Reactions between bacterial exopolymers and goethite: A combined macroscopic and spectroscopic investigation

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**ABSTRACT**

The adsorption to goethite of extracellular polymeric substances (EPS) isolated from Pseudomonas putida was investigated using batch adsorption experiments, electrophoretic mobility (EM) measurements, Fourier transform infrared (FTIR) spectroscopy, isothermal titration calorimetry (ITC) and X-ray absorption fine structure (XAFS) spectroscopy. The adsorption of EPS decreased the point of zero charge of goethite from 7.6 to 3.2, suggesting the formation of negatively charged inner-sphere surface complexes. The adsorption isotherms of EPS on goethite conformed to the Langmuir equation. The adsorption energy constant (\(K\)) of EPS on goethite was in the sequence of EPS phosphate-containing moieties > nitrogen-containing moieties > carbon-containing moieties, indicating those containing phosphate were the most strongly adsorbed. FTIR showed ligand exchange of phosphate groups of EPS with surface hydroxyls on goethite. Extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES) spectroscopy results further demonstrated phosphate groups of EPS can form monodentate inner-sphere complexes at lower pH 3.0, while form bidentate inner-sphere complexes at higher pH 9.0. The oxidation state of iron in goethite was not changed after the reaction with EPS at different pH values. The information obtained in this study is of fundamental significance for the understanding of the interaction mechanisms between bacteria and minerals in soil and aquatic environments.

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1. Introduction

Growth of a wide variety of Gram-negative and Gram-positive bacteria is accompanied by the production of extracellular polymeric substances (EPS). The EPS are bound to the cell surface (“capsular”), released into solution (“free”) or associated with the hydrated matrix of biofilms (Wingender et al., 1999). In soil or aquatic environments, the interactions of EPS with inorganic colloids have significant effects on bacteria adhesion and transport, biofilm formation, mineral dissolution, biomineralization and heavy metals accumulation (Banfield et al., 1999; Chan et al., 2004; Herzberg et al., 2009; Kim et al., 2009; Fang et al., 2010; Ying et al., 2010).

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EPS are a heterogeneous mixture composed dominantly of polysaccharides and proteins, with nucleic acids and lipids as minor constituents. EPS contain various weakly acidic functionalities (carboxyl, phosphoryl, amide, amino, hydroxyl) that ionize in response to changes in environmental pH or ionic strength (Wingender et al., 1999; Cao et al., 2011a). The mechanisms of bacterial EPS adsorption on mineral surfaces including hydrophobic, electrostatic, covalent, and polymer–polymer interactions have been studied by several soil and environmental chemists over the past years (Tsuneda et al., 2003; Parikh et al., 2004). For example, adsorption of EPS from Bacillus subtilis on goethite increased with the decrease of pH from 9.0 to 3.0 and NaCl concentrations from 100 to 1 mM, suggesting electrostatic interactions contribute to EPS adsorption (Omoike and Chorover, 2006). The atomic force microscope (AFM) and electronic structure calculations suggested the phosphate-bearing polymers are the major components in EPS responsible for the adhesion on silica surfaces at low pH, and H-bonds and electrostatic interactions as the dominant forces (Kwon et al., 2006). Quartz crystal microbalance with dissipation (QCM-D) revealed that deposition efficiencies of EPS from four bacterial strains on bare silica surfaces increased with increasing ionic strength in both monovalent and divalent solutions (Zhu et al., 2009). Macromolecules may bind to the mineral surface by inner-sphere (directly bonded to the surface) or outer-sphere (qua-ion surrounded by water molecules and thus held to the surface of the sorbent by electrostatic attraction) complexation (Sparks, 1995). Using attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy, Omoike et al. (2004) found that EPS from Pseudomonas aeruginosa and B. subtilis bind to Fe centers (FeⅢ is in the center of the octahedron) on goethite via inner-sphere complexation of phosphate-containing macromolecules. Quantum chemical calculations results further revealed that the reaction is monodentate rather than bidentate complexation at pH 6.0 and 10 mM NaCl. However, no direct experimental data were used to support their assumptions on complexation forms.

