Published in final edited form as:

Environ Sci Process Impacts. 2014 February ; 16(2): 194–202. doi:10.1039/c3em00527e.

Deposition and disinfection of *Escherichia coli* O157:H7 on naturally occurring photoactive materials in a parallel plate chamber†

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Abstract

Dissolved organic matter in combination with iron oxides has been shown to facilitate photochemical disinfection through the production of reactive oxygen species (ROS) under UV and visible light. However, due to the extremely short lifetime of these radicals, the disinfection efficiency is limited by the successful transport of ROS to bacterial surfaces. This study was designed to quantitatively investigate three collector surfaces with various potentials to produce ROS [bare quartz, hematite (α -Fe₂O₃) coated quartz, and Suwannee River humic acid (SRHA)] and the effects of extracellular polymeric substance (EPS) (full or partial coating) and solution chemistry (ionic strength, IS) on the interactions between bacteria and the ROS-producing substrates. With few exceptions, bacterial deposition studies in a parallel plate (PP) flow chamber have revealed increasing cell adhesion with IS. Furthermore, interactions between collector surfaces and cells can be explained by electrostatic forces, with negatively charged SRHA reducing and positively charged α -Fe₂O₃ enhancing bacterial deposition significantly. Increased deposition was also observed with full EPS content, indicating the ability of EPS to facilitate interaction between cells and surfaces in the aquatic environment. In complementary disinfection studies conducted with simulated light, viability loss was observed for cells fully coated with EPS when attached to α -Fe₂O₃ under all IS conditions. Based upon our prior study in which EPS was found to not inhibit hydroxyl radical activity toward bacteria, we proposed that EPS might therefore promote disinfection by facilitating cell attachment to ROS-producing surfaces where higher concentrations of ROS are expected at closer proximities to reactive substrates (*e.g.*, SRHA and α -Fe₂O₃). Our findings on the mechanism and controlling factors of cell interactions with photoactive substrates provide insight as to the role of ionic strength in photochemical disinfection processes.

†Electronic supplementary information (ESI) available: Additional details on material and methods are mentioned. See DOI: 10.1039/c3em00527e

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1. Introduction

Escherichia coli O157:H7 is a notorious strain that has been involved in numerous outbreak events causing foodborne and waterborne diseases, resulting in hemolytic-uremic syndrome.¹ In the United States alone, O157:H7 causes an average of 75 000 infections and 61 deaths each year.² *E. coli* O157:H7 can be associated with various farm animals as sources for contamination of water and food,^{3,4} as well as causing impairment to surface water associated with agricultural run-off.

Energy from the sun can be exploited to drive photochemical disinfection, a natural attenuation process critical to pathogen persistence in surface waters. Previous work has looked at indirect photochemical disinfection processes (*i.e.*, those involving reactive oxygen species, or ROS, generated from sensitizers like dissolved organic matter) and quantified disinfection rates as a function of various bacterial and solution parameters (cell type, cell polymer presence, sensitizer type, solution chemistry, *etc.*). For example, our prior work revealed the insensitivity of disinfection rate to cellular extracellular polymeric substances (EPS) levels in a homogeneous (*i.e.*, constituents fully dissolved) aquatic system with nitrate photolysis as a source of hydroxyl radical.⁵

In heterogeneous systems, however, such as those with insoluble natural organic matter present, studies have suggested the existence of microenvironments near the sensitizer interface where ROS concentrations can be more than two orders of magnitude greater than the bulk, resulting in much faster disinfection⁶ and inactivation⁷ rates. Due to the short life span of the ROS, successful disinfection requires prompt transport of ROS from point of production to cell surfaces.^{8,9} It has been shown that bacterial EPS have the ability to initiate adhesion processes to form aggregates or biofilms.^{10,11} Accordingly, EPS should contribute to cell interaction with photoactive surfaces and possibly influence ROS access to the cell surface. There is a need, therefore, for a study to explore the contribution of cell adhesion to photoactive surfaces to the ultimate disinfection of the cells, and the contribution of EPS levels to this process.

