

## Role of microbial exopolymeric substances (EPS) on chromium sorption and transport in heterogeneous subsurface soils: II. Binding of Cr(III) in EPS/soil system

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### ABSTRACT

Laboratory batch sorption and column experiments were performed to investigate the effects of microbial EPSs isolated from *Pseudomonas putida* P18, *Pseudomonas aeruginosa* P16 and *Pseudomonas stutzeri* P40 on Cr(III) mobility in heterogeneous subsurface soils. Our batch and column results indicate that microbial EPS may have a pronounced effect on Cr(III) sorption and transport behavior depending on system conditions (e.g., pH, type of EPS). While EPS had no effect on Cr(III) sorption at pH < 5, it led to a significant decrease in Cr(III) sorption under slightly acidic to alkaline pH range. Column experiments performed at pH 7.9 suggest that, in the presence of EPS, chromium(III) was significantly mobilized relative to non-EPS containing system due to the formation less sorbing and highly soluble Cr–EPS complexes and competition of EPS against Cr for surface sites.

A two-site non-electrostatic surface chemical model incorporating a discrete ligand approach for the description of Cr–EPS interactions accurately predicted Cr(III) sorption and transport behavior in the presence of EPS under variable chemical conditions. Our simulations show that an accurate description of Cr(III) transport in the presence of EPS requires incorporation of proton and Cr(III) binding by EPS, EPS binding by soil minerals, Cr(III) binding by soil minerals, and ternary Cr(III)–EPS surface complexes into the transport equations. Although this approach may not accurately describe the actual mechanisms at the molecular level, it can improve our ability to accurately describe the effects of EPS on Cr(III) mobility in subsurface environment relative to the use of distribution coefficients ( $K_d$ ).

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### 1. Introduction

The sorption and transport behavior of chromium (Cr) in subsurface environment depends highly on several chemical conditions including oxidation state, pH and presence of other inorganic and organic materials. Chromium can exist in oxidation states ranging from Cr(–II) to Cr(VI), although Cr(III) and Cr(VI) are the most dominant oxidation states in natural systems. While chromium typically occurs in the hexavalent form (Cr(VI)) under standard environmental (oxidizing) conditions, Cr(VI) can be reduced to Cr(III) in reducing environments. The Cr<sup>3+</sup> ion is the dominant Cr(III) species at pH < 3.5, but it hydrolyzes and forms sparingly soluble oxides and hydroxides (e.g., Cr(OH)<sub>3</sub>(s)) under alkaline pH conditions (Palmer and Puls, 1994).

Batch sorption studies, primarily performed with pure mineral phases, suggest that while chromium(III) is highly immobile under slightly acidic to alkaline pH conditions due to its low solubility and highly sorptive characteristics (Karthein et al., 1991; Fendorf

et al., 1994; Fendorf and Sparks, 1994; Csoban and Joo, 1999), chromium(VI) is relatively mobile in the environment due to the fact it exhibits weak to medium binding affinity for metal oxides such as Fe- and Al-oxides depending on the environmental conditions (e.g., pH, organic matter content) (Palmer and Puls, 1994; Davis et al., 2000; Kantar et al., 2008). Relatively, little information is available on reaction mechanisms for Cr(III) sorption onto mineral surfaces. For example, studies by Karthein et al. (1991) and Fendorf et al. (1994) suggest that Cr(III) binding with metal oxides mainly occurs through the formation of monodentate (e.g., SOCr<sup>2+</sup>) surface complexes.

An important chemical property of Cr(III) with respect to its environmental behavior is its ability to form complexes with natural organic ligands (Bartlett and Kimble, 1976; Puzon et al., 2008; Cetin et al., 2009). The formation of such organo–Cr complexes has a pronounced impact on the solubility, toxicity, bio-availability, complexation and sorption behavior of chromium in subsurface environment (Kantar et al., 2008; Puzon et al., 2008; Cetin et al., 2009). For example, Puzon et al. (2005) found that Cr(VI) reduction in the presence of cellular organic metabolites (e.g., citrate, ascorbate, malate) formed highly soluble organo–Cr(III) complexes, which are very stable over a broad pH range. Similarly,

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Puzon et al. (2008) observed that complexation reactions between Cr(III) and organic ligands, e.g., citrate led to a significant decrease in Cr(III) sorption to soils, thereby increasing its mobility in subsurface systems. Cetin et al. (2009) found that alginic and galacturonic acids lowered Cr(III) sorption to soils by forming highly soluble and less sorbing Cr(III)–ligand complexes.

Exopolymeric substances (EPS) are one of the major components of natural organic matter (NOM) in natural systems (Lamelas et al., 2006). EPS is produced by microbes for a variety of purposes in response to environmental stresses, e.g., Cr(VI). Microbial EPSs contain multiple functional groups (e.g., carboxylic, phosphate, amine, hydroxyl functional groups), available for binding with metal ions, e.g., Cr (Guibaud et al., 2005, 2008; Harper et al., 2008). The nature of Cr(III) complexation with EPS varies with the composition of EPS (Priester et al., 2006). Several researchers have also reported on the effect of microbial EPS for metal ion speciation and behavior in natural and engineered systems. For example, Priester et al. (2006) found that Cr(III) that formed after microbial reduction with *Pseudomonas putida* was partly associated with EPS which forms due to the toxic effect of Cr on microbial cells. Their results also demonstrate that cellular lysis, cellular association and extracellular DNA binding of Cr(III) led to the localized biotic stabilization of Cr in contaminated vadose zones.

Despite the overwhelming evidence on the production of microbial EPS during microbial Cr(VI) reduction, little is known about the role of EPS on Cr(III) sorption and transport in subsurface systems. In our previous study (Part I of this publication series), we describe the development of a discrete ligand model for the description of aqueous phase Cr(III)–EPS interactions in which EPS is conceptualized as being composed of monoprotic acid ligands ( $HL_n$ ) with arbitrarily assigned  $pK_a$  values of 4, 6, 8 and 10. These functional groups can be operationally defined as carboxyl, carboxyl/phosphoric, phosphoric, and hydroxyl/phenolic sites, respectively. The development of such simple mechanistic modeling approaches allow the modelers to incorporate metal–NOM interactions (e.g., Cr–EPS complexation) into surface complexation models (SCM). In this study, batch sorption and column experiments were conducted to determine the effects of microbial EPS isolated from *P. putida* P18, *Pseudomonas stutzeri* P40 and *Pseudomonas aeruginosa* P16 on Cr(III) sorption and subsequent mobility or immobility in heterogeneous subsurface systems contaminated with Cr(III). In addition, the effects of EPS on Cr(III) sorption to soil minerals in the presence of EPS under a wide range of environmental conditions (e.g., pH) were simulated by linking the discrete ligand approach for EPS with a non-electrostatic surface complexation model (SCM) based on the Generalized Composite (GC) approach. As stated above, although this approach has been successfully applied to natural sediments by several authors (e.g., Kent et al., 2000; Curtis et al., 2004), to our knowledge data regarding the application of the GC SCM approach to natural systems containing EPS is not available in the literature. The predictive ability of SCM parameters derived from batch sorption data was also tested in a transport code by simulating breakthrough curves from column experiments.