Although some attempts have been made to elucidate the mechanism of EPS adsorption on silica and Fe-oxo(hydr)-oxides, the interactions between minerals and EPS remain poorly resolved (Omoike and Chorover, 2006; Kwon et al., 2006; Zhu et al., 2009). The information on EPS-mineral complexes at the molecular level is lacking, particularly in terms of directly measured data. X-ray absorption fine structure (XAFS) spectroscopy is one of the most powerful methods to study the local structure and bonding of metal complexes/compounds (Karlsson and Persson, 2010). Recent research on the binding of Fe(III) to organic soils or humic substances studied by XAFS indicated that carboxylic groups are the dominant functional groups involved in the complex of Fe(III) (Gustafsson et al., 2007; Karlsson et al., 2008; Karlsson and Persson, 2010). The extended X-ray absorption fine structure (EXAFS) measurements on the reactions between iron and fulvic acid showed the longer Fe–O distance (2.10 A) of fulvic acid–Fe(III) complex at low pH, which suggests that fulvic acid promoted the reduction of Fe(III) to Fe(II) and the process was enhanced with the decrease of pH (van Schaik et al., 2008). To our knowledge, no XAFS studies have been performed on iron in biomolecules complexes with oxide minerals. In addition, very few studies have been conducted on the thermodynamics of EPS adsorption on minerals in terms of direct calorimetric measurements (Perry et al., 2005). Isothermal titration calorimetry (ITC) is a highly accurate and automated technique that directly measures the enthalpy change (ΔH) associated with reactions in solution or colloidal systems (Jódar-Reyes et al., 2001; Blasie and Berg, 2003; Cai et al., 2006; Rong et al., 2010). In the present work, we investigated the adsorption of EPS isolated from Pseudomonas putida on goethite in a novel combination of these macroscopic and molecular-scale methods (FTIR, ITC and XAFS). The study aimed to better understand the nature of interactions between EPS and environmental media.

2. Material and methods

2.1. Bacterial EPS isolation and purification

P. putida was isolated from the waste soil adjacent to Academy of Hubei Agricultural Sciences, Wuhan, China (Fu et al., 2008). P. putida was cultivated aerobically in Luria broth at 28 °C to early stationary growth phase (24 h). The cells were removed from the culture solution by centrifugation (5000 g, 15 min, 4 °C) and the supernatant solution was then centrifuged at higher force (12,000 g, 15 min, 4 °C) to remove residual cells. The unbound EPS was precipitated from the supernatant solution by adding cold reagent-grade ethanol to the supernatant solution at a volumetric ratio of 3:1, and the mixture was then stored at 4 °C for 18 h (Omoike and Chorover, 2006). The precipitate was separated from the ethanol suspension by centrifugation. The pellet obtained after centrifugation was dialyzed against Milli-Q water using cellulose membranes (3500 MWCO from Spectrum) to remove low molecular weight impurities including ethanol. After dialysis for 72 h against two changes of Milli-Q water per day, the EPS solution was freeze-dried and stored at 4 °C until use.

The chemical components of EPS were characterized as follows: the total organic C (TOC) and N (TN) concentrations of EPS were determined using a TOC/TN analyzer (multi N/C 3100, Analytik Jena, Germany); total content of EPS-P was digested with potassium peroxydisulfate (K2S2O8) and measured by molybdenum blue spectrophotometric method (Johnes and Heathwaite, 1992); proteins were measured by bicinchoninic acid (BCA) assay (Boisynthesis Co., Ltd, Beijing, China); polysaccharides were measured according to the Dubois method (Dubois et al., 1956) using glucose as standard; and the nucleic acid content was determined with a U-0080D photodiode array spectrophotometer (Hitachi High-Technologies, Tokyo, Japan).