The interactions between cells and surfaces depend on many factors. Common controlling factors influencing cell–surface interactions or biofilm formation in water include classical Derjaguin–Landau–Verwey–Overbeek (DLVO) type forces (van der Waals and electrostatic interaction),^{12,13} gravity,^{14,15} steric repulsion,¹⁶ electrosteric force,^{17,18} hydrophobic properties,¹⁹ and hydration conditions of cells and solid surfaces.²⁰ The interactions between cells and surfaces in water also depend on solution chemistry including pH, ionic strength (IS), and valence.^{21,22} Other than these physicochemical factors, the interactions also depend on biological factors like quorum sensing,²³ and the presence of bacterial surface macromolecules including flagella, pili, fimbriae, proteins, lipopolysaccharides (LPS), and EPS.^{1,24} Previous studies on *E. coli* O157:H7 transport and adhesion in porous media revealed deviation from DLVO predictions due to electrosteric repulsion,²⁵ pH associated electrosteric stabilization,²⁶ and the presence of EPS.^{25–27} However, the mechanisms involved in interactions between O157:H7 cells and photoactive substrates responsible for ROS production (such as $\cdot\text{OH}$, $^1\text{O}_2$, and H_2O_2) have not been clearly determined.

Many studies have focused on the role of surface macromolecules in regards to cell interaction and adhesion behavior in systems such as batch adhesion tests,²⁸ packed-bed columns,^{29–33} radial stagnation point flow (RSPF) systems,^{17,18,32,34} parallel plate (PP) flow chamber,³⁵ and direct observation through atomic force microscopy (AFM).^{36–38} A number of these studies demonstrated surface macromolecules play a significant role in well controlled adhesion experimental systems. However, results deviate on the contribution of macromolecules where some suggest an increase in adhesion,^{28,30,34} and others observed hindrance.^{17,18,28,29} Due to the discrepancy in the literature, a straightforward experimental system with well-defined conditions focusing on a specific macromolecule is needed to investigate the disinfection scenario where cells interact with photoactive surfaces.

This study was designed to investigate the role of the adhesion between cells and naturally occurring photoactive materials on the disinfection of bacteria. Specifically, we examined the adhesion behavior of *E. coli* O157:H7 on representative photoactive substrates in a PP flow chamber while also conducting complementary disinfection experiments with these photoactive materials using a solar simulator.^{5,39–41} The PP flow chamber system simulates the condition where the flow stream is parallel to collector surfaces in porous media, and the fundamental mechanisms influencing bacteria adhesion to solid surfaces can be revealed in a well-defined hydrodynamic flow field. This technique can provide fundamental insight through direct observation of the bacterial interactions with model sensitizing agents Suwannee River humic acid (SRHA) and iron oxide (hematite, α -Fe₂O₃) under a microscope with cells of varying EPS levels. The working hypothesis for this study was that greater levels of interaction between the cell and solid collector surfaces (made of the desired ROS-generating material) will lead to a higher potential for successful disinfection under UV and visible light.

2. Materials and methods

2.1 Cell selection, preparation, and characterization

A pathogenic strain *E. coli* O157:H7/pGFP strain 72 with ampicillin resistance was chosen as a model organism in this study.^{39,40} This strain was selected because it is a rod-shaped, Gram-negative bacteria with very good fluorescence expression.⁴⁰ Details on cell preparation and culture are mentioned in ESI.† The concentrations of bacteria were determined by direct visualization under a light microscope (Fisher Scientific Micromaster) with a cell counting chamber (Buerker-Türk chamber, Marienfeld Laboratory Glassware, Lauda-Königshofen, Germany). The cell concentrations used in the deposition and disinfection portions of this study were 10⁸ cells per mL and 5 × 10⁸ cells per mL, respectively. The final cell suspensions were made in the background solution of the subsequent disinfection or deposition experiment (1, 10, or 100 mM KCl). All solutions used were made from reagent grade chemicals (Fisher Scientific) and deionized (DI) water (Milli-pore, Billerica, MA). A wide range of characterization was conducted to determine the surface properties of bacterial cells mentioned in detail in the ESI.† In brief, zeta potentials of *E. coli* were determined through electrokinetic properties, while cell size was measured from microscopic image analysis. Hydro-phobicity of bacteria was determined by a partitioning test referred to as Microbial Adhesion to Hydrocarbons (MATH).

