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Cd sequestration by bacteria–aluminum hydroxide composites

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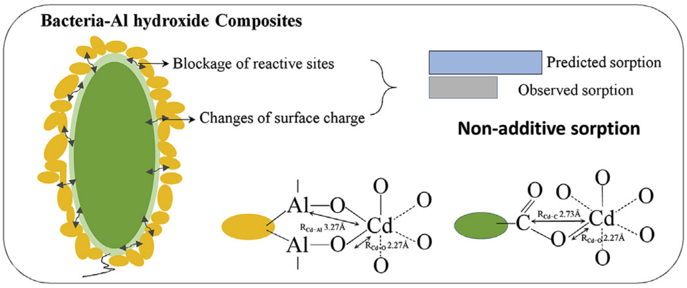
HIGHLIGHTS

- First report of Cd sorption by bacteria–aluminum hydroxide composite.
- Changes of surface charge and sorption site account for the non-additive sorption.
- Cd sequestration in the composites depends on pH and bacteria-to-Al mass ratio.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbe-associated aluminum (Al) hydroxides occur naturally in aquatic and geologic environments and they might play a crucial role in the sequestration of trace metals because these composite solids comprise both reactive mineral and organic surface, but how they do it still remains unknown. Here we replicate Al hydroxide organo–mineral composite formation in soil and sediments by synthesising composites using *Pseudomonas putida* cells, during coprecipitation with Al hydroxide. Morphological and ATR-FTIR analysis show closely attached nano-sized Al hydroxides on the bacterial surface. For composites dominated by either bacteria or Al hydroxide, an enhanced metal adsorption is observed on the composites than on pure Al hydroxide at $pH < 6$. Cd uptake by the mainly Al mineral composite is approximately additive, i.e., the sum of the end-member metal adsorptivities, whereas that on the mainly bacteria composite is non-additive. This non-additive sorption is not only due to the blockage of surface reactive sorption sites, but more importantly the changes of surface charge when bacteria and Al mineral are interacted. EXAFS results show that Cd is predominately sorbed as a bidentate corner-sharing complex on the amorphous Al hydroxide surface and a carboxyl-binding on the bacterial surface. This study has important implications for understanding both Al and trace metal cycling in microbe-rich geologic environments.

1. Introduction

Amorphous hydroxide minerals of iron, aluminum and manganese (Fe/Al/Mn) occur naturally in aquatic and geologic environments (Trivedi and Axe, 2001). They have small particle size,

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large surface area and abundance of hydroxyl functional groups, which make them effective scavengers of dissolved metals (Lee et al., 2002; Rahmanian et al., 2018). In the soil environment, soluble Fe, Al and Mn ions are commonly found to precipitate with the organic substances such as small molecule organic ligands, dissolved organic matter, bacteria or/and extracellular polymeric substances (EPS), to produce organic–hydroxide assembles (Mikutta et al., 2011; Pena et al., 2011; Chen et al., 2014). These products comprise both mineral and organic surfaces that can participate in metal sorption reactions (Martinez et al., 2003; Soltani et al., 2017).

Bacterial cell walls contain a variety of surface organic metal-binding groups of carboxyl, phosphate, amino, sulfydryl and hydroxyl (Fein et al., 1997), therefore, they can greatly influence the interfacial binding behaviors of trace metals. Heretofore, numerous efforts have been made to clarify the binding characteristics of trace metals on the Fe hydroxide–bacteria composites. Small et al. (1999) observed significant enhanced strontium sorption on the bacteria–Fe hydrous oxide composites over pure Fe oxides. Moreover, there was a reduction in the bacterial surface binding sites in the composites, which caused a lower sorptive capacity than was calculated. Kulczycki et al. (2005) showed that the sorption of Cd and Pb by the *Bacillus subtilis*/*Escherichia coli*–ferrihydrite composites was strongly pH-dependent, and the observed composites adsorptivity was lower than predicted upon available site additivity. They also proposed a blockage of reactive surface sites by the interaction of bacteria and ferrihydrite. Works by Moon and Peacock (2012) have elucidated the molecular binding mechanism of Cu on the ferrihydrite–*Bacillus subtilis* composites, where Cu was sorbed as a bidentate edge-sharing complex with the ferrihydrite fraction and a monodentate Cu-Carboxyl complex with the bacterial fraction of the composites. Moreover, composites composed mainly of bacteria expressed significant deviation from Cu sorption additivity whereas that on the mainly ferrihydrite composites could be modelled in a component additivity approach (Moon and Peacock, 2013). Recently, Franzblau et al. (2016) discovered the bacterial ligand–Cu–oxide ternary surface complexes formation when they investigated Cu removal by the composites of *Anoxybacillus flavithermu* and Fe oxide during the addition, oxidation and hydrolysis of Fe^{2+}. Moreover, Cu^{2+} was reduced to Cu^{+} by the bacterial surface ligands and the oxidation of sorbed Fe^{2+}.

