Impacts of Goethite Particles on UV Disinfection of Drinking Water

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A unique association between bacterial cells and small goethite particles (~0.2 by 2 μm) protected Escherichia coli and Pseudomonas putida from UV inactivation. The protection increased with the particle concentration in the turbidity range of 1 to 50 nephelometric turbidity units and with the bacterium-particle attachment time prior to UV irradiation. The lower degree of bacterial inactivation at longer attachment time was mostly attributed to the particle aggregation surrounding bacteria that provided shielding from UV radiation.

Particles may inhibit chemical disinfection of water and wastewater and enhance the regrowth of pathogens in drinking water distribution systems (14, 26, 27), thus causing outbreaks of waterborne diseases (10, 29). Mechanisms for the reduced chemical disinfection efficiency, analogous to the biofilm system (3, 4, 8), could be through colonization of particle surfaces by bacteria, where the disinfectant is present at a lower concentration due to (i) the high reactivity of particle surfaces for disinfectant degradation and (ii) limitation of mass transfer to the interface. Particle-attached bacteria are known to be more resistant to chemical disinfection than suspended bacteria (23, 30).

UV radiation has been increasingly used in the United States as an alternative approach for drinking water disinfection, promoted by the need of reducing potentially carcinogenic disinfection by-products (11, 21, 22, 31). Similar to chemical processes, UV disinfection is also negatively affected by the presence of suspended particles (6, 15, 19). However, particles can protect bacterial cells from UV radiation via shielding, absorbing, scattering, and blocking (25, 31), not necessarily through bacterial attachment to particles as in chemical disinfection. Microorganisms can be present in the “shadow” cast by attaching to particles, thus allowing escape from full exposure to the UV radiation (6, 9, 15). The protective effects against UV inactivation of attached bacteria depend on particle sizes (9, 15) and turbidity levels (6, 7). Past research on particle-associated bacteria and UV shielding mostly used particles greater than 10 μm (12, 28).

Little information is available on the effects of small-sized particles on bacterial inactivation in drinking water. Predominant mechanisms of UV attenuation in such systems are unknown. Although research conducted using wastewater provides useful information on UV attenuation in drinking water, particles in drinking water and wastewater are different in nature, likely affecting UV transmission differently (6). For example, suspended particles in water supplies can be smaller and have more mineral components, including clays and metal oxides. Studies have shown that Na-montmorillonite clay can neither prevent viruses from being inactivated by chlorine (26) nor protect bacteriophage (MS2) from UV disinfection at low concentrations (24). Very little information is available on the effect of other clays and mineral oxides such as goethite (α-FeOOH), which is one of the most common metal oxides present in natural systems and can also be produced by corrosion of drinking water distribution systems. Goethite particles are known to enhance the formation of free radicals (17) but do not protect Sphingomonas bacteria from inactivation by chlorine in drinking water, unlike the particles in wastewater that inhibited chlorine disinfection (12). The objective of this work was to investigate the effect of freshly generated small-sized goethite particles on UV disinfection of drinking water.

Two strains of bacteria were employed in this study: Escherichia coli B (ATCC catalog no. 11303, kindly provided by the Missouri Department of Health) and Pseudomonas putida (ATCC catalog no. 23483). E. coli is the predominant fecal coliform in the environment, and its presence is used to indicate whether water is contaminated, and P. putida is also widely present in natural systems. E. coli was grown at 30°C on LB broth (Miller), and P. putida was grown at 26°C on modified LEP medium as described previously (1, 5). The pure strain suspension was prepared by harvesting cells using centrifugation (8,000 × g for 20 min at 4°C), followed by washing with sterile phosphate-buffered saline (137 mM NaCl, 2.5 mM KH₂PO₄, 6.9 mM K₂HPO₄, pH 7.2) to eliminate broth nutrients. Cells were resuspended in phosphate-buffered saline and incubated for 24 h at 20°C to acclimate them to low-nutrient conditions prior to use.

Goethite (α-FeOOH) particles were synthesized by reaction of Fe(NO₃)₃ with KOH, following a previously reported procedure (16). The precipitate was washed with 0.01 M HNO₃ solution three times and then Milli-Q water three times. The resulting yellow-brownish precipitate was resuspended in 240 ml of Milli-Q water and stored as a 1 M goethite stock suspension. Scanning electron microscopy (SEM) images showed that the particles were rod shaped, mostly with a width of around 0.2 μm and a length of 2 μm.