Potentiometric titration was utilized to determine the acidity and surface charge densities of *E. coli*. Fourier transform infrared spectroscopy (FT-IR) was used to determine functional groups on full and partial EPS coated bacteria.

2.2 Surface preparation and characterization

Quartz slides (9 mm × 20 mm) (Electron Microscopy Sciences, Ft. Washington, PA) were used for the deposition and disinfection studies. Prior to coating or direct use in experiments they were cleaned following the protocol described in the ESI[†] and elsewhere.⁴¹ For select experiments with organic matter, the quartz surfaces were cleaned with the procedure mentioned above, and subsequently were coated with SRHA according to a previously established flow through method.⁵

For iron oxide (α -Fe₂O₃) disinfection experiments, the clean glass slides (9 mm × 20 mm) (Fisherbrand®) were hand coated with 25 μ L of the α -Fe₂O₃ solution for an even distribution. The slides were allowed to dry and were kept in the dark until experiments. Subsequent analysis *via* XRD confirmed the deposited phase as hematite (data not shown). The detailed coating procedure is mentioned in the ESI.[†]

2.3 Parallel plate experiments

Deposition behavior of *E. coli* O157:H7 was observed in real time with a custom-made PP flow cell system, inserted on the stage of an upright fluorescence microscope BX-51 (Olympus, Tokyo, Japan).⁴² Images were taken by a 40× objective lens passing through a fluorescent filter set (Chroma Technology Corp., Brattleboro, VT). The flow cell is rectangular shaped with an inner chamber 6 cm × 1 cm × 0.0762 cm in size, formed by attaching the deck, rubber gasket, and a microscope glass slide with a thin layer of vacuum grease. The flow deck was made with a pocket in the center, 9 mm in width, 20 mm in length (parallel to flow direction), and 1.5 mm in depth. This pocket allows for different collector surface samples (PP coupons) of this size to be inserted into the pocket and be fixed with vacuum grease. The inlet of the flow cell was attached to a 60 mL syringe pump where the flow rate of the bacterial solution was kept at 0.1 mL min⁻¹ for all conditions. The concentration of bacterial solution was optimized to be 10⁸ cells per mL in the flow cell for all conditions tested. Images of bacterial deposition on desired surfaces were taken by QImaging Retiga EXi digital camera (QImaging, Surrey, BC, Canada) every 30 s over the course of 30 min and then analyzed with the supplied software (SimplePCI, Precision Instruments, Inc., Minneapolis, MN). The number of cells attached to the surface for five or more consecutive images were counted and the particle flux was determined from the linear slope of the number of cells deposited *versus* time and normalized by the microscope viewing area (230 mm × 170 μ m). Resulting bacterial transfer rate coefficients k (m s⁻¹) were calculated from the deposition flux J (cells per s × m²), and injection concentration C_0 (cells per m³).

$$k = \frac{J}{C_0} \quad (1)$$

2.4 Bacterial disinfection experiments

Cell preparation and characterization for the disinfection study followed protocols mentioned in Sections 2.1 and 2.2. Disinfection experiments were conducted with a solar simulator, following protocols utilized in previous work.⁵ Briefly, a collimated beam of light from a 450 W O₃-free Xe arc lamp (Newport Corporation, Irvine, CA) was passed through a 320 nm filter to generate light in the UV and visible range. A 50 mL (37 mm ID) jacketed beaker kept at 25 °C via a circulating water bath (Thermo Scientific, Waltham, MA) was employed for all experiments. Experiments used quartz and α -Fe₂O₃-coated quartz in the presence and absence of light (*i.e.*, dark) across a range of IS. Experiments were conducted with the jacketed beaker containing three quartz or α -Fe₂O₃-coated slides in the jacketed beaker. Experiments at 1 and 10 mM KCl were conducted over 80 minutes and sampled in 20 minutes intervals (*i.e.*, 0, 20, 60, and 80 min) while experiments at 100 mM KCl were conducted up to 140 min with 20 min sampling intervals because more time was needed to observe an effect. Dark control experiments were conducted under the same parameters; however, the beakers were covered in foil to eliminate light exposure and allowed to sit in a dark room for 20 min intervals at 25 °C.