2.2. Goethite (α-FeOOH) synthesis and characterization

Goethite was synthesized by neutralization of a 0.15 M Fe(NO3)3 solution in a high-density polyethylene bottle according to the method of Atkinson et al. (1967). The mineral was identified by X-ray diffraction (XRD) analysis and the results are shown in Figure S1 of the Supporting Information. The diffraction data of our sample matched well with the corresponding standard XRD data for goethite (JCPDS 00-29-0713). The specific surface area of the goethite, determined by
N$_2$ BET adsorption, was 41.26 m$^2$ g$^{-1}$. The hydrophobicity of the goethite, measured by contact angle, was 10.68$^\circ$. Field Emission Scanning Electron Microscopy (FESEM) shows that goethite is 1 µm long needle-shaped crystals (Figure S2). The details of the experimental approach are given in the Supporting Information.

2.3. Electrophoretic mobility (EM) measurement

The EM was determined using ZetaPALS analyzer (Brookhaven Instruments Corporation, Holtsville, NY). The pH of EPS and the suspension containing 1 mg mL$^{-1}$ goethite in 10 mM NaCl was adjusted to between 3.0 and 10.0 using dilute NaOH and HCl solutions. Goethite suspensions without or with 0.001, 0.01, 0.1 or 1 mg mL$^{-1}$ EPS were placed on a rotating shaker for 2 h. Control experiment was conducted with individual EPS suspension (1 mg mL$^{-1}$). The final EM of the mixed samples was measured. Three different samples were prepared for each solution pH condition and averages values of 10 runs were obtained for one sample. EM was converted to zeta potential using Smoluchowski equation (Elimelech et al., 1995).

2.4. Adsorption of EPS

Stock solution of EPS was prepared in a background solution of 10 mM NaCl (pH 5.0). Adsorption experiments were also conducted in this background solution. Forty milligram of goethite was mixed with 8 mL of EPS salt solution (pH 5.0) containing 0, 0.8, 1.6, 3.2, 4.8, 6.4, 8, 9.6, 12, 16 mg of EPS. EPS-C, -N and -P initial concentrations ranged from 0 to 773.6, 0 to 9.0 mg L$^{-1}$, respectively. The mixture was gently shaken at 25 ºC for 2 h (kinetic experiment showed that adsorption reached equilibrium in 120 min, Figure S3) and centrifuged at 20,000 g for 30 min. The amount of EPS-C, -N and -P adsorbed was calculated by the difference between the amount of EPS added and that remaining in the supernatant. Adsorption data were analyzed with the Langmuir isotherm (Elimelech et al., 1995; Rong et al., 2010; Lin et al., 2001):

$$ Q_{ads} = m q_{m} \Delta H_{ads} $$

where $Q_{ads}$ (mg) is the net heat for the interactions of EPS with goethite, $m$ (mg) represents the amount of adsorbents and $q$ (mg g$^{-1}$) is the amount of EPS adsorbed.

2.7. X-ray absorption spectra measurement

Iron K-edge X-ray absorption spectra at 7112 eV were recorded on beamline U7c at the National Synchrotron Radiation Laboratory (NSRL, China). The electron beam energy was 0.8 GeV and the mean stored current was 100 mA. The energy of X-ray was detuned by using a fixed-exit double-crystal Si (111) monochromator. Ionization chambers with N$_2$ atmosphere were used to collect the Fe K-edge spectra at room temperature. All sorption samples and solid standard compounds (FePO$_4$, H$_2$O, FeC$_6$O$_7$, 2H$_2$O) were measured by transmittance. The XAFS data analysis was performed with the NSRL-XAFS software (Zhong et al., 2001). The XAFS oscillations were isolated from the raw, averaged data by removal of the pre-edge background, approximated by a first-order polynomial. The extracted XAFS spectra, obtained via spline fitting techniques and normalized using a Victoreen function, were Fourier transformed (FT) using the k range 2.0–12.6 Å$^{-1}$ (Fan et al., 2009). The theoretical scattering phases and amplitudes used in data analysis were calculated with the scattering code FEFF 7 (Zabinsky et al., 1995) using the crystal structures of α-FeOOH (Szytula et al., 1968) and C$_2$H$_4$Fe$_2$O$_6$P$_4$ (La Force and Fendorf, 2000).