Aluminum is the most abundant metallic elements in the earth's crust and occurs mostly in its stabilized forms (Grim, 1982). However, under acidic conditions or the presence of strong ligands, elevated concentrations of Al may be mobilized to the aquatic systems (Sánchez-España et al., 2016). In acid soils and peatlands, soluble Al ions exist in soil waters, and interact frequently with various soil components such as the organic substances (Helmer et al., 1990; Mikutta et al., 2011). In these low pH environments, heavy metal pollution is more prominent (Liao et al., 2005; Du et al., 2016a). It is critical, therefore, to study the aluminum–organic–heavy metal system, in order to assess both the cycling and availability of Al and heavy metals. Coprecipitation of Al with bacterial biomass is a common process in microbe-rich soil-root interface (Mikutta et al., 2011). The formation of these biogenic aluminum hydroxides with microbial cell surface and extracellular polymeric substance (EPS), therefore, play a substantial role in the stabilization of trace metals. Surprisingly, little is known about the environmental reactivity of bacteria–Al hydroxide composites towards trace metals, compared to the well-studied bacteria–iron hydroxide composites (Small et al., 1999; Kulczycki et al., 2005; Moon and Peacock, 2012), despite that the prevalence of dissolved and colloidal Al species exceed that of Fe in soil pore water due to the higher solubility of Al minerals. Recently, Mikutta et al. (2011) suggested that coprecipitation of EPS with Al fundamentally change the composition and reactivity of the EPS-Al precipitates where the organic P compounds were preferentially bound, and the sorbed EPS was stabilized against microbial decay. However, a gap still remains in our understanding of how bacteria–Al hydroxide interactions affect the sorption behaviors towards trace metals such as cadmium.

In this study, we investigate the binding characteristics of Cd on bacteria–Al hydroxide composites by batch sorption experiments coupled with Cd K-edge EXAFS. Our bacteria–Al hydroxide composites are synthesized via rapid Al^{3+} hydrolysis in the presence of *Pseudomonas putida* cells, a heavy metal-resistant bacterium isolated from a contaminated soil (Du et al., 2017b). We provide the first evidence for Cd sequestration by both the mineral and bacterial fraction in an amorphous Al hydroxide–organic composite; we show that the distribution of total sorbed Cd between the end-member components is a function of the bacteria-to-Al mass ratio and environmental pH. The new results present here will help us to develop thermodynamic models for Cd sorption on Al hydroxide composites, and also contribute to a new understanding of the biogeochemical cycling of both Al and Cd in soil and sediments.

2. Materials and methods

2.1. Amorphous Al hydroxide and bacteria–Al hydroxide composites preparation and characterization

Pseudomonas putida X4 (CCTCC M209319) is an aerobic Gram-negative bacterium, preserved in the China Center for Type Culture Collection (CCTCC; http://www.cctcc.org/). It is a heavy metal-resistant bacterium isolated from a multi-metal contaminated soil (Du et al., 2017b). The bacterial cells were cultured in a Luria-Bertani medium ($10.0\,g\,L^{-1}$ tryptone, $5.0\,g\,L^{-1}$ yeast extract, $5.0\,g\,L^{-1}$ NaCl) as reported in Du et al. (2016b) following the methods of Wu et al. (2014). The cell pellets were separated from the nutrient solution by centrifugation and were rinsed with the electrolyte (0.1 M KNO_3). The resulting bacterial cells were resuspended in the electrolyte and stored at 4 °C before the preparation of bacteria–Al hydroxide composites and metal sorption experiments.

Amorphous Al hydroxide (HAO) and bacteria–Al hydroxide composites were prepared via hydrolysis of Al^{3+} salt solution in the absence and presence of bacterial cells, respectively. Briefly, pure HAO were prepared by dope-wise addition of 1 M KOH to 0.1 M aluminum nitrate solution until pH 7, whereas bacteria–Al hydroxide composites were synthesized by hydrolysis 0.1 M aluminum nitrate solution made with *Pseudomonas putida* suspensions. The mixed bacteria–Al^{3+} solutions were stirred for ~30 min before the addition of 1 M KOH. Two bacteria–HAO composites were prepared: one where the dry mass of bacterial cells is ~6.7 times the mass of Al, and a second where the dry mass of bacterial cells is ~1.3 times of the mass of Al. The products of the bacteria–HAO composites, therefore would be approximately 70:30 and 30:70 bacteria:HAO mass ratios, respectively [assuming the structure of HAO of $Al(OH)_3$ (Huang et al., 1997; Mikutta et al., 2011)]. Herein, the 70:30 bacteria:HAO composite was abbreviated to PAl_70:30 whereas the 30:70 one was abbreviated to PAl_30:70. The HAO and bacteria–HAO composites were washed with deionized water to remove exceed salt and were resuspended in the electrolyte. The fresh precipitates were employed in the metal sorption experiments.

Atomic force microscope (AFM) were applied to obtain the morphology of the HAO, bacteria and bacteria–HAO composites. AFM can provide nanoscale surface characterization of bacteria–mineral associates with minimal pretreatment compared