(VI) on *C. vulgaris*. *Journal of Chemical Technology and Biotechnology* 70 (24), 368–378.


Casas, J. M., Crisóstomo, G. & Cifuentes, L. 2005 Speciation of the Fe(II)–Fe(III)–$\text{H}_2\text{SO}_4–\text{H}_2\text{O}$ system at 25 and 50 °C. *Hydrometallurgy* 80, 254–264.

Corzo, J., León-Barrios, M., Hernando-Rico, V. & Gutiérrez-Navarro, A. M. 1994 Precipitation of metallic cations by the acidic exopolysaccharides from *Bradyrhizobium japonicum* and *Bradyrhizobium (Chamaecytisus)* strain BGA-1. *Applied and Environmental Microbiology* 60 (12), 4531–4536.


McLean, R. J., Beauchemin, D. & Beveridge, T. J. 1992 Influence of oxidation state on iron binding by Bacillus licheniformis capsule. Applied and Environmental Microbiology 58 (1), 405–408.


Quintelas, C., Rocha, Z., Silva, B., Fonseca, B., Figueiredo, H. & Tavares, T. 2009 Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an E. coli biofilm supported on kaolin. Chemical Engineering Journal 149, 519–324.


Volesky, B. 2003 Sorption and Biosorption. BV-Sorbex Inc., St. Lambert (Quebec), Canada.


First received 3 December 2010; accepted in revised form 8 April 2011