3. Results and discussion

3.1. Electrophoretic mobility

Fig. 1 shows the zeta potentials of goethite in both the presence and the absence of EPS. The point of zero charge pH (pH$_{pzc}$) of goethite was 7.6 in the absence of EPS. The pH$_{pzc}$ was derived by linear interpolation of the two points above and
below the x-axis. The pH_{pzc} of goethite was shifted from 7.6 to 6.8, 4.1 and 3.2 in the presence of 0.001, 0.01 and 0.1 mg mL\(^{-1}\) EPS, respectively. The pH_{pzc} of metal oxides is determined by protonation and deprotonation of surface hydroxyl groups (Pena et al., 2006). The formation of outer-sphere surface complexes cannot shift the pH_{pzc} because there are no specific chemical reactions between the adsorbate and the surface that could change the surface charge (Stumm, 1999). The shift of pH_{pzc} to a lower pH range is evidence of the formation of anionic negatively charged surface complexes (Stumm, 1999; Pena et al., 2006). Therefore, the results of the EM measurements imply that EPS formed negatively charged inner-sphere complexes on goethite.

### 3.2. Equilibrium adsorption of EPS on goethite

The adsorption isotherms of EPS-C, -N and -P on goethite are shown in Fig. 2. EPS-C, -N and -P adsorption on goethite increased rapidly at low EPS equilibrium concentration and reached a plateau, which suggested that EPS adsorption on goethite is a monolayer adsorption phenomenon. EPS adsorbed by goethite was fitted by the Langmuir equation

\[ X = \frac{X_m K C}{1 + K C}, \]

where \( X \) is the amount of EPS adsorbed per unit area of goethite (\( \mu g \text{ m}^{-2} \)), \( X_m \) is the maximum amount of EPS adsorbed (\( \mu g \text{ m}^{-2} \)), \( K \) is a constant related to the adsorption energy (L m\(^{-2} \)) and \( C \) stands for the concentration of EPS in the equilibrium solution (mg L\(^{-1} \)). The adsorption of EPS by goethite increased steadily with the increase of EPS concentration in solution. Based on the isotherms, the maximum amount of EPS-C, -N and -P adsorption on goethite were 683.65, 222.04 and 96.35 \( \mu g \text{ m}^{-2} \), respectively (Table 1). The greater the \( K \) value, the higher the affinity between goethite and EPS molecules. The \( K \) values for EPS elements adsorption on goethite were in the order of EPS-P (20.56) > EPS-N (1.50) > EPS-C (1.07), which indicates that the affinity of EPS-P-containing moieties on goethite is higher than that of EPS-C and -N.

Distribution coefficient (\( K_d \) values), where \( K_d = X/C \), are plotted as a function of EPS concentration in Figure S4. At lower EPS concentrations (0.1–0.6 mg mL\(^{-1} \)), a strong preference for adsorption of EPS-P relative to -N and -C was observed, consistent with the much higher Langmuir \( K \) values for EPS-P in Table 1. However, there were no significant differences among the \( K_d \) values for EPS-C, -N and -P at higher EPS concentrations (0.8–2.0 mg mL\(^{-1} \)).

### 3.3. FTIR spectral analysis

The FTIR spectra (2000–700 cm\(^{-1} \)) of EPS, goethite and EPS–goethite complex at pH 5.0 are shown in Fig. 3. The FTIR spectrum of EPS shows two high frequency bands (1792 and 1652 cm\(^{-1} \)) corresponding to overtones of OH vibrations, and two low frequency bands located at 887 and 794 cm\(^{-1} \) from Fe–OH in plane and out-of-plane bending vibrations, respectively (Ruan et al., 2001). The peak assignments of EPS are as follows: near 1653 cm\(^{-1} \) (C=O stretching in amide I group) and 1543 cm\(^{-1} \) (N–H bending and C–N stretching in amide II group); near 1240 cm\(^{-1} \) and 1128 cm\(^{-1} \) (P=O stretching of phosphate groups), 1057 cm\(^{-1} \) (symmetric stretching of O–P–O) and 970 cm\(^{-1} \) (asymmetric ester O–P–O stretching modes from nucleic acids) (Cao et al., 2011a, b; Jiang et al., 2004; Ueshima et al., 2008; Cagnasso et al., 2010).