To quantify the percentage of viable *E. coli* that adhered to the slides, the Live/Dead BacLight kit (L-13152; Molecular Probes, Eugene, OR) and a fluorescent microscope (BX 51, Olympus, Japan) with a red/green fluorescence filter set (Chroma Technology Corp., Brattleboro, VT) was utilized. Additionally, a planktonic sample was removed from the jacketed beaker and viewed with the Live/Dead kit to quantify effects of the light and/or α -Fe₂O₃ on the free floating cells.

3. Results and discussion

3.1 Characterization of *E. coli* O157:H7 and collector surfaces

Both *E. coli* O157:H7 and coated surfaces were characterized over a wide range of solution chemistry and the results are reported in Table 1. The streaming potential of all three types of collector surfaces (bare quartz, SRHA-coated, and α -Fe₂O₃-coated) became less negative (or transitioned to positive) with greater IS. The zeta potentials of bare quartz appeared to be the most negative at 1 mM KCl and increased with IS towards neutral. For SRHA coated surfaces, the coating seemed to result in slightly more negative zeta potential (-23.4 ± 1.0 mV) in 10 mM KCl than bare quartz (-19.8 mV). However, in 100 mM KCl, the charge of SRHA coated surface was reversed to a positive value (19.7 ± 24.4 mV) due to charge screening at high IS. The α -Fe₂O₃ coating on the glass surface resulted in positive zeta potentials in both 10 and 100 mM KCl (pH 5.6) and a more positive value was observed in 100 mM (44.8 ± 25.4 mV) than in 10 mM (0.1 ± 1.89 mV) KCl.

Bacterial surfaces appear to be near neutral according to electrokinetic characterization; whereas, most nonpathogenic *E. coli* are more negatively charged at similar pH and IS conditions.^{5,48} The zeta potential of O157:H7 also showed less sensitivity to the changing IS^{25–27} as compared to other nonpathogenic *E. coli* strains.^{43,44} Similar values of O157:H7 zeta potentials were found with full and partial EPS coated cells in 1 and 10 mM KCl where no significant difference was detected between full EPS bacteria and their partial EPS

counterpart ($p > 0.1$). In 100 mM KCl, zeta potential of full EPS cells was observed to be significantly higher than in the partial EPS counterpart ($p < 0.1$).

Additional characterization of bacterial cell surfaces was conducted to determine the difference in surface characteristics between full and partial EPS-coated O157:H7. Potentiometric titration results are summarized in Table 1 and Fig. 1. Fig. 1 shows that acidity as well as surface charge density of partial EPS-coated O157:H7 were notably lower than full EPS-coated O157:H7. This indicates that sonication successfully removed the surface polymers of bacteria. However, the shape of potentiometric titration curve remained identical for both partial and full EPS-coated O157:H7, indicating that the sonication protocol used in this study did not create an artifact biasing collection of only certain surface polymers of the bacteria. FT-IR was conducted to determine the functional groups removed during sonication. FT-IR results (Fig. S2[†]) indicate that the major functional group removed due to sonication was C=O stretching from proteins (1650 cm^{-1}).

3.2 Adhesion behavior of O157:H7 in the parallel plate flow chamber

Experimental results from O157:H7 transport experiments with the three test collector surfaces as a function of ionic strength and with full or partial EPS bacteria are reported in Fig. 2.

3.2.1 Effect of collector surface on adhesion behavior of O157:H7—Surface coating was found to impact deposition trends across the range of IS conditions tested. For example, the bacterial transfer rate coefficient (k) on the bare quartz in 10 mM KCl was $1.32 \times 10^{-8} \pm 4.93 \times 10^{-9}\text{ m s}^{-1}$ and $2.98 \times 10^{-9} \pm 1.42 \times 10^{-10}\text{ m s}^{-1}$ for full and partial EPS, respectively. Full EPS-coated cells attached to both bare and SRHA-coated quartz similarly at 10 mM ($1.32 \times 10^{-8} \pm 4.93 \times 10^{-9}\text{ m s}^{-1}$ and $1.31 \times 10^{-8} \pm 2.87 \times 10^{-9}\text{ m s}^{-1}$ respectively). This indicates that the SRHA-coated surface did not provide additional steric hindrance towards the full EPS-coated bacteria. Partially coated EPS bacteria, on the other hand, attached less on SRHA ($k = 1.19 \times 10^{-9} \pm 6.46 \times 10^{-10}\text{ m s}^{-1}$) than on quartz, whereas k on quartz was 2.5 times greater than that on the SRHA-coating. Hence, removal of EPS from the bacterial cell surface influenced the cellular interactions with SRHA-coated surfaces.