## 2. Materials and methods

### 2.1. Materials

Unless stated otherwise, all chemicals used in the experiments were reagent grade or better. Water for all experiments was supplied from Millipore (Simplicity 185) UV-water system. Chromium(III)-nitrate-nonahydrate (Merck) was used as the source for Cr(III) in all experiments. All stock solutions, including NaOH and HCl for pH adjustments, NaCl for ionic strength adjustment were prepared using UV-water and stored in amber glass

bottles in the dark at 4 °C. Stock solutions of ‘dissolved EPS’ were prepared using EPSs isolated from *P. aeruginosa* P16, *P. putida* P18, and *P. stutzeri* P40, as described by Hung et al. (2005). Detailed information regarding EPS isolation and purification from bacteria can be found in the Supporting information, page S2.

### 2.2. Soil samples

Soil samples were collected at a depth of 0–30 cm from uncultivated and unpolluted agricultural fields located in Mersin, Turkey (36° 50'N, 34° 24'W). The samples were taken from a depth of 0–30 cm. The samples were transported to the laboratory in plastic bags. All samples, mixed and homogenized in the laboratory, were air-dried at room temperature, and passed through a 2-mm sieve. Samples were then stored at room temperature in plastic bags until required. Soil physical and chemical properties have been previously determined by Kantar et al. (2009). In general, the soils were moderately calcareous (5.02%), and slightly alkaline (pH 7.57). The texture class of the soils was sandy loam (SL) with a high quartz and silt content according to USDA classifications. The soils also contained very high concentrations of Al- and Fe-oxides, mainly in the amorphous form (Kantar et al., 2009). Using a titration procedure, Kantar et al. (2009) estimated a site concentration of 0.133 mmol g<sup>-1</sup> for the soil.

### 2.3. Batch sorption experiments

Batch scale sorption experiments were performed using 50 mL polycarbonate Oak Ridge centrifuge tubes at a soil/solution ratio of 3 g L<sup>-1</sup>, 10<sup>-5</sup> M Cr(III) and/or 50 mg L<sup>-1</sup> EPS concentration. Samples were run in triplicate for each experimental condition. The tube lids were loosely covered to allow for gas exchange; hence all sorption experiments were open to the environment to facilitate equilibration with atmospheric pressure. In all of the batch experiments, sufficient NaCl was added to the tubes to obtain desired ionic strength ( $I = 0.01$  M). In the batch sorption experiments at pH values greater than 7.0, increasing volumes of NaHCO<sub>3</sub> were added to the tubes to facilitate solution equilibration with atmospheric CO<sub>2</sub>.

After pre-equilibration with the background solution for 24 h, aliquots of Cr(III) and/or EPS were added to obtain the final desired total Cr(III) and/or EPS concentration. The pH of the samples was adjusted with HCl or NaOH, and sufficient UV-water was added to bring all the samples to the desired volume (20 mL). The samples were then placed on a shaker table in the dark, and allowed to come to equilibrium for 48 h. A reaction time of 48 h was chosen since nearly 100% of adsorption occurred within this time. After equilibration, the sample pH was checked, and the solids were separated by centrifugation at 7600 rpm for 10 min. The aqueous Cr concentration was determined using ICP-MS (Agilent 7500ce) with a detection limit of  $4.06 \times 10^{-10}$  M, and the Cr bound to the soil was calculated from the difference between the Cr concentration in these samples and the Cr concentration prepared without soil. Similarly, the amount of EPS sorbed to soil was determined by analyzing the samples with or without soil for their P contents with ICP-MS.

### 2.4. Column experiments

Column experiments were performed to study the effects of microbial EPS on Cr(III) mobility under advective conditions similar to those that might be observed in the field. The laboratory column studies were conducted using liquid chromatography columns with Teflon fittings with an inner diameter of 2.2 cm. A reciprocating dual-piston high performance liquid chromatography pump (Alltech Model 301 HPLC Pump with inert PEEK heads)

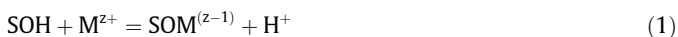
was used to deliver solutions. Samples were collected and analyzed for Cr contents after elution through the column using an ISCO Instruments Retriever II Fraction Collector. All column experiments were conducted at  $22 \pm 2$  °C and a flow rate of  $0.076 \text{ mL min}^{-1}$ .

The columns were packed with the soil in such a manner as to provide a uniform flow. Column packing entailed filling the column with a suspension of 6 g of the soil in 30 mL UV-water. Prior to performing experiments, the columns were preconditioned by pumping several pore volumes of NaCl electrolyte solution until inflow and outflow pH values differed by 0.02 or less. All solutions were prepared in 0.01 M NaCl with a pH of 7.9, and contained 0.58 mM  $\text{NaHCO}_3$  to minimize equilibration times and pH drift. Following equilibration, about 300 pore volumes of  $10^{-5}$  M Cr(III) solutions prepared in 0.01 M NaCl with a pH of 7.9 and/or  $100 \text{ mg L}^{-1}$  EPS were pumped through the column, and the samples collected with the fraction collector were analyzed for their Cr and EPS contents.

To characterize the columns, bromide ( $\text{Br}^-$ ) was injected as a conservative tracer. The breakthrough curves of bromide were analyzed with CXTFIT (Toride et al., 1995) in order to estimate the linear pore velocity ( $v$ ) and hydrodynamic dispersion coefficient ( $D_h$ ). At a flow rate of  $0.076 \text{ mL min}^{-1}$ , the average linear velocity was  $48.01 \text{ cm d}^{-1}$  with a corresponding dispersion coefficient of  $5.56 \text{ cm}^2 \text{ d}^{-1}$ . The pore volume was estimated to be 4.4 mL using the methods of Kantar and Honeyman (2006). All experiments were performed in duplicate.

## 2.5. Batch sorption modeling

Surface complexation modeling of sorption data was done in a chemical equilibrium based computer program, FITEQL 4.0 (Herbelin and Westall, 1999) using a non-electrostatic GC surface complexation model incorporating a discrete ligand approach for the description of binary Cr–EPS interactions. Here, our goal was a parsimonious model fit to the Cr(III) and/or EPS sorption data to determine the best fit of various Cr(III) and EPS surface reactions or combinations of reactions in the non-electrostatic GC model calculations. As outlined by Kantar et al. (2009), our modeling strategy was to construct the ternary system (Cr(III)/EPS/soil) models through the combination of appropriate binary sub-models (e.g., Cr(III)/soil). Detailed information on GC SCM approach can be found elsewhere (e.g., Davis et al., 1998). In short, it is based on mass action expressions between surface sites and dissolved ions (Kent et al., 2000), e.g.:



where  $\text{M}^{z+}$  is the free metal ion in solution, SOH is a protonated surface site and  $\text{SOM}^{(z-1)}$  is a possible metal surface complex. Moreover, this approach allows the modeler to couple solution-phase reactions with surface-phase reactions, as exemplified by the formation of the aqueous metal organic ligand ( $\text{L}^{m-}$ ) complex, i.e.:



where  $\text{M}(\text{L})_n^{(z-m)}$  is the solution phase metal–ligand complex.