FTIR spectra of the free EPS changed after it was bound by goethite. The EPS spectrum of amide I (1653 cm\(^{-1} \)) and amide II (1543 cm\(^{-1} \)) shifted to 1641 cm\(^{-1} \) and 1534 cm\(^{-1} \) for the EPS–goethite complex, indicating that proteins of EPS were involved in EPS adsorption on goethite (Jiang et al., 2004; Cao et al., 2011b). Preferential adsorption of proteins in EPS from B. subtilis on goethite was also reported (Omoike and Chorover, 2006). Furthermore, the stretching vibration of PO\(_4^2-\) located at 1240 cm\(^{-1} \), 1128 cm\(^{-1} \) and 970 cm\(^{-1} \) in the spectrum of bulk EPS was observed.

![Fig. 2 – Adsorption isotherms of EPS on goethite at pH 5.0.](image)

![Fig. 1 – Zeta potential of 1 mg mL\(^{-1} \) goethite as a function of pH and total EPS concentration (mg mL\(^{-1} \)) in 10 mM NaCl solution. Control experiment was conducted with individual EPS suspension (1 mg mL\(^{-1} \)).](image)

### Table 1 – Langmuir parameters for adsorption of EPS-C, -N and -P on goethite.

<table>
<thead>
<tr>
<th>Element adsorbed by goethite</th>
<th>( X_m ) (( \mu g \text{ m}^{-2} ))</th>
<th>( K ) (L m(^{-2} ))</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPS-C</td>
<td>683.65</td>
<td>1.07</td>
<td>0.98</td>
</tr>
<tr>
<td>EPS-N</td>
<td>222.04</td>
<td>1.50</td>
<td>0.98</td>
</tr>
<tr>
<td>EPS-P</td>
<td>96.35</td>
<td>20.56</td>
<td>0.99</td>
</tr>
</tbody>
</table>
is apparently disappeared after complexing with goethite. This result suggests that the electronic density of the phosphorous atom is weakened upon complexation and that the free $P=O$ is absent (Barja et al., 1999). Most important is the emergence of one new band located at 1030 cm$^{-1}$ that is consistent with stretching vibrations of $P=O–Fe$ bonds as indicated by both empirical and molecular modeling studies (Barja et al., 1999; Sheals et al., 2002; Omoike et al., 2004). Emergence of the new band and the disappearance stretching vibration of $PO_2^-$ are consistent with inner-sphere complexation of EPS phosphate groups (deriving principally from phosphodiester of nucleic acids and proteins) at goethite surface hydroxyls (Omoike and Chorover, 2006). Therefore, FTIR results indicate that amide groups in proteins and phosphate groups in phosphodiester bridges of nucleic acids likely play an important role in EPS adsorption on goethite.