Solid surfaces with $\alpha\text{-Fe}_2\text{O}_3$ coating appeared to be more favorable for bacterial deposition than bare quartz surfaces in 10 mM KCl for both full and partial EPS-coated bacteria. Specifically, deposition on $\alpha\text{-Fe}_2\text{O}_3$ resulted in 2.3 and 8.7 times greater mass transfer ($k = 3.04 \times 10^{-8} \pm 9.36 \times 10^{-10}\text{ m s}^{-1}$ for full EPS; $k = 2.60 \times 10^{-8} \pm 2.05 \times 10^{-9}\text{ m s}^{-1}$ for partial EPS) than on quartz, respectively.

The bacterial transfer rate coefficients (k) on the bare quartz in 100 mM KCl were $2.79 \times 10^{-8} \pm 3.25 \times 10^{-9}\text{ m s}^{-1}$ and $1.83 \times 10^{-8} \pm 2.77 \times 10^{-9}\text{ m s}^{-1}$ for full and partial EPS, respectively. The SRHA coating made the surface less favorable than bare quartz in 100 mM KCl for both full and partially EPS-coated bacteria ($k = 1.36 \times 10^{-8} \pm 5.42 \times 10^{-10}\text{ m s}^{-1}$ for full EPS and $k = 8.53 \times 10^{-9} \pm 1.36 \times 10^{-9}\text{ m s}^{-1}$ for partial EPS). Comparing the bacterial transfer rate coefficients for full and partial EPS in 100 mM KCl, the transfer rate coefficients in quartz were about 2 times greater than on SRHA. Similar to what was observed in 10 mM

KCl, the α -Fe₂O₃ coating caused an increased transfer rate to the surface (1.5 times for full EPS and 1.1 times for partial EPS) as compared to the bare quartz.

Addition of a surface coating, either natural organic matter or α -Fe₂O₃, generally resulted in significant differences in transfer rate coefficients, as determined with a Student's *t* test. Insignificant differences were only found between (1) quartz and SRHA-coated quartz in 10 mM KCl with full EPS; (2) quartz and α -Fe₂O₃-coated glass in 100 mM KCl with partial EPS. The reason the surface coatings impacted the extent of attachment is due to a combination of mechanisms. Notably, electrostatic interactions were crucial. For example, the addition of SRHA coating on the quartz increased the negativity of quartz surface, thus reducing the attractive forces between bacterial surfaces and the solid. The greater deposition on α -Fe₂O₃-coated surfaces than on bare quartz is likely due to the positively charged coating (as confirmed by zeta potential measurements) leading to greater electrostatic attraction with the negatively charged bacteria (as compared to cellular interaction with the negatively charged bare quartz). A number of previously reported studies corroborate these trends. For example, negatively charged latex colloids were found to deposit less in porous media when both the silica 0.2 mm spherical soda-lime glass beads and colloids were coated with humic acid.⁴⁵ Similarly, transport studies in porous media which reported substantial bacterial attachment on α -Fe₂O₃, which was subsequently reduced by the addition of both SRHA and soil humic acid at a concentration of 2.5 mg L⁻¹ dissolved organic content.^{46,47} Another study found increased bacterial deposition rates occurred with Fe-coated sand and enhanced bacterial surface coverage of sediment surfaces.⁴⁸