The goethite-cell suspensions were prepared by mixing 250 ml of the following constituents for 0.5 to 24 h (20°C, 220 rpm) in a series of conical flasks: (i) different concentrations of goethite suspension with measured turbidities of 0, 2, 10, 20, 40, and 100 nephelometric turbidity units (NTU), respectively,
and (ii) a bacterial suspension ($1 \times 10^9$ cells/ml) acclimated to low-nutrient conditions. The final concentrations of goethite particles were approximately 0, 1, 5, 10, 20, and 50 mg/liter with a turbidity contribution of 0, 1, 5, 10, 20, and 50 NTU, respectively.

UV inactivation experiments were performed by dosing a petri dish containing 20 ml of goethite-cell suspension with a UV lamp (15 W; Spectronics Corp.) for a designated time under stirring conditions after cells and goethite particles were mixed for 0.5 to 24 h. Enumeration of cultivable bacteria was performed by pour plating with 1.0-ml samples of appropriate dilutions into the nutrient agar. Dispersion of attached or aggregated bacteria was performed by sonicating subsamples (FS20; Fisher Scientific) for 60 s or by mixing on a Vortex Genie. Since the results of both mixing methods were similar in our preliminary tests, only the Vortex Genie mixing was subsequently used in this study. Every experiment had three replicates, and the average results are presented in this report.

SEM analysis was used to examine bacterial cell attachment to particles after mixing biological cells and particles for different times. The goethite-cell suspension was removed for primary fixation with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3), washed with the buffer solution, dehydrated with an increasing ethanol concentration series, mounted onto SEM stubs, and sputter coated with platinum (20 nm for 60 s). The specimens were examined in a Hitachi S-4700 field emission scanning electron microscope. Approximately 50 bacterial cells were randomly selected to determine the degree of bacterial attachment to the goethite particles.

The UV dose responses of *E. coli* in the presence of various concentrations or turbidities of goethite particles are illustrated in Fig. 1. The data were obtained by using the bacterial cells after 24 h of attachment. At the low UV dosages, the log reduction values were similar under different turbidity conditions caused primarily by goethite. At a UV dosage of 10 mJ/cm² or higher, increasing the concentration of goethite particles significantly lowered the log reduction values for the bacteria. There were also more pronounced tailings, i.e., deviations from the first-order kinetics with increasing UV dosages, when the concentrations of goethite particles increased. At a UV dosage of 15 mJ/cm², for example, the log reduction values were 5.8, 5.2, 4.8, 4.4, 4.1, and 3.8 as the turbidity increased from 0, 1, 5, 10, 20, and 50 NTU, respectively. These results indicated that the presence of goethite particles significantly affected the efficiency of UV inactivation, even at a low concentration of particles with a turbidity range of 1 to 5 NTU.

The efficiency of UV inactivation was also affected by time of attachment to goethite particles (Fig. 2). In the experiments with 50 mg/liter of goethite particles at a UV dose of 14.7 mJ/cm², the log reduction values for *P. putida* and *E. coli* inactivation decreased with increasing attachment time. The log reduction values for *P. putida* and *E. coli* with 24 h of attachment time were 1.6 and 1.4, respectively, less than those with 0.5 h of attachment time. The t-test statistics confirmed that the difference between these values with the two different attachment times was significant ($P \approx 0.01$). This phenomenon was more apparent in the experiments with higher concentrations of goethite particles and at higher doses of UV radiation (data not shown).

Further study examined the UV inactivation of *E. coli* after dispersion of the suspension with sonication. The ultrasound treatment did not affect the efficiency of UV inactivation of bacteria mixed with goethite particles for 0.5 h but significantly

![FIG. 1. Effects of goethite turbidity on UV efficiency [log (Nt/No), where Nt is the cell number at the designated time for UV disinfection and No is the initial number of cells before UV disinfection] for inactivation of *E. coli*. The error bars represent standard deviations. Turbidity values: T0, turbidity is close to 0 and no goethite particles; T1, turbidity is 1 and goethite particles are present at 1 mg/liter; T5, turbidity is 5 and goethite particles are present at 5 mg/liter; T10, turbidity is 10 and goethite particles are present at 10 mg/liter; T20, turbidity is 20 and goethite particles are present at 20 mg/liter; T50, turbidity is 50 and goethite particles are present at 50 mg/liter.](http://aem.asm.org/)

