Supporting Information

Deposition and Disinfection of *Escherichia coli* O157:H7 on Naturally Occurring Photoactive Materials in a Parallel Plate Chamber

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1. Introduction

In this Supporting Information section, additional materials and methods are described including cell selection, preparation, characterization, extraction of EPS, cell surface characterization, and surface preparation for deposition and disinfection experiments.

2. Additional 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.[1] This strain was selected because it is a rod-shaped, Gram-negative bacteria with very good fluorescence expression.[1b, 2] Bacterial cells were pre-cultured by inoculating bacterial colonies streaked from a Luria-Bertani (LB) agar plate into 5 mL LB media (Fisher Scientific, Fair Lawn, NJ). Cells were grown in the presence of 0.1 g/L ampicillin the day before the experiment and incubated at 37˚C in an incubator overnight (16 hours). The pre-culture step was followed by a culturing process in which 2 mL of the pre-culture was added to 200 mL of LB media (1:100 v/v) and incubated at 37˚C for 3.5 hours to reach mid-exponential growth phase. After reaching this growth stage, the culture was harvested by centrifugation (5804R; Eppendorf, Hamburg, Germany) with a fixed-angle rotor (F-34-6-38; Eppendorf) at 4˚C 3700 g for 15 min to separate the cells from the growth media. The centrifugation step was at 4˚C 3700 g for 15 min, followed by decanting the supernatant, and resuspending in 10 mL of electrolyte (10 mM KCl, Fisher Scientific). This was repeated twice to remove all traces of growth media. Next, a stock suspension

was prepared by resuspending the pellet in 5 mL of electrolyte, at the same ionic strength required for subsequent transport or cell characterization steps.

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 resulting cell concentrations in this particular study were in between 4×10^{10} and 6×10^{10} bacteria/mL. All solutions used were made from reagent grade chemicals (Fisher Scientific) and deionized (DI) water (Millipore, Billerica, MA).

2.2 Extraction of EPS by Sonication Technique.

Bacterial EPS was extracted through a previously established protocol applying probe sonication (Omni-Ruptor 250, Omni International, Kennesaw, GA) and centrifugation (Eppendorf).[3] Bacteria in stock solution were re-suspended in 10 mL of 1, 10 or 100 mM KCl. A 5/32" Micro-Tip sonicating probe (Omni International) was immersed 10 cm into the solution in a 15 mL centrifuge tube. The temperature was maintained below 4˚C by immersing the tube in an ice bath throughout the sonication process. Sonication was repeatedly applied to the bacterial cells by way of five sec duration pulses with five sec rest periods in between at a fixed intensity (30% of the 150 Watt = 45 Watt) for a total of 300 sec. After sonication, the bacterial solution was centrifuged at 4°C and 4000g for 20 min (Eppendorf), and the supernatant was collected and passed through a 0.22 μm filter (Millipore, Fisher Scientific) for further EPS

composition analysis. Finally, the pellet was resuspended in electrolyte for subsequent characterization and transport experiments.

The sugar and protein content of the EPS were evaluated through the Phenol-Sulfuric Acid (PSA) method[4] and the Lowry method[5] with Xanthan gum (Sigma-Aldrich corporation, St. Louis, MO) and bovine serum albumin (BSA, 1mg/mL) (Fisher BioReagents, Fisher Scientific) as the standards, respectively. Both are colorimetric methods where the chemical reaction induced color change can be measured by spectroscopy (BioSpec-mini, Shimadzu Corp. Kyoto, Japan) and compared with a standard curve for sugar or protein concentration. The sugar level was determined by adding 50 µL of 80% (v/v) phenol and then 5 mL of highly concentrate sulfuric acid (95.5% Fisher Scientific) into 2 mL of an extracted EPS sample. The mixing of these chemicals produces heat and a color change. Therefore, the solution required 10 min at room temperature to cool down, followed by immersion in a water bath (Lab-Line Instruments, Inc., Melrose Park, IL) at 25-30°C for 20 min, and finally, room temperature incubation for 4 hr to stabilize the color before measuring the absorbance at 480 nm (Biospec-mini, Shimadzu Corp.). The protein level was evaluated by adding 1.5 mL of alkaline copper reagent (made by mixing 1 mL of 2% $Na_2C_4H_4O_6$, 1 mL of 1% $CuSO_4$, and 98 mL of 2% $NaCO_3$ in 0.1 M NaOH) to 0.3 mL of EPS sample. After adding the alkaline copper reagent, the sample required incubation at room temperature for 10 min. Next, 75 µL of Folin reagent (Folin and Ciocalteu's Phenol Reagent, MP Biomedicals, LLC, Germany) was added into the mixture, the solution was vortexed (AutoTouch Mixer Model 231, Fisher Scientific), and incubated at room temperature for 30 min

before measuring absorbance at 500 nm (Biospec-mini, Shimadzu Corp.). All chemicals used were ACS grade (Fisher Scientific).

2.3 Cell Surface Characterization.

Bacterial cell sizes, surface charge densities, hydrophobicity, and zeta potentials were determined according to previous established protocols.[6] The size and shape of bacteria were evaluated by taking images of 10^{10} bacteria/mL with a light microscope (Fisher Scientific Micromaster). Images with more than 50 cells were processed through MATLAB software (Matlab, the MathWorks, Inc., Natick, MA), and individual bacteria widths and lengths were determined to calculate the effective diameters.

Surface charge densities were evaluated by potentiometric titrations in an auto titrator (798 MPT Titrino, Metrohm, Switzerland). Briefly the amounts of base added into bacterial solution for the pH value to increase from 4 to 10 were measured. The bacterial concentration in