3.2.2 EPS effect on adhesion behavior of *E. coli* O157:H7—Generally, lower bacterial transfer rate coefficients (*k*) were observed for cells with partial EPS than with full EPS across all conditions tested. For example at 100 mM KCl, the *k* values were $2.54 \times 10^{-8} \pm 2.95 \times 10^{-9} \text{ m s}^{-1}$ and $1.66 \times 10^{-8} \pm 2.52 \times 10^{-9} \text{ m s}^{-1}$ for full and partial EPS on quartz surfaces, respectively, where the rate coefficient *k* for cells with full EPS is 1.5 times greater than for cells with partial EPS in the same condition. At 10 mM KCl, significantly lower deposition was observed with partial EPS than full EPS, with $k = 2.71 \times 10^{-9} \pm 1.29 \times 10^{-10} \text{ m s}^{-1}$ (partial EPS) and $1.20 \times 10^{-8} \pm 4.48 \times 10^{-9} \text{ m s}^{-1}$ (full EPS), where the rate coefficient *k* for cells with full EPS is 4.3 times that for cells with partial EPS in the same solution. With SRHA coated surfaces, *k* values for cells with full EPS in 10 and 100 mM accounts for 11 and 1.6 times greater than partial EPS. With the α -Fe₂O₃ coating, *k* values for cells with the full EPS in 10 and 100 mM resulted in 1.2 and 2.1 times more than with partial EPS. This indicates that EPS association with cell surfaces may facilitate the interaction between cells and collector surfaces. Previous studies reported adhesion of bacteria increase with more EPS produced and present around the cells,^{10,49,50} which is in agreement with these reported trends (Fig. 2). Overall, the data suggest that EPS facilitates the association between bacteria and solid surfaces through a combination of mechanisms. These include polymer bridging^{51–53} in lower IS and an increase of heterogeneity^{54,55} on bacterial surfaces.

3.2.3 Adhesion behavior of *E. coli* O157:H7 as a function of ionic strength—Increasing IS generally was found to enhance the amount of deposition. This was the case

for cells with partial EPS on quartz and SRHA, as well as cells with full EPS on α -Fe₂O₃. Specifically, the k values for cells with partial EPS onto quartz and SRHA increased by ~6 and ~7 times when the IS was increased from 10 to 100 mM. The value of k for cells with the full EPS onto the α -Fe₂O₃ increased just above 30% over that same change in solution chemistry.

The difference between bacterial cells deposited on quartz surfaces with full EPS at 1 and 10 mM KCl was insignificant ($p > 0.1$). Bacterial cells with full EPS attached similarly on SRHA coated quartz in 10 and 100 mM KCl with no significant difference ($p > 0.1$). The only deviation from this trend of increasing IS leading to greater deposition was for cells with partial EPS depositing on α -Fe₂O₃ surfaces. Bacterial cells with partial EPS attached less on α -Fe₂O₃ in 100 mM KCl ($k = 1.75 \times 10^{-8} \pm 3.18 \times 10^{-9} \text{ m s}^{-1}$) than in 10 mM KCl ($k = 2.36 \times 10^{-8} \pm 1.86 \times 10^{-9} \text{ m s}^{-1}$). Possible mechanisms include the stabilization of the surface polymers by the increased IS,^{41,56} or the extraction of EPS from cells may have altered the local charge heterogeneity,^{54,57,58} such that the electrostatic attractive force of the surface may be reduced in 100 mM KCl.

3.3 Bacterial disinfection

To relate deposition to disinfection, a viability analysis was performed in an attempt to relate cell death with sensitizer exposure under varying IS (Fig. 3). As might be expected, generally irradiated samples (open symbols) tended to show more viability loss than dark controls (solid symbols), consistent with photodisinfection. Interestingly, data also suggest that association with a surface can also promote viability loss. This behavior is most obvious at the highest ionic strength condition considered (100 mM), where cells adhered to quartz and hematite exhibited (i) greater viability loss compared to planktonic cells under identical conditions (compare Fig. 3E and F), and (ii) the largest loss of viability observed under any IS condition. At lower IS (*i.e.*, 1 and 10 mM), little if any difference in viability loss was observed between attached and planktonic cells. This is consistent with greater attachment achieved at higher IS assisting in light-facilitated disinfection mechanisms. Finally, trends in disinfection with respect to light *versus* dark and attached *versus* planktonic cells were essentially identical between quartz and hematite systems, suggesting that the nature of the surface may be of limited importance – as compared to the degree of attachment and proximity to the sensitizer – when considering such heterogeneous photo-chemical disinfection processes.