In GC SCM approach, adsorption is assumed to occur on generic surface sites that represent average properties of the sediment surfaces rather than specific mineral surfaces. Model parameters are calibrated to adsorption data for postulated reaction stoichiometries, and different model formulations are selected on the basis of simplicity and goodness of fit (Davis et al., 1998, 2004; Curtis et al., 2004). Electrostatic terms are usually not included in such semi-mechanistic approaches because of the difficulties in quantifying the electrical field and charge at the mineral–water interface in the mixture of mineral phases and associated surface coatings (Curtis et al., 2004; Davis et al., 2004). In addition, the surface acid-

ity constants for surface sites were not included to reduce the number of fitted parameters in the model (Kohler et al., 1996; Davis et al., 1998; Curtis et al., 2004) due to the fact that the surface site speciation is mainly dominated by the uncharged surface species (SOH) under a wide range of environmental conditions (e.g., pH) (Davis et al., 1998; Kantar et al., 2009).

## 2.6. Cr(III)–EPS interactions

It is surprising to note that there have been relatively few attempts to simulate the sorption of metal ions to mineral surfaces in the presence of natural organic matter (NOM) using the surface complexation approach (Lenhart and Honeyman, 1999). The main obstacle has been the development of a method for describing NOM in such a fashion that the inherent polyfunctional nature of NOM can be adapted to the SCM framework. The development of new chemical 'mechanistic' modeling approaches for the description metal–NOM interactions has recently offered a means of linking solution-phase and surface-phase reactions in a manner conducive to the application of surface complexation modeling (e.g., Purdue et al., 1984; Westall et al., 1995; Kinniburgh et al., 1996; Lenhart and Honeyman, 1999; Tipping, 2002). Here, the Cr(III)–EPS interactions were studied using a non-electrostatic discrete approach in which EPS was conceptualized as being composed of four different monoprotic ligands with  $\text{pK}_a$  values of 4, 6, 8 and 10 (Table 1). Although this approach may not correspond to a strict physical or chemical model of natural organic matter due to complex structure and reactivity of natural organic molecules, it provides a consistent evaluative framework for the simulations of interactions of EPS with soil surfaces and ions. Detailed information on the derivation of discrete ligand approach for Cr–EPS interactions can be found in Part 1 of this publication series.

## 2.7. Reactive transport modeling

Predictive ability of batch-derived SCM parameters was tested in a transport model by simulating breakthrough curves from column experiments. Simulations were created using the reactive multicomponent transport code, PHREEQC (Parkhurst and Appelo, 1999). The extensive data base in PHREEQC enables the speciation of solutes as well as consideration of dissolution/precipitation,

**Table 1**  
EPS solution-phase reactions (from Part 1 of the publication series).

Reaction	$T_{\text{HL}i}$ ( $\text{mmol g}^{-1}$ )	$\log K$ ( $I = 0$ )
<i>P. stutzeri</i> P40		
$\text{HL}_1 = \text{L}_1 + \text{H}^+$	0.5460	–4
$\text{HL}_2 = \text{L}_2 + \text{H}^+$	0.5380	–6
$\text{HL}_3 = \text{L}_3 + \text{H}^+$	0.1220	–8
$\text{HL}_4 = \text{L}_4 + \text{H}^+$	0.5920	–10
$\text{Cr}^{3+} + \text{HL}_3 = \text{Cr}(\text{L}_3)^{2+} + \text{H}^+$		1.994
$\text{L}_i^- + \text{Na}^+ = \text{NaL}_i$		1.195
<i>P. putida</i> P18		
$\text{HL}_1 = \text{L}_1 + \text{H}^+$	0.96	–4
$\text{HL}_2 = \text{L}_2 + \text{H}^+$	0.72	–6
$\text{HL}_3 = \text{L}_3 + \text{H}^+$	0.356	–8
$\text{HL}_4 = \text{L}_4 + \text{H}^+$	0.626	–10
$\text{Cr}^{3+} + \text{HL}_2 = \text{Cr}(\text{L}_2)^{2+} + \text{H}^+$		1.225
$\text{L}_i^- + \text{Na}^+ = \text{NaL}_i$		1.006
<i>P. aeruginosa</i> P16		
$\text{HL}_1 = \text{L}_1 + \text{H}^+$	0.773	–4
$\text{HL}_2 = \text{L}_2 + \text{H}^+$	0.673	–6
$\text{HL}_3 = \text{L}_3 + \text{H}^+$	0.380	–8
$\text{HL}_4 = \text{L}_4 + \text{H}^+$	0.254	–10
$\text{Cr}^{3+} + 2\text{HL}_2 = \text{Cr}(\text{L}_2)_2^+ + 2\text{H}^+$		1.199
$\text{L}_i^- + \text{Na}^+ = \text{NaL}_i$		0.727

sorption, complexation and redox reactions. Reactions not in the existing database can be added to the database.

### 3. Results and discussion

#### 3.1. Batch experiments

##### 3.1.1. Cr(III) sorption to soils

Batch sorption experiments were performed to investigate the effects of pH on Cr(III) sorption to soils. These experiments were open to the atmosphere, and were carried out by using samples adjusted from pH 3–8.2, 3 g L<sup>-1</sup> soil, an ionic strength of 0.01 M NaCl, and 10<sup>-5</sup> M Cr(III)<sub>T</sub> (Fig. 1). Chromium(III) sorption increases with increasing pH, as is characteristic of cation or metal-like sorption. The increase in Cr(III) sorption with an increase in pH is thought to result from the sorption of the positively charged Cr<sup>3+</sup> and Cr(OH)<sup>2+</sup> onto negatively charged mineral surfaces in soils especially at pHs < 8. Table 2 shows aqueous phase Cr(III) hydrolysis species. These results are in good agreement with the previously published data of Csoban and Joo (1999) who observed a similar behavior for Cr(III) sorption onto pure mineral phases (e.g., alumina (Al<sub>2</sub>O<sub>3</sub>) and silica gel).

##### 3.1.2. EPS sorption to soils

Fig. 2 shows the sorption of EPS isolated from *P. putida*, *P. aeruginosa* and *P. stutzeri* onto soil minerals as a function of pH. The EPS sorption experiments were conducted in the same manner as the batch Cr(III) sorption experiments. All EPSs studied exhibit a typi-

