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Adhesion of *Escherichia coli* to nano-Fe/Al oxides and its effect on the surface chemical properties of Fe/Al oxides

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ABSTRACT

We investigated the adhesion of *Escherichia coli* to α-Fe_2O_3 and γ-Al_2O_3 and the effects of adhesion on the surface properties of the oxides in batch experiments, where we conducted potentiometric titration, zeta potential measurements, and FTIR spectroscopy. The adhesion isotherms fitted a Langmuir equation well. γ-Al_2O_3 had a higher adhesion capacity than α-Fe_2O_3 because of the higher positive charge on γ-Al_2O_3. The adhesion of *E. coli* to Fe/Al oxides decreased with increasing pH. Adhesion increased with increasing NaCl concentration, reaching its maximum at 0.05 M for α-Fe_2O_3 and at 0.1 M for γ-Al_2O_3, after which it decreased with further increases in NaCl concentration. Therefore, the electrostatic force plays an important role in the adhesion of *E. coli* to Fe/Al oxides. The zeta potential–pH curves of the binary-system fell between that for bacteria and those for Fe/Al oxides. Thus, overlapping of the diffuse layers of the electric double layers on the negatively-charged *E. coli* and positively-charged Fe/Al oxides reduced the effective surface charge density of the minerals and bacteria. *E. coli* adhesion decreased the point of zero salt effect and the isoelectric point of the Fe/Al oxides. The FTIR spectra indicated that non-electrostatic force also contributed to the interaction between *E. coli* and Fe/Al oxides, in addition to the electrostatic force between them.

1. Introduction

There is an abundance of microorganisms in the soil, approximately 80%–90% of which are tied to the solid surface [1]. In soil and water environments, the interactions between bacteria and minerals play important roles in the transport and fate of bacteria, as well as the in situ remediation of waste water [2–5]. In the saturated region, bacterial transport is mainly dominated by the adhesion of bacteria to matrix surfaces [5,6]. Thus, bacteria move rapidly in soil columns with low clay content [7]. The interaction between bacteria and soil has a significant effect on the physical, chemical and biological properties of soil, such as mineral weathering, organic matter decomposition, and aggregate formation [8,9]. Organic and inorganic pollutants can be adsorbed to bacterial surfaces, and these pollutants can move with the bacteria in soil and water. Thus, the interactions between bacteria and soil can affect the mobility and transformation of the pollutants in soil and water. For example, bacteria may affect the bioavailability, speciation, and mobility of heavy metals in soil [10]. The adhesion of bacteria can also be used for mineral flotation [11].

Many factors can affect the interactions between bacteria and minerals. Electrostatic interactions, van der Waals forces, hydrophobicity, surface tension and surface roughness dominate the deposition of bacteria on mineral surfaces in water–mineral systems [12–16]. Bacterial adhesion influences bacterial surface properties, soil solid properties and soil solution composition [17–22]. The force that dominates the deposition of bacteria varies with the ionic strength of the medium [23]. The size of soil particles also affects bacterial adhesion. The silt fractions have the highest affinity for *Pseudomonas putida* among all soil size fractions, while the soil organic matter plays a suppressive role in the adhesion of *P. putida* onto soil particles [24]. *Escherichia coli* is preferentially attached onto 16–30 μm particles of a clay loam soil. For soil particles > 2 μm, *E. coli* showed at least 3.9 times greater preference to association with the 16–30 μm than any other fractions [25]. Non-ionic surface active agents are preferentially adsorbed by the clay fraction of soil, so they can compete with *E. coli* for adsorption sites on soil clay, which decreases the retention of *E. coli* by soils [26]. In addition, steric hindrance plays a role in the interactions between bacteria and minerals. Long chain bacterial surface polymers generate a repulsive force because polymers resist compression, which prevents the bacterial core from reaching the substratum. However, surface polymers can also be attractive because of their small dimensions and since their charged surface

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polymers rarely encounter any electrostatic barriers to their adhesion, which essentially reduces the electric double layer repulsion and forms a bridge between surfaces.

The interactions between bacteria and minerals have been investigated extensively using dolomite, apatite [11], hematite, corundum, quartz, kaolinite, and montmorillonite [27–30]. However, few studies have investigated the interactions between bacteria and Al oxides, or the interactions between bacteria and nano-Fe/Al oxides. Moreover, the effects of bacterial adhesion on the surface chemical properties of minerals are not well understood. Therefore, we tested nano-particles of α-Fe_2O_3 and γ-Al_2O_3 to investigate the adhesion of *E. coli* under different conditions and the effects of *E. coli* adhesion on the surface chemical properties of Fe/Al oxides. Because Fe/Al oxides are the main components of variable charge soils from tropical and subtropical regions and nano-oxides exist in these types of soils [31,32], the results obtained in the present study will help our understanding of the interaction between *E. coli* and variable charge soils.

2. Materials and methods

2.1. Mineral preparation

The nano-scale α-Fe_2O_3 and γ-Al_2O_3 mineral powders used in the experiments were obtained from the Nanjing Emperor Nano Material Co., Ltd (Nanjing, China). The diameters of α-Fe_2O_3 and γ-Al_2O_3 were not more than 30 nm and 40 nm, respectively. These oxides were washed using 95% ethanol until the supernatant exhibited a constant electric conductivity of 10 μS/cm for α-Fe_2O_3 and 0.5 μS/cm for γ-Al_2O_3. The oxides were then dried at 60 °C and ground to pass through a 100-mesh sieve (0.15 μm) before storage and subsequent utilization. The isoelectric points (IEP) of α-Fe_2O_3 and γ-Al_2O_3 were 6.73 and 8.65, respectively, in an electrolyte solution of 1 mM NaCl at 25 °C.