3.4 Mechanistic interpretations

3.4.1 Predicted interactions between *E. coli* O157:H7 cells and collector surfaces—To understand the interactions between *E. coli* O157:H7 and the collector surfaces, zeta potential measurements were performed at the same solution chemistries used in transport experiments. These values were used to predict the interaction forces between cells and surfaces according to DLVO theory^{12,13} (Table S2†). The DLVO calculations based on resulting zeta potential values (Table S2†) indicated there were no energy barriers to cell interactions with all three surfaces, for cells with either full and partial EPS at 10 and 100 mM KCl. The energy barrier in 1 mM KCl for both full and partial EPS coated cells was very low, below 10 kT in value, which may be overcome by other attractive forces like

Brownian motion⁵⁹ and diffusion.⁴⁵ These predictions suggest chemically favorable conditions for O157:H7 interaction with the surfaces under all conditions tested in the PP system. This indicates that the differences observed in cellular attachment are mainly due to changes in surface macromolecules in bacterial cells (full EPS vs. partial EPS) and deposition surfaces (bare quartz vs. SRHA-coated).

3.4.2 Disinfection of attached and planktonic cells—Previous results⁴⁶ suggest that planktonic lag phases in photo-chemical disinfection may take up to two hours under 10 mM KCl conditions; for 100 mM in this study, a delayed lag phase of 130 min was established for both attached and planktonic experiments. The greater lag time is correlated with an increase in IS (Fig. 3E and F), which seems to suggest the bacteria have a greater tolerance to the light and α -Fe₂O₃ presumably due to enhanced bacterial aggregation at high IS.

At high IS, cells can also attach to appropriate surfaces to a greater extent. While our data certainly appears to indicate that surface attachment promotes viability loss upon irradiation, the mechanism of disinfection remains unclear and merits further investigation. It is tempting to attribute the viability loss observed in hematite systems to reactive oxygen species (ROS) that can be generated in the near surface region from Fe(II) generated *via* the photoreduction of structural Fe(III) in the hematite lattice. However, hematite photoreduction to yield Fe(II) is usually quite slow at neutral pH and is likely not sufficient to produce appreciable concentrations of ROS necessary to drive disinfection, although we cannot rule out the possibility that EPS could promote Fe(II) formation if able to form a complex with lattice Fe(III) that is a chromophore.

Evidence against an ROS-mediated disinfection route is also obtained from results with irradiated quartz slides, which tended to produce comparable, and occasionally greater, extents of viability loss than in hematite-containing systems. As ROS will not be generated in non-photoactive SiO₂ systems, other mechanisms responsible for viability should be considered, including the role that surfaces may play in concentrating or reflecting light and heat, both of which would be expected to promote cell die off.

4. Implications for photodisinfection

The decrease of the bacterial EPS level lowered the deposition of cells as expected. This indicates the bacterial macromolecules enhance interactions with other surfaces in aquatic systems. The SRHA surface coating lowered the rate of deposition in both 10 and 100 mM KCl as compared to bare quartz. In contrast, the α -Fe₂O₃ surface coating resulted in greater attachment on the surface than on quartz. These observations clearly demonstrate that electrostatic forces play a significant role on O157:H7 deposition as demonstrated in the PP system.

Deposition of bacteria with full and partial EPS increased with greater IS. The general trend of increased attachment with IS agreed with DLVO calculations. The few exceptions suggest that some behaviors are not captured by classic DLVO theory, specifically caused by the presence of EPS,⁶⁰ which causes surface heterogeneity^{61,62} and facilitates steric interactions.^{28,36}

While more work is warranted, we conclude that increased association between bacteria and surfaces promote photo-chemical disinfection by generating a microenvironment less hospitable for cell survival. For photoactive substrates, the near surface region could be rich in ROS, thereby increasing the likelihood of reaction with cell surfaces. In addition, surfaces may also concentrate light and *via* absorption produce localized regions of higher temperature that may facilitate greater rates of photodisinfection. Through enhancing the production of EPS, the effect of EPS may be exploited to maximize interactions with surrounding agents. Approaches to alter EPS production, and hence cell–sensitizer interactions, include modifying carbon sources, the carbon to nitrate ratio, phosphate, and nutrient levels.^{63,64} The formation of bacterial surface macromolecules and their composition can also be optimized for greater photo-chemical disinfection by means of modifying related solution chemistry conditions, *e.g.*, IS,³⁶ pH,⁶⁵ and temperature.^{66,67} Additionally, the more favorable interactions between bacteria and α -Fe₂O₃ surfaces suggest the presence of colloidal iron may also facilitate photochemical disinfection in natural waters.