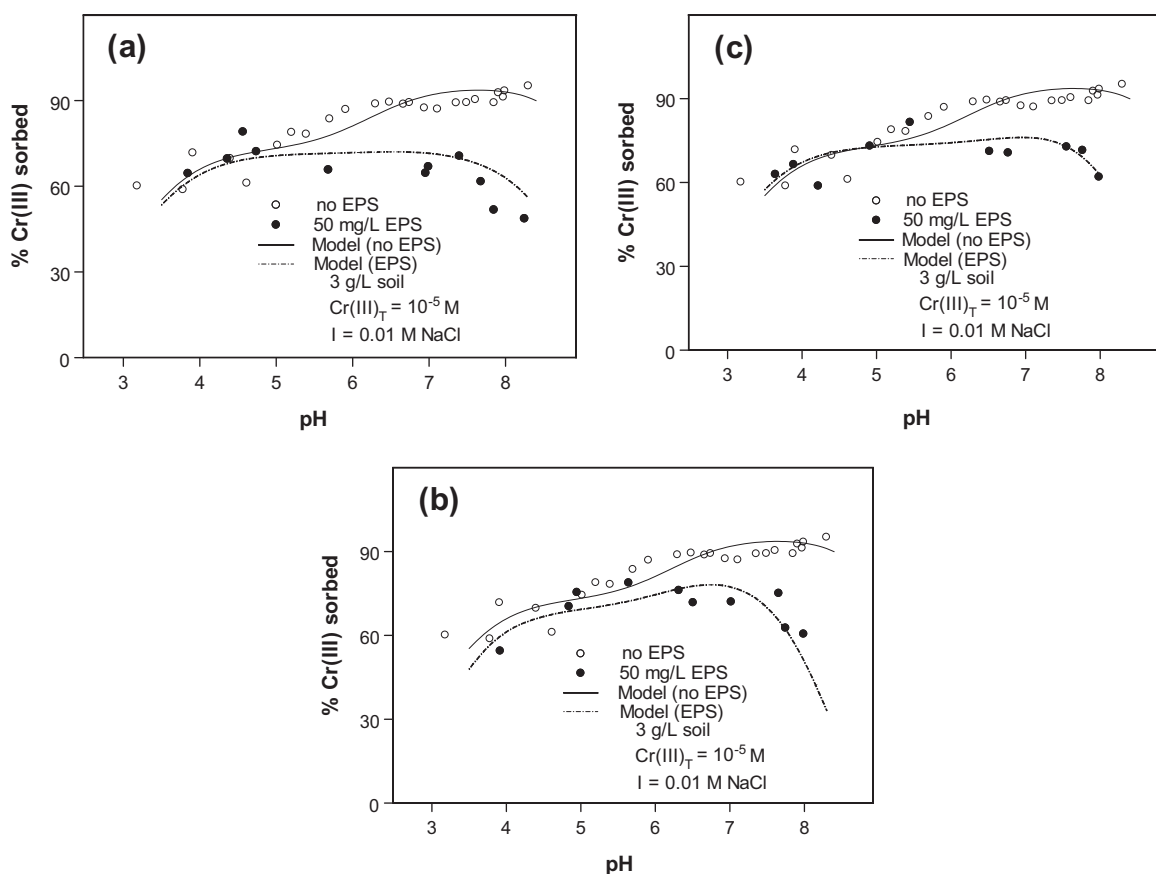
**Table 2**  
Chromium(III) hydrolysis reactions.

Reaction	log K(I = 0)	Reference
Cr <sup>3+</sup> + H <sub>2</sub> O = Cr(OH) <sup>2+</sup> + H <sup>+</sup>	-3.486	Cetin et al. (2009)
Cr <sup>3+</sup> + 2H <sub>2</sub> O = Cr(OH) <sub>2</sub> <sup>+</sup> + 2H <sup>+</sup>	-10.4	Pettit and Powell (1995)
Cr <sup>3+</sup> + 3H <sub>2</sub> O = Cr(OH) <sub>3</sub> + 3H <sup>+</sup>	-18.7	Pettit and Powell (1995)
Cr <sup>3+</sup> + 4H <sub>2</sub> O = Cr(OH) <sub>4</sub> <sup>-</sup> + 4H <sup>+</sup>	-27.8	Pettit and Powell (1995)

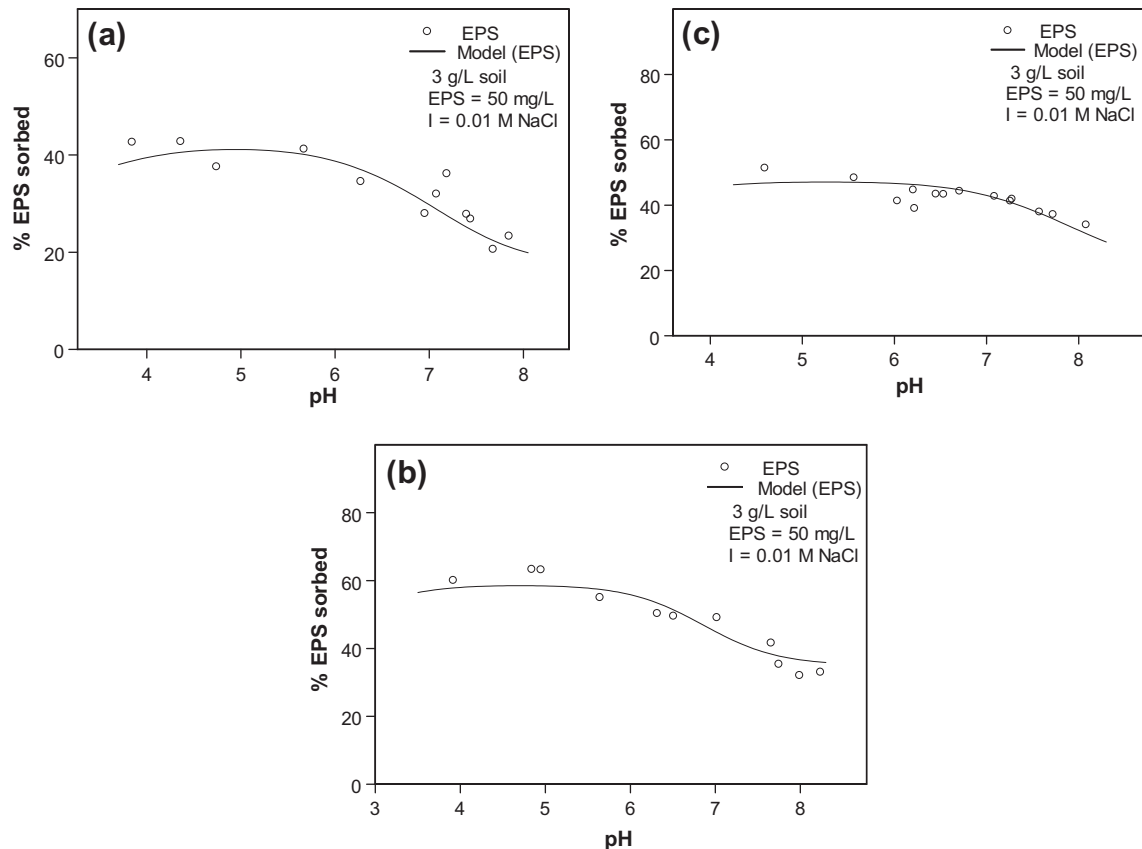
cal “ligand-like” behavior, with the sorption gradually decreasing with increasing solution pH. The shallow slope observed in EPS sorption toward alkaline conditions may be attributed to the poly-functional nature of EPSs. Table 1 shows EPS acid–base reactions. Note that EPS contains multiple functional groups with pK<sub>a</sub> values ranging from 4 to 10 under experimental conditions studied. This kind of sorption behavior has also been reported for the sorption of humic substances (Schlautman and Morgan, 1994; Lenhart and Honeyman, 1999). The relatively high sorption of EPS onto soil minerals may be explained through the strong interaction of EPS with Fe- and Al-oxides (Kantar et al., 2009). The decrease in the EPS sorption at high pHs may be partly caused by the electrostatic repulsion between deprotonated surface groups and EPS.

##### 3.1.3. Cr(III) sorption to soils in the presence of EPS

Ternary system batch sorption experiments were conducted to examine the effects of EPS on Cr(III) sorption by soil minerals (Fig. 1). These experiments were open to the atmosphere, and were carried out by using samples with a pH range of 3–8.2, 3 g L<sup>-1</sup> soil,



**Fig. 1.** Experimental results and FITEQL simulation of percent Cr(III) sorbed vs. pH in the absence or presence of 50 mg L<sup>-1</sup> EPS isolated from: (a) *P. aeruginosa* P16, (b) *P. putida* P18 and (c) *P. stutzeri* P40. All samples had equivalent initial total Cr(III) (Cr(III)<sub>T</sub> = 10<sup>-5</sup> M) and constant ionic strength (I = 0.01 M NaCl), and were in equilibrium with atmospheric CO<sub>2</sub>. Symbols represent experimental data and lines represent non-electrostatic surface complexation model (SCM) fit values using the surface reactions given in Table 3. Solution phase EPS and Cr hydrolysis reactions are given in Tables 1 and 2.