### 3.4. ITC analysis

The power–time curves for the titration of EPS into goethite suspension and 10 mM NaCl at different pH values are shown in Figures S5 and S6, respectively. By integrating the area under the peak resulting from each injection and subtracting the dilution heat of EPS, the net heat of EPS adsorption was obtained (Rong et al., 2010) and presented in Table 2. The negative $\Delta Q_{ads}$ values (from $-3.30$ to $-11.00$ J g$^{-1}$) revealed an exothermic process for the binding of EPS on goethite. It was worth noting that the values of $Q_{ads}$ and $\Delta H_{ads}$ increased with the increase pH from 3.0 to 7.0, whereas decreased with increasing pH from 7.0 to 9.0. The changes of $Q_{ads}$ and $\Delta H_{ads}$ values with solution pH implied that the adsorption behavior appears to be controlled by the overall surface charges of both EPS and goethite. EPS functional groups are mostly protonated at pH $< 2.0$, but become progressively more negative with increasing pH due to proton dissociation of carboxyl (pH 2.0–6.0), phospholipids (pH 2.4–7.2) and phosphodiester (pH 3.2–3.5) groups (Martinez et al., 2002). The results of zeta potential experiments showed that $pH_{zpc}$ values of goethite and EPS were 7.6 and <2.0, respectively (Fig. 1 and Table S1). Therefore, more functional groups such as carboxylic and phosphoric groups on EPS are deprotonated with the increase pH from 3.0 to 7.0, and these negatively charged groups reacted with positively charged groups of the goethite surfaces. The sharp increase in $\Delta H_{ads}$ with the increasing pH values from 3.0 to 7.0 suggested that EPS interaction with goethite through electrostatic attraction, followed by the formation of inner-sphere complexes on goethite surfaces. Furthermore, the complexation was strongly dependent on deprotonation of the functional groups on EPS. FTIR results also showed that organic phosphate groups were involved in EPS adsorption on goethite via inner-sphere $P=O–Fe$ surface complexation. Therefore, the increased $Q_{ads}$ and $\Delta H_{ads}$ values with the increase pH from 3.0 to 7.0 may be attributed to the formation of more phosphoric inner-sphere complexes. However, at high pH 9.0, the similar surface negative charges on goethite and EPS may cause the repulsion between EPS and goethite, which restrain the formation of inner-sphere complexes. Therefore, the dramatic decrease of $Q_{ads}$ and $\Delta H_{ads}$ values was observed at pH 9.0 as compared to that at pH 7.0. In addition, SEM images of EPS–goethite complexes showed that EPS just like an outer envelope shielding the goethite surface, and goethite particles connect with each other looser at pH 9.0 than that at pH 7.0 (Figure S7). Our previous studies also showed that the mass fraction of goethite-adsorbed EPS-C, -N, -P decreased with the increase of initial pH from 3.0 to 9.0 (Cao et al., 2011b). Based on the above results, it suggests that electrostatic interaction played an important role in the formation of inner-sphere complexes between EPS and goethite.

#### 3.5. X-ray absorption fine structure (XAFS) data analysis

The X-ray absorption near edge structure (XANES) has been used extensively and successfully to determine the oxidation state and the chemical coordination environment of ions in different systems (Higashi and Takahashi, 2009). Fe K-edge XANES spectra of all reference compounds, goethite and EPS–goethite complex at pH 3.0 are shown in Figure S8. Fe K-edge XANES spectra of complex at pH 3.0 were the same as those at pH 5.0, 7.0 and 9.0 (figure not shown). The edge for Fe(II) was represented by solid standard compound FeC$_2$O$_4$2H$_2$O and those for Fe(III) were denoted by FePO$_4$4H$_2$O and goethite. The adsorption edge of EPS–goethite complex was very similar to that of Fe(III) model compounds, which indicates that the oxidation state of iron was not changed during the adsorption process of EPS on goethite surface. Fig. 4 shows the $k^3$-weighted (to enhance the higher $k$ space data) Fe K-edge EXAFS spectra of EPS–goethite complex at different pH (3.0, 5.0, 7.0 and 9.0) and iron model compounds.

<table>
<thead>
<tr>
<th>$pH$</th>
<th>$Q_{ads}$ (mg)</th>
<th>$\Delta H_{ads}$ (J g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>$-0.66$</td>
<td>$-3.30$</td>
</tr>
<tr>
<td>5.0</td>
<td>$-0.75$</td>
<td>$-5.00$</td>
</tr>
<tr>
<td>7.0</td>
<td>$-1.54$</td>
<td>$-11.00$</td>
</tr>
<tr>
<td>9.0</td>
<td>$-0.69$</td>
<td>$-9.86$</td>
</tr>
</tbody>
</table>

**Table 2 – Net heat ($Q_{ads}$) and adsorption enthalpy ($\Delta H_{ads}$) changes of EPS on goethite at different pH values.**