2.2. Bacteria preparation

The *E. coli* strain (species number: 1.2389) used in the experiments was obtained from the China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences. *E. coli* was initially cultured in beef extract peptone for 15.5 h at 37 °C. Cells were collected from the nutrient medium by centrifugation at 8000 × *g*, after which they were rinsed with distilled deionized (DDI) water three times. Following the centrifugation at 8000 × *g*, the wet weight of washed cells was determined, after which the washed cells were suspended in DDI water. The suspension was then diluted with DDI water to concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL. Next, the absorbance of the *E. coli* suspension was determined using an ultraviolet-visible spectrophotometer (Mapada, UV-3000) at 420 nm and a standard curve of the absorbance against the concentrations of *E. coli* was obtained. Different calibration curves were used for different salt concentrations and pH values because the size of the bacteria will differ in different solutions and give a different absorbance. The enumerated density of the *E. coli* suspension with a concentration of 2.5 mg/mL was about 1.144×10^9 CFU/mL according to the plate count method.

2.3. Bacterial adhesion experiments

α-Fe_2O_3 (35 mg) was added to 10 mL of bacterial suspension while γ-Al_2O_3 (15 mg) was added to 20 mL of bacterial suspension. The suspensions were diluted to produce a total volume of 25 mL using DDI water. The pH of the mixtures was adjusted to 3.5–10.0 using NaOH or HCl and the mixture was shaken with an end-over-end shaker at 20 rpm and 25 °C for 70 min because the preliminary experiments indicated that the adhesion of *E. coli* could reach reaction equilibrium after 60 min. Isolation of unattached bacteria was accomplished by injecting 3 mL of sucrose solution (60% by wt.) into the bottom of the mixture [24]. The suspension was then centrifuged at 4000 × *g* for 10 min, after which unattached bacteria in the supernatant were removed and measured by spectrophotometry at 420 nm. Next, the amount of the adhered bacteria was calculated by subtracting the final unattached bacteria from the initial bacteria added. Any reduction in the attached bacterial concentration was assumed to have been caused by *E. coli* adhesion to Fe/Al oxides. Similar experiments were carried out in the presence of 0, 0.01, 0.05, 0.1, 0.3, and 0.5 M NaCl solution to study the effects of electrolyte concentration on adhesion.

The adhesion of *E. coli* to the tube was examined at pH 3.68, 6.03, 6.74, 8.23, and 9.49. When 26.94 mg of *E. coli* was added, the amount of the bacteria adhered to the tube was less than 1% of that added. Similarly, when 3.06, 5.96, 8.77, 11.64, and 14.48 mg of *E. coli* were added, the amount of bacteria adhered to the tube was also less than 1% of that added at pH 6.12. Thus the adhesion of *E. coli* to the tube is negligible. Any reduction in bacterial concentrations in the suspensions was assumed to be caused by *E. coli* adhesion to Fe/Al oxides.

2.4. Zeta potential measurements

For single colloidal suspensions, *E. coli*, α-Fe_2O_3 or γ-Al_2O_3 were suspended in 1 mM NaCl solution at a concentration of 0.25 mg/mL. The Fe/Al oxides were then dispersed by treatment in an ultrasonic bath at 25 °C for 1 h, after which the pH values of the suspensions were adjusted to 4–10 using NaOH or HCl several times and maintained for 48 h. The bacterial suspensions were dispersed by magnetic stirring at 25 °C for 1 h, after which the pH values of the suspensions were adjusted to 4–10 using NaOH or HCl several times and maintained for 4 h. After the pH values of all suspensions were measured, the zeta potentials were determined using a Zetaplus (Brookhaven Instruments Corporation, USA). For the binary-colloid suspensions, a single suspension of *E. coli*, α-Fe_2O_3, or γ-Al_2O_3 was prepared in a similar way. The pH values of suspensions containing α-Fe_2O_3 or γ-Al_2O_3 were adjusted to 4–10 using NaOH or HCl several times and then maintained for 44 h, after which the suspension of *E. coli* was added to the mineral suspensions and subjected to magnetic stirring to produce a total concentration of 0.25 mg/mL with the mass ratios of *E. coli* to Fe/Al oxides of 4:1, 3:2, 2:3, and 1:4. The pH values of the binary-colloid suspensions were adjusted to 4–10 using NaOH or HCl several times and maintained for 4 h, after which the zeta potentials for binary systems containing *E. coli* and α-Fe_2O_3 (or γ-Al_2O_3) were measured using the same method.

2.5. Determination of the surface charge

The surface charges were determined by potentiometric titration [33]. A colloid suspension containing 1 mM or 0.1 M NaCl as the background electrolyte was prepared at a concentration of 20 mg/mL α-Fe_2O_3 or γ-Al_2O_3 and the surface charges were determined using an automatic potentiometric titrator (Radiometer TIM 854). First, the suspensions were titrated to pH 3.0 for α-Fe_2O_3 and pH 4.0 for γ-Al_2O_3 using HCl solution. After equilibration for 30 min, the suspensions were titrated to pH 10.0 using NaOH solution. For the binary-colloid suspensions, a single suspension of *E. coli*, α-Fe_2O_3, or γ-Al_2O_3 was prepared first. The suspension of *E. coli* was mixed with the suspension of α-Fe_2O_3 or γ-Al_2O_3 to give a total composite concentration of 20 mg/mL where the mass ratio of *E. coli* to the Fe/Al oxides was 1:1. The same titration procedures as the single Fe/Al oxide system were then used to obtain charge–pH curves for the binary systems containing Fe/Al oxides