**Fig. 2.** Experimental results and FITEQL simulation of percent EPS sorbed vs. pH. (a) *P. aeruginosa* P16, (b) *P. putida* P18 and (c) *P. stutzeri* P40. All samples had equivalent initial total EPS (EPS = 50 mg L<sup>-1</sup>) and constant ionic strength (*I* = 0.01 M NaCl), and were in equilibrium with atmospheric CO<sub>2</sub>. Symbols represent experimental data and lines represent non-electrostatic surface complexation model (SCM) fit values using the surface reactions given in Table 3.

ionic strength (*I*) of 0.01 M NaCl and 10<sup>-5</sup> M Cr(III)<sub>T</sub>. As shown in Fig. 1, while EPS had no impact on Cr(III) sorption at pHs < 5, the addition of 50 mg L<sup>-1</sup> EPS led to a significant decrease in Cr(III) sorption especially at pHs > 5 relative to non-EPS containing systems. This decrease in Cr(III) sorption with EPS may be explained through: (1) the complexation of Cr(III) with EPS, (2) competition of EPS and Cr(III) for surface sites and (3) alteration of surface sites by EPS, as also suggested by Logue et al. (2004). Table 1 shows Cr(III)–EPS complexation reactions. The effectiveness of EPS on Cr(III) sorption decreases at pH < 5 due to the fact that EPS becomes mostly protonated toward more acidic pH values. Fig. 1 also shows that the extent of Cr(III) sorption varies from one EPS to another with the least amount of Cr(III) sorbing to soil minerals in the presence of EPS isolated from *P. aeruginosa*.

### 3.2. Column experiments

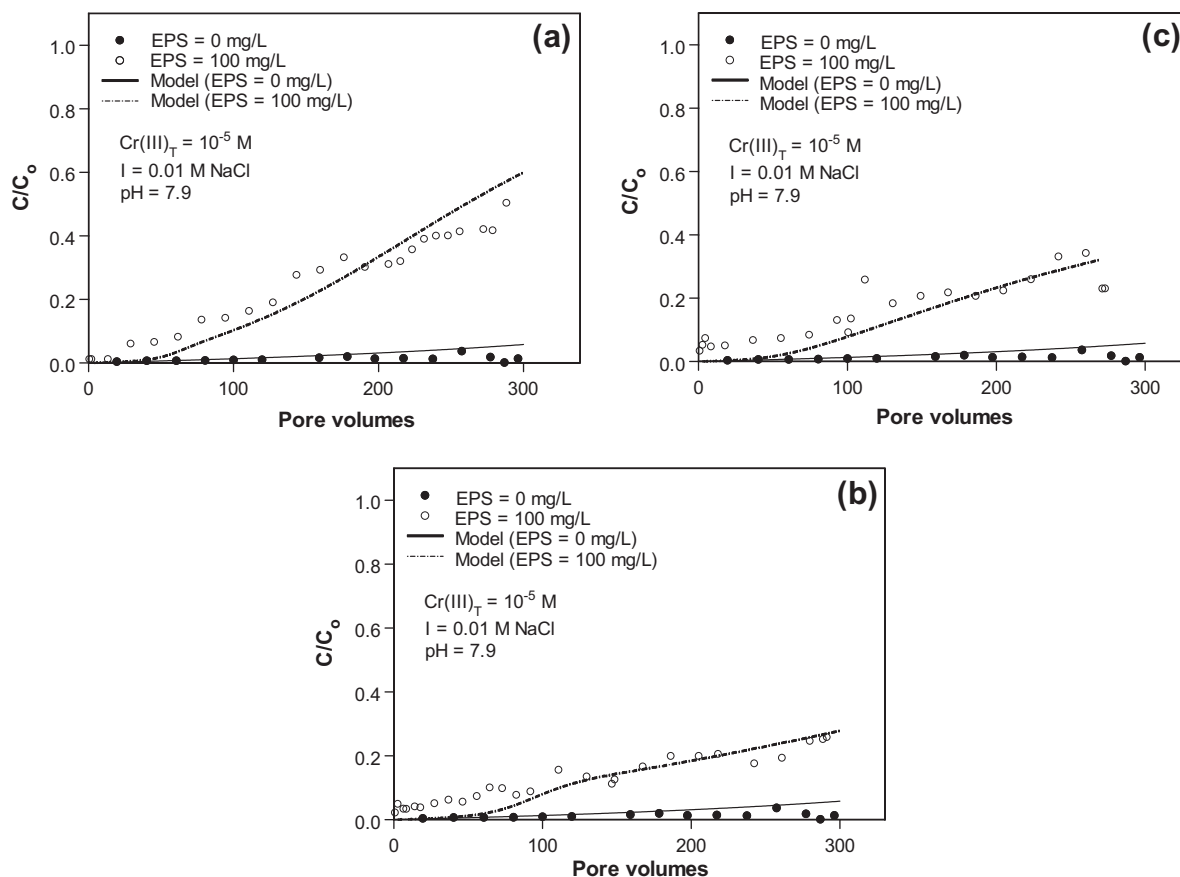
The mobility and toxicity of Cr(VI) in subsurface environment can be reduced by converting it to Cr(III) using soil bacteria. However, many bacteria species release EPS during microbial Cr(VI) reduction due to the toxic effect of chromium on microbial cells. Microbial EPS contains multiple functional groups for complexation with Cr(III) (Part 1 of this publication series). Here, column experiments were conducted to determine the effects of EPS on Cr(III) stability in subsurface environment. Fig. 3 shows the breakthrough curves for 10<sup>-5</sup> M Cr(III) transport in the absence or presence of 100 mg L<sup>-1</sup> EPS at pH 7.9. The corresponding breakthrough curves for 100 mg L<sup>-1</sup> EPS in the presence of 10<sup>-5</sup> M Cr(III) at pH 7.9 are also presented in Fig. 4. Note that in the absence of any EPS, no chromium exits the columns, even up to 300 pore volumes (Fig. 3). This is due to the fact chromium(III)

has very low solubility, and strongly sorbs to the soil minerals under the experimental conditions (pH 7.9), as was the case observed in batch sorption experiments (Fig. 1). However, in the presence of EPS, chromium(III) was highly mobilized relative to non-EPS containing systems. The increase in Cr(III) mobilization in the presence of EPS can be explained through the formation of less sorbing and highly soluble Cr(III)–EPS complexes (see Part 1 of this publication series), and competition of EPS against Cr(III) for mineral surface sites. As given in Fig. 4, all EPSs studied were highly mobile under the experimental conditions investigated (pH 7.9), indicating that a significant portion of Cr(III) complexed with microbial EPS was transport along with the EPS.