![Fig. 3 – FTIR spectra of (a) EPS, (b) goethite, (c) EPS–goethite complex at pH 5.0.](image-url)
It is observed that spectrum of EPS–goethite complex is distinct from that of FeC2O4·2H2O, whereas it is similar to that of FePO4·4H2O, suggesting that the P–O–Fe bond may occur in EPS–goethite complex. Fig. 5 shows the corresponding radial structure functions (RSF) as Fourier transform (FT) versus radial distance. The FT of the EXAFS spectra isolates the contributions of different coordination shells, in which the peak positions correspond to the inter-atomic distances. As shown in Fig. 5, the first peak in the FT was the result of backscattering from the nearest neighbor Fe–O shell, and the second wide peak and a distinct shoulder were attributed to Fe–Fe and Fe–P shells (Omoike et al., 2004; Gustafsson et al., 2007). To gain more insight into the molecular structure of Fe on EPS–goethite complex, the EXAFS spectrum was fitted and presented in Figure S9. The coordination number (CN), atomic separation distance (R), and EXAFS Debye–Waller factor (σ²) obtained are listed in Table 3. As for goethite, Fe was coordinated by approximately 6 O atoms at an average distance of 1.96 Å in the first shell. However, the Fe–O distance of EPS–goethite complex increased from 1.96 to 2.06 Å with the increase of pH from 3.0 to 9.0. As for the second shell, the Fe–P distance decreased from 3.40 to 3.28 Å and the coordination number decreased from 3.38 to 2.84, respectively with the increase of pH from 3.0 to 9.0. These Fe–P distances are in close with those measured by X-ray absorption spectroscopy of colloidal phosphate–iron complexes (3.05–3.39 Å) (Rose et al., 1997) and phosphate minerals such as vivianite (3.19–3.43 Å) (Rose et al., 1996). Based on correspondence between spectral data and quantum chemical calculations, Omoike et al. (2004) indicated that these phosphate groups emerge from the EPS mixture to form monodentate complexes with Fe centers on the goethite surface. Quantum chemical calculations results also showed the distances of Fe–OP (between Fe and Oᵢ) and Fe–P were 1.97 Å and 3.39 Å in the monodentate complex, while those in bidentate complex were 2.06 Å and 3.22 Å, respectively. Compared with their calculation results, our experimental data suggested the phosphate groups of EPS can mainly form monodentate inner-sphere complexes at lower pH 3.0, while predominantly form bidentate inner-sphere complexes at higher pH 9.0. Both monodentate (via one of the EPS phosphate-containing O atoms) and bidentate (via both EPS phosphate O atoms) structures were showed in Figure S10. Therefore, it is inferred that the coordination structure of EPS-P adsorbed to goethite surface changed from monodentate inner-sphere complexes to bidentate inner-sphere complexes with the increase of pH. It is similar to the investigation of phosphate bound to goethite surface based on XAFS, FTIR studies or

![Fig. 4 – k^3-weighted EXAFS spectra for Fe in solid standard compounds, goethite and EPS–goethite complex at different pH values. (a) EPS–goethite complex at pH 9.0, (b) EPS–goethite complex at pH 7.0, (c) EPS–goethite complex at pH 5.0, (d) EPS–goethite complex at pH 3.0, (e) goethite, (f) FePO4·4H2O, (g) FeC2O4·2H2O.](image)

![Fig. 5 – k^3-weighted Fourier transforms of EXAFS data (uncorrected for phase shifts) for EPS–goethite complex at different pH values. (a) pH = 3.0, (b) pH = 5.0, (c) pH = 7.0, (d) pH = 9.0. Vertical dashed lines indicate the peak position for the first and second oscillations in goethite complexes.](image)

### Table 3 – EXAFS fit results of goethite and EPS–goethite complexes.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Interaction</th>
<th>R (Å)</th>
<th>σ² (Å²)</th>
<th>CN</th>
<th>Rexp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goethite</td>
<td>Fe–O</td>
<td>1.96</td>
<td>0.0081</td>
<td>6.07</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>Fe–Fe</td>
<td>3.25</td>
<td>0.0122</td>
<td>4.21</td>
<td>0.040</td>
</tr>
<tr>
<td>EPS–goethite (pH 3)</td>
<td>Fe–O</td>
<td>1.96</td>
<td>0.0096</td>
<td>6.07</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Fe–Fe</td>
<td>3.22</td>
<td>0.0016</td>
<td>8.66</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Fe–P</td>
<td>3.40</td>
<td>0.0066</td>
<td>3.38</td>
<td>0.042</td>
</tr>
<tr>
<td>EPS–goethite (pH 5)</td>
<td>Fe–O</td>
<td>1.98</td>
<td>0.0100</td>
<td>6.03</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Fe–Fe</td>
<td>3.22</td>
<td>0.0025</td>
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<tr>
<td></td>
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<td>0.0056</td>
<td>2.68</td>
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<tr>
<td>EPS–goethite (pH 7)</td>
<td>Fe–O</td>
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<td>0.0121</td>
<td>6.13</td>
<td>0.062</td>
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<td>Fe–Fe</td>
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<td>0.0022</td>
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<td>2.89</td>
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<td>EPS–goethite (pH 9)</td>
<td>Fe–O</td>
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<td>0.0100</td>
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<td>0.061</td>
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<td>0.0019</td>
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<td>Fe–P</td>
<td>3.28</td>
<td>0.0064</td>
<td>2.84</td>
<td>0.048</td>
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</table>

| a | Atomic separation distance. |
| b | Debye–Waller factor coefficient. |
| c | Coordination number. |
| d | Residual factor = \( \frac{\sum k^2 x_{\text{calc}} - k^2 x_{\text{exp}}}{\sum k^2 x_{\text{calc}}} \), which measures the quality of the model Fourier-filtered contribution \( x_{\text{calc}} \) with respect to the experimental contribution \( x_{\text{exp}} \). The amplitude reduction factor \( S_k \) was set to 0.90 and 0.95 for the shell 1 and shell 2 fit, respectively. |
CD–music model fitting by Persson et al. (1996) and Antelo et al. (2005). Their studies demonstrated that phosphate can form monodentate or bidentate inner-sphere complexes with goethite surface sites and the structure of complexes is sensitive to changes in pH. Based on the results from this work and relevant previous studies, it is proposed that the two different inner-sphere structure may occur for the adsorption of EPS on goethite surface: 1) the phosphate groups of EPS form a monodentate inner-sphere complex, where one oxygen of the anion binds directly the Fe atom of a FeOH\(_{1/2}\)-group, releasing the attached OH\(^-\); and 2) a bidentate inner-sphere complex, where two oxygens of the anion bind two Fe atoms of two adjacent FeOH\(_{1/2}\)-groups (Figure S11). Extracted EPS from \(P.\ putida\) are a complex mixture of biomacromolecules consisting primarily of polysaccharides (363.1 ± 11.8 mg g\(^{-1}\)) and proteins (209.1 ± 3.3 mg g\(^{-1}\)), with smaller amounts of nucleic acids (16.2 ± 0.4 mg g\(^{-1}\)) (Table S1). These constituents containing phosphate groups may provide different energetically stable bonds for further EPS or cell adhesion depending on environmental solution pH (Figure S12) and also play a vital role in conditioning film formation on Fe oxide surfaces.

4. Conclusions

EPS adsorption may result in the formation of P–O–Fe bonds between phosphoryl groups and goethite surface Fe metal centers. Inner-sphere complexes types changed from monodentate to bidentate with increasing pH from 3.0 to 9.0. The goethite–EPS interaction is mediated by formation of P–O–Fe bonds, which provides a strong anchor mechanism and contribute to bacterial adhesion leading to cell immobilization and biofilm formation in the near-surface environments.

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Appendix A. Supplementary material

Supplementary material related to this article can be found in the online version at http://dx.doi.org/10.1016/j.watres.2012.07.046.

REFERENCES