![FIG. 2. Effects of attachment time on UV efficiency for inactivation of *E. coli* and *P. putida*. Goethite, 50 mg/liter; turbidity, 50 NTU; UV dose, 14.7 mJ/cm².](http://aem.asm.org/)
enhanced the efficiency of UV inactivation of *E. coli* with 24 h of attachment time (Fig. 3). It was likely that few bacterial cells were attached to goethite particles in 0.5 h but more bacteria were bound to the particles at 24 h, resulting in less cell inactivation. This conclusion was supported by SEM evidence (Fig. 4). In a test with 0.5 h of attachment prior to UV irradiation, less than 10% of the bacterial cells were attached to the goethite particles (Fig. 4a). With a longer incubation time (24 h), a much closer association of bacterial cells and particles was observed, as illustrated in Fig. 4b. Approximately 40% of the bacterial cells were bound by some goethite particles in tests with 24 h of attachment. Some polymer-like products linking cells and particles were observed by SEM. It was possible that bacterial cells exuded extracellular polymers for closely binding goethite particles.

SEM analysis showed that the freshly synthesized goethite particles were mostly 0.2 by 2 µm in size, smaller than bacterial cells. Such small particles were ignored or excluded in the previous studies on the protective effects of particles against UV disinfection (18, 25). However, our results showed that bacteria were protected by these particles from a UV dose of 10 to 30 mJ/cm², which is comparable to the range of 21 to 36 mJ/cm² recommended by the U.S. Environmental Protection Agency for drinking water treatment (20). The inactivation of *E. coli* (Fig. 1) seemed to follow first-order kinetics as reported previously (9, 18), and no significant difference in inactivation was observed under different concentrations of goethite particles in the range of UV doses of 2.5 to 10 mJ/cm². After a 3 log₁₀ value of inactivation of *E. coli* was achieved, a pronounced deviation from the first-order kinetics occurred at a relatively high concentration of goethite particles. This is similar to previous studies with large particles from wastewater solids (9, 18). It suggests that small-sized goethite particles protect bacteria from UV disinfection even at very low levels of turbidity (1 to 5 NTU). The results extended the previous studies (6, 7) at high turbidities to a much lower turbidity level (0 to 5 NTU) relevant to drinking water supply systems.

Several potential mechanisms exist for the enhanced protection by goethite particles in the long bacterium-goethite attachment time experiments (Fig. 2), including (i) protection of particles by absorbing, scattering, and blocking UV light (25, 31); (ii) change of radical formation under UV irradiation (17); (iii) change of bacterial aggregation (12); and (iv) change of microbial resistance resulting from bacterial attachment (2, 13). In the inactivation experiments, the concentrations and turbidities of goethite particles are the same and thus their capacity for providing bacteria with protection from UV exposure by absorbing, scattering, and blocking UV light should be similar. The presence of goethite particles could potentially enhance the formation of radicals under UV irradiation (17), thus improving the efficiency of UV disinfection. Our experimental data have, however, shown that an increase in goethite particle concentrations decreases UV disinfection efficiency. This implies that disinfection through enhanced radical formation by goethite could not be significant in the system.

Bacterial aggregation is likely to be an important factor resulting in a decrease in disinfection efficiency, as previously reported (12). Our experiments on UV disinfection in the absence of goethite particles have, however, found no significant difference regarding the effects of bacterial aggregates on
UV disinfection for the same bacterial suspension at the two different attachment times. Bacterial aggregates seem to have no impact on UV disinfection in our system, although both single and aggregate cells could attach to the particles as previously reported (12). Instead, it appeared that bacteria were provided with additional protection from UV radiation by a unique association of a bacteria cell with a clump of goethite particles following 24 h of particle attachment (Fig. 4b). This unique association, different from cell attachment to a large particle, can be boosted by bacterial exudates and by prolonging the attachment time. It suggests that the attachment to a cell of many small goethite particles after an adequate time could be an important mechanism for bacterial protection from UV disinfection. Because the attachment is expected to be dependent on the surface properties of particles and cells, this mechanism may not be extrapolated to all matrix particles and organisms. For example, a recent study showed that Na-montmorillonite clay particles had no effect on UV disinfection efficiency for MS2 (24), but the bacteriophage (MS2) used in that study is considered to be more resistant to UV disinfection (24). Further study is needed to illustrate the effect of particle surface properties on UV disinfection efficiency for different organisms.

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REFERENCES