Fig. 3 also suggests that the extent of Cr(III) mobilization is also highly dependent on the type of EPS used, with more Cr(III) being mobilized in the presence of EPS isolated from *P. aeruginosa* compared to the EPSs from *P. putida* and *P. stutzeri*. This confirms the results obtained in batch sorption experiments (Fig. 1). Studies by Spaulding et al. (2004a,b) show that metal ions such as Cd, Cu and Pb can be mobilized in systems containing microbial EPSs due to the formation of metal–ligand complexes. Similarly, Puzon et al. (2005) and Priester et al. (2006) determined highly soluble Cr–ligand complexes in systems containing organic metabolites (e.g., EPS). In a column study with Cr(III), Puzon et al. (2008) observed that cellular organic metabolites (e.g., citrate) increase the solubility of Cr(III), thereby leading to the mobilization of Cr(III) species in subsurface environment.

### 3.3. Data simulations and model results

A two-site [strong (S<sub>1</sub>OH) and weak binding affinity(S<sub>2</sub>OH)] non-electrostatic SCM based on the GC approach was developed



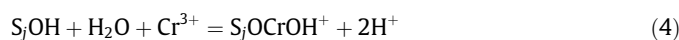
**Fig. 3.** Observed and PHREEQC simulation of the breakthrough curves for Cr(III) in the absence or presence of 100 mg L<sup>-1</sup> EPS isolated from: (a) *P. aeruginosa* P16, (b) *P. stutzeri* P40 and (c) *P. putida* P18. All experiments were performed at pH 7.9, and had equivalent initial total EPS concentration of 100 mg L<sup>-1</sup>, a total Cr(III) concentration of 10<sup>-5</sup> M and constant ionic strength (*I* = 0.01 M NaCl). Symbols represent experimental data and lines represent non-electrostatic surface complexation model (SCM) predictions with the batch-derived SCM parameters given in Table 3. Solution phase EPS and Cr hydrolysis reactions are given in Tables 1 and 2.

as an alternative to the partition coefficient ( $K_d$ ) approach to describe Cr(III) sorption to heterogeneous subsurface soils under variable chemical conditions (e.g., pH) and in the presence of microbial EPS. Soil was assumed to contain a weak binding site comprising 95% of the total site concentration and a strong binding site comprising 5% of total surface sites. Our main goal in using such a simple semi-empirical SCM was to accurately describe the experimental data over a range of environmental conditions (e.g., pH and EPS concentration) by using a minimum number of adjustable parameters. As indicated by Kantar et al. (2009), such models are more easily incorporated into reactive transport codes compared to most electrostatic SCMs.

### 3.3.1. Modeling Cr(III) sorption

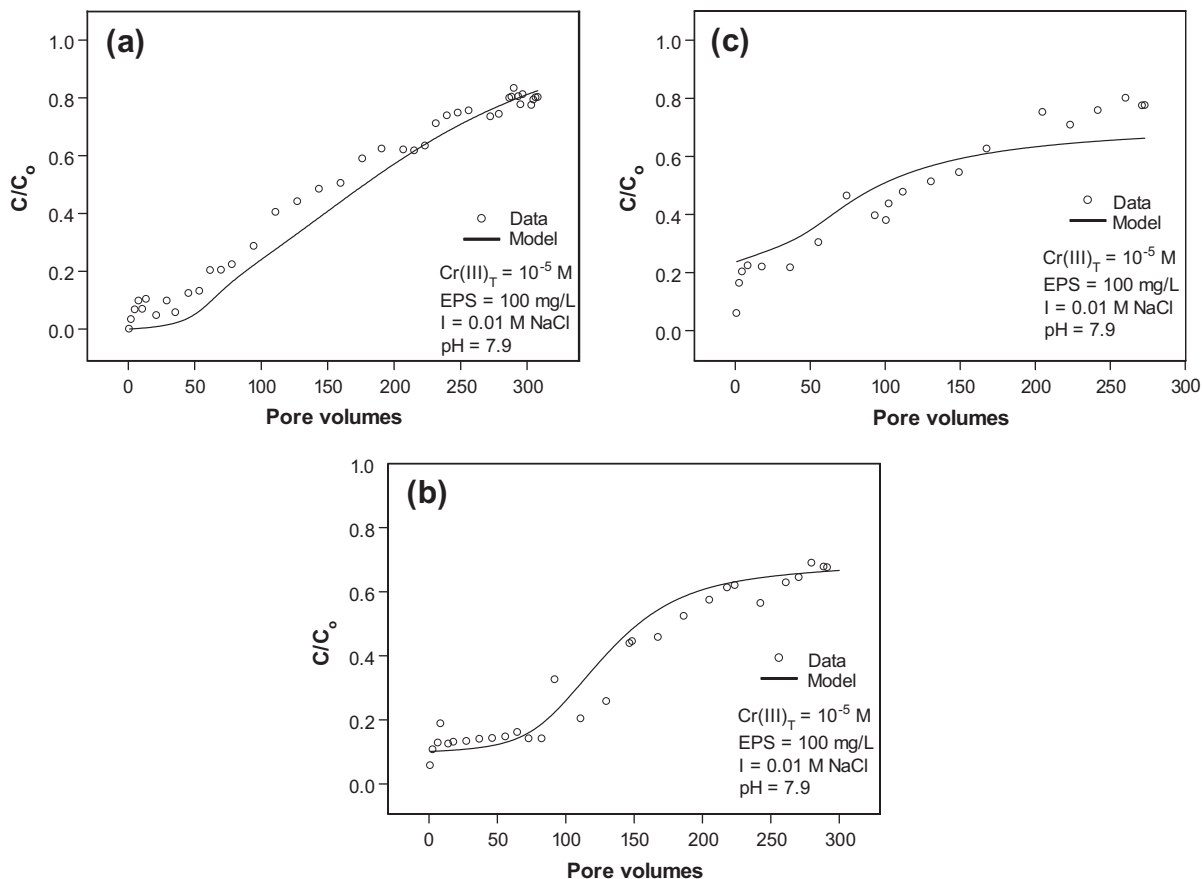
Several spectroscopic and modeling studies suggest that Cr(III) binds with metal oxides (e.g., silica) through the formation of monodentate (e.g.,  $\text{SO}_2\text{Cr}^{2+}$ ,  $\text{SO}_2\text{CrOH}^+$ ) surface complexes (Karthein et al., 1991; Fendorf and Sparks, 1994; Fendorf et al., 1994, 1996; Csoban and Joo, 1999). For example, the extended X-ray absorption fine structure spectroscopy (EXAFS) data by Charlet and Manceau (1992) and Fendorf et al. (1994) showed Cr(III) to form monodentate inner-sphere complexes with silica, goethite and hydrous ferrous oxide (HFO). Similarly, in a study with a multitude of molecular level experimental techniques, including diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy and high-resolution transmission electron microscopy (HRTEM), Fendorf and Sparks (1994) found that chromium(III) formed a monodentate surface complex on silica at surface coverages less than 20%, while at

greater surface coverages discrete chromium hydroxide surface clusters were discerned. Based on data gathered from electron spin resonance (ESR) and electron spin-echo envelope modulation (ESEEM) spectroscopies, Karthein et al. (1991) proposed bidentate ( $\text{SO}_2\text{Cr}$ ) surface complexes between Cr(III) and hydrous  $\delta\text{-Al}_2\text{O}_3$  surface. Here, a systematic evaluation of surface complexes between surface sites ( $\text{S}_1\text{OH}$ ,  $\text{S}_2\text{OH}$ ) and Cr(III) was performed, invoking the formation of monodentate and bidentate surface complexes with  $\text{Cr}^{3+}$  and surface sites. After a detailed examination of the model complexes between  $\text{Cr}^{3+}$  and all reasonable permutations of the surface sites, the best fit to the sorption data was obtained by postulating two surface reactions with reaction stoichiometries given below:



where  $j$  represents the strong ( $\text{SOH}_1$ ) or weak surface sites ( $\text{SOH}_2$ ),  $\text{S}_j\text{OCr}^{2+}$  and  $\text{S}_j\text{OCrOH}^+$  are the binary Cr(III) surface complexes.