Environmental impact

Providing clean, safe drinking water is a priority for maintaining the health of the world's population; therefore, understanding the mechanisms which lead to bacterial deposition in such systems can inform better processes for eliminating microorganisms. In this study, the role of the collector surface, extracellular polymeric substance (EPS) of the bacteria, and ionic strength were examined to determine bacterial interaction with and attachment to reactive oxygen producing substrates. We determined that solution chemistry (ionic strength) plays an important role in the disinfection process. Optimizing bacterial surface molecules (EPS) by modifying the solution chemistry may help facilitate photochemical disinfection.

Acknowledgements

This study was financially supported through the USDA CSREES NRI water and watershed grant (no. 2008-01768). Amy Gong was partially supported through a U.S. Department of Education GAANN Grant (no. P200A060047). We would also like to thank Risa Guysi (NSF Career Award no. CBET-0954130) and Hailey Wilder (Walker's John Babbage Chair in Environmental Engineering) for their assistance in the lab as well as the funding sources supporting their involvement with this research.

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Fig. 1.

Potentiometric titration of *E. coli* 0157:H7 with full and partial EPS. Solution chemistry used was 10 mM KCl. Titration was conducted from pH 3 to 11 using 0.1 M NaOH as a titrant. Procedure of EPS removal was mentioned in the ESI.[†]

Fig. 2.

O157:H7 bacterial mass transfer rate coefficient (k) as a function of IS observed in the PP flow chamber with quartz, SRHA, and α -Fe₂O₃ as solid collectors. Full or partial refers to EPS levels. Error bars indicate one standard deviation.

Fig. 3.

Survival of *E. coli* 0157:H7 with coated and uncoated α -Fe₂O₃ quartz slides as a function for attached and planktonic bacteria at 1 (A and B), 10 (C and D), and 100 (E and F) mM KCl. Experiments were conducted under both light (open symbols) and dark (solid symbols) conditions where A, C, and E report data for attached bacteria and B, D, and F show results for the planktonic cells. Error bars indicated one standard deviation.

Table 1
Characterization of *E. coli* O157:H7 and collector surfaces as a function of solution chemistry

Collector surface zeta potential ^d (mV)		O157:H7 zeta potential (mV)		Cell size ^f (mm)	Hydrophobicity ^g	Acidity ^h (meq per 10 ⁸ cells) x 10 ⁻⁶	Charge density ^h (μCcm ⁻²)
IS (mM)	Quartz ^b	SRHA-coated	α-Fe ₂ O ₃ -coated	Full EPS ^d	Partial EPS ^e		
1	-24.7	ND ^c	ND	-2.7 ± 0.3	-3.44 ± 0.6	0.55	Full: 329.9 ± 35.9;
10	-19.8	-23.4 ± 1.0	0.1 ± 1.5	-0.2 ± 2.1	-0.15 ± 1.2	0.58	Partial: 215.7 ± 17.9
100	-3.4	19.6 ± 24.4	44.8 ± 25.7	2.3 ± 1.9	-3.35 ± 3.7	0.53	

^a Collector surface zeta potential measured using a streaming potential analyzer (EKA).

^b Quartz surface zeta potentials from ref. 41.

^c ND: not determined.

^d "O157:H7 with full EPS" zeta potentials measured with freshly harvested cells diluted in test electrolytes.

^e "O157:H7 partial EPS" zeta potentials measured with cells after applying probe sonication for EPS extraction.

^f Cell size was determined from microscopic image analysis.

^g Hydrophobicity of bacterial cells was determined by Microbial Adhesion to Hydrocarbons (MATH) test.

^h Acidity and surface charge densities were calculated from potentiometric titration results in 10 mM NaCl.