Simulations of the Cr(III) sorption data are given in Fig. 1, and were accomplished using the solution phase Cr(III) hydrolysis reactions (Table 2) and surface-phase Cr reactions presented in Table 3. The surface reaction constants ( $\log K$ ) are optimized by simultaneously fitting the constants for the postulated surface reactions to the sorption data given in Fig. 1. Note that postulating surface reactions with only strong surface sites ( $\text{SOH}_1$ ) was adequate to describe the Cr(III) sorption data under variable chemical conditions (e.g., pH). This is not surprising since the spectroscopic data



**Fig. 4.** Observed and PHREEQC simulation of the breakthrough curves for EPS isolated from: (a) *P. aeruginosa* P16, (b) *P. stutzeri* P40 and (c) *P. putida* P18. All experiments were performed at pH 7.9, and had equivalent initial total EPS concentration of  $100 \text{ mg L}^{-1}$ , a total Cr(III) concentration of  $10^{-5} \text{ M}$  and constant ionic strength ( $I = 0.01 \text{ M NaCl}$ ). Symbols represent experimental data and lines represent non-electrostatic surface complexation model (SCM) predictions with the batch-derived SCM parameters given in Table 3. Solution phase EPS and Cr hydrolysis reactions are given in Tables 1 and 2.

gathered by Karthein et al. (1991) shows that Cr(III) complexation takes place only at specific surface sites.

### 3.3.2. Modeling EPS sorption

Several spectroscopic and modeling studies suggest that a variety of processes/mechanisms including ligand exchange, cation bridging and entropy driven physical adsorption (Lenhart and Honeyman, 1999; Kantar, 2007) play an important role on the sorption of natural organic matter (NOM) to mineral phases. Using Fourier transform infrared spectroscopy (FTIR), Fu and Quan (2006) found that the ligand exchange involving carboxylic functional group was responsible for the sorption of fulvic acid onto the surface sites of goethite and hematite. For phthalic acid/hematite system, Hwang et al. (2007) present infra red (IR) spectral evidence to support the formation of three fully deprotonated phthalate surface complexes (one outer-sphere complex and two inner-sphere complexes). Their results indicate that the two inner-sphere complexes were bidentate (chelating and bridging) structures involving both carboxylic groups. Similarly, in a CIR-FTIR spectroscopy involving salicylate sorption to goethite, Yost et al. (1990) found a surface chelate structure involving binding of the phenolic oxygen and one carboxylic oxygen with one surface iron atom. Benoit et al. (1993) found that sorption reactions for a series of monodentate and bidentate organic ligands are highly influenced by the number of functional groups on the ligand. As indicated in Fig. 2, the polyfunctional nature of EPSs studied was described by combining a discrete ligand approach with a non-electrostatic SCM. The four ligands invoked, HL<sub>1</sub>, HL<sub>2</sub>, HL<sub>3</sub> and HL<sub>4</sub> with pK<sub>a</sub> values of 4, 6, 8 and 10, respectively can operationally be defined as

carboxyl (HL<sub>1</sub>), phosphoric/carboxyl (HL<sub>2</sub>), phosphoric (HL<sub>3</sub>) and hydroxyl/amin/phenolic (HL<sub>4</sub>) based on their pK<sub>a</sub> values (Table 1). It is assumed that these ligands not only provide binding sites for complexation with cations in solution but also take part in complexation with soil surface sites (Lenhart and Honeyman (1999). The simulation of EPS sorption data required two different types of reactions with reaction stoichiometries as shown below:



where  $i$  represents the ligand type ( $i = 1-4$ ),  $S_j\text{L}_i$  and  $S_j\text{OHL}_i^+$  are the binary EPS surface complexes with surface sites.

In this approach, the number of fitting parameters depends on the number, and the types of sites used in the model. The apparent stability constants for these surface reactions were determined with FITEQL by the best fit to the sorption data given in Fig. 2, with the log  $K$  values as well as the model stoichiometry given in Table 3. The EPS acid-base reactions used in the simulations are given in Table 1. Note that the accurate description of EPS sorption requires a two-site surface chemical model with surface reactions involving the ligands with pK<sub>a</sub> values of 4 (HL<sub>1</sub>) and 6 (HL<sub>2</sub>) indicating that the carboxylic and phosphoric sites are the primary functional groups responsible for the sorption of EPS onto soil minerals under the experimental conditions studied (e.g., pH).

### 3.3.3. Modeling Cr(III) sorption to soil in the presence of EPS

The effects of EPS on Cr(III) sorption to soils was modeled by combining discrete ligand model for Cr(III) and proton binding by

**Table 3**  
Surface reactions for Cr(III) and EPS.

Reaction <sup>a,b</sup>	Log <i>K</i> ( <i>I</i> = 0)	WSOS/DF <sup>c</sup>
<i>Cr(III) surface-phase reactions</i>		
$S_1OH + Cr^{3+} = S_1OCr^{2+} + H^+$	2.015 <sup>d</sup>	0.241
$S_1OH + H_2O + Cr^{3+} = S_1OCrOH^+ + 2H^+$	-4.073 <sup>d</sup>	
<i>P. aeruginosa P16 EPS surface-phase reactions</i>		
$S_1OH + HL_1 = S_1OHL_1^- + H^+$	0.73 <sup>e</sup>	0.412
$S_1OH + HL_2 = S_1OHL_2^- + H^+$	-0.34 <sup>e</sup>	
$S_2OH + HL_2 = S_2L_2 + H_2O$	4.825 <sup>e</sup>	
$S_1OH + Cr^{3+} + HL_1 = S_1OCrL_1^+ + 2H^+$	2.5 <sup>d</sup>	
$S_2OH + Cr^{3+} + HL_1 = S_2OCrL_1^+ + 2H^+$	0.3 <sup>d</sup>	
<i>P. putida P18 EPS surface-phase reactions</i>		
$S_1OH + HL_1 = S_1OHL_1^- + H^+$	4 <sup>e</sup>	0.31
$S_2OH + HL_2 = S_2L_2 + H_2O$	4.6 <sup>e</sup>	
$S_2OH + HL_1 = S_2OHL_1^- + H^+$	0.65 <sup>e</sup>	
$S_2OH + Cr^{3+} + HL_1 = S_2OCrL_1^+ + 2H^+$	2.45 <sup>d</sup>	
$S_2OH + Cr^{3+} + HL_3 = S_2OCrL_3^+ + 2H^+$	-2.0 <sup>d</sup>	
<i>P. stutzeri P 40 EPS surface-phase reactions</i>		
$S_1OH + HL_1 = S_1OHL_1^- + H^+$	1.603 <sup>e</sup>	0.21
$S_1OH + HL_2 = S_1OHL_2^- + H^+$	0.042 <sup>e</sup>	
$S_2OH + HL_2 = S_2L_2 + H_2O$	5.764 <sup>e</sup>	
$S_1OH + Cr^{3+} + HL_1 = S_1OCrL_1^+ + 2H^+$	3.8 <sup>d</sup>	
$S_2OH + Cr^{3+} + HL_1 = S_2OCrL_1^+ + 2H^+$	1.6 <sup>d</sup>	

<sup>a</sup> Total site density of 0.133 mmol g<sup>-1</sup> (from Kantar et al., 2009).

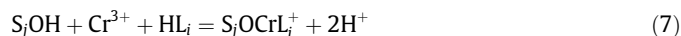
<sup>b</sup> S<sub>1</sub>OH and S<sub>2</sub>OH represent the strong (5% of the total sites) and weak surface sites, respectively.

<sup>c</sup> In all cases, the values of the weighted sum of squares divided by degrees of freedom (WSOS/DF) for the models were less than 1. Herbelin and Westall (1999) suggest that the values of WSOS/DF between 0.1 and 20 are required for the model to be acceptable.

<sup>d</sup> Based on model fit of chromium(III) sorption edge given in Fig. 1.

<sup>e</sup> Based on model fit EPS sorption edge given in Fig. 2.

EPS with the binary Cr(III) and EPS surface chemical models (Table 3). However, simulations with the combined binary systems underestimated the Cr(III) sorption. The discrepancy between the model and actual sorption data can be explained through the formation of ternary surface complexes. For example, Lenhart and Honeyman (1999) presented evidence for the formation of ternary surface complexes in ternary hematite/U(VI)/humic acid system. Using an in situ and ex situ infrared spectroscopy, Orsetti et al. (2006) observed that the presence of Pb(II) led to an increase in humic acid sorption to goethite due to formation of ternary goethite-Pb-surface complex. Similarly, in a spectroscopic study (Electron Spin Resonance and Electron Spin-Echo Spectroscopies), Karthein et al. (1991) proposed a ternary inner-sphere surface complex (SOCrL) for the description of chromium(III) sorption to hydrous aluminum oxide in the presence of oxalate (L). Thus, we postulated Type A ternary surface complexes (soil/Cr/EPS) at soil surface sites:



where S<sub>j</sub>OCrL<sub>i</sub><sup>+</sup> is the ternary surface complex. The apparent binding constants (log *K*) for Eq. (7) were determined by simultaneously fitting the constants for reactions to the experimental data with 3 g L<sup>-1</sup> soil, 10<sup>-5</sup> M Cr(III) and 50 mg L<sup>-1</sup> EPS given in Fig. 1. Table 3 shows the best fit log *K* values for these ternary surface reactions. Fig. 1 shows the resulting simulations of Cr(III) sorption to soil in the presence of EPS including ternary surface complexes to supplement the binary system models given in Table 3. Note that the new model with the ternary surface complexes accurately simulates the effect of EPS on Cr sorption under variable chemical conditions, with no variations in constants.

### 3.3.4. Sensitivity analysis for formation constants of surface reactions

A sensitivity analysis was also performed with FITEQL to determine how well the experimental data constrains the constants

(log *K*) presented by the model given in Table 3. Here, the values of log *K* were varied systematically and the WSOS/DF plotted on the y axis (Supporting information, Fig. S1). Parameter sensitivity coefficients were determined for the sensitivity of Cr sorption with respect to log *K* values using a method outlined by Zheng and Bennett (2002). The results indicate the amount of Cr sorbed is most sensitive to the formation constants for surface reactions involving strong surface sites (S<sub>1</sub>OH) due to the fact these reactions are the most dominant Cr surface reactions. For example, among all the surface reactions presented in Table 3, Cr sorption is most sensitive to the equilibrium constant of the surface complexation reaction for S<sub>1</sub>OCr<sup>2+</sup>, which is also the surface complex that is present at the highest concentration. Additional details on sensitivity analysis can be found on Pages S2–7 of Supporting information.

### 3.3.5. Transport predictions with batch-derived surface chemical model

Predictive ability of non-electrostatic SCM parameters directly derived from batch sorption data was tested in a reactive transport code PHREEQC using the reactions given in Tables 1–3. Figs. 3 and 4 show a comparison of observed breakthrough curves for Cr(III) and EPS with the model predictions for 10<sup>-5</sup> M Cr(III) in the absence or presence of 100 mg L<sup>-1</sup> EPS at 7.9. Note that the batch derived non-electrostatic SCM parameters, with no variations in constants, accurately predict the breakthrough curves for both Cr(III) (Fig. 3) and EPS (Fig. 4).

A number of studies suggest that the discrete ligand models combined with electrostatic SCMs (e.g., triple layer) can be effectively used to accurately describe the influence of NOM on metal ion sorption to soils and minerals (Lenhart and Honeyman, 1999; Weirich et al., 2002). However, the application of such models to complex mineral systems requires detailed information on the distribution and composition of subsurface soils as well as specific interactions between soil components and ligand/NOM (Davis et al., 1998). On the other hand, the semi-empirical surface chemical model as used here is relatively simple, and can be more easily incorporated into reactive transport codes compared to most electrostatic SCMs since it does not require electrostatic correction factors for surface and metal-NOM complexes (Kantar et al., 2009). However, such non-electrostatic models are only applicable to the site specific materials and environmental conditions used in the simulations due to the fact they may not provide an accurate representation of complexation and sorption reactions at the molecular level (Davis et al., 1998).

## 4. Conclusions

Subsurface systems contaminated with Cr(VI) can be remediated with microbial Cr(VI) reduction by soil microorganisms. However, toxic substances such as Cr(VI) may stimulate the production of exopolymeric substances (EPS) by some soil microorganisms in subsurface systems. In this study, laboratory batch sorption and column experiments were performed to better understand the role of EPS extracted from *P. putida* P18, *P. aeruginosa* P16 and *P. stutzeri* P40 on Cr(III) mobilization or immobilization in heterogenous subsurface soils under variable chemical conditions (e.g., pH). Our batch results indicate that while microbial EPS had no effect on Cr(III) sorption at pHs < 5, it led to a decrease in Cr(III) sorption under slightly acidic to alkaline pH range. Column experiments performed at pH 7.9 suggest that the presence of EPS increased the mobility of Cr(III) due to the formation less sorbing and highly soluble Cr-EPS complexes and competition of EPS against Cr for surface sites. The extent of Cr sorption and transport also depends highly on the type of EPS used with more Cr(III) being



mobilized in the presence of EPS from *P. aeruginosa* P16 compared to the EPS from *P. putida* P18 and *P. stutzeri* P40.

A two-site non-electrostatic surface chemical model (SCM) combined with a discrete ligand approach in EPS is conceptualized as being composed of four different monoprotic acids with arbitrarily assigned  $pK_a$  values of 4, 6, 8 and 10 was used to model the effects of EPS on Cr(III) sorption and mobility in subsurface soils. The SCM parameters directly derived from batch sorption data improved our ability to accurately simulate the effects of Cr(III)/EPS complexes on Cr(III) transport. To simulate the ternary soil/Cr(III)/EPS systems, the following interactions need to be explicitly considered: (1) proton and Cr(III) binding by EPS, (2) EPS binding by soil minerals, (3) Cr(III) binding by soil minerals and (4) ternary Cr(III)–EPS surface complexes. The good agreement between experimental results from column experiments and model predictions with batch-derived parameters indicates that the conceptual models based on a non-electrostatic GC SCM approach can be used as an important tool to design and monitor in situ microbial remediation techniques for the treatment of subsurface system contaminated with Cr(VI).

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2010.11.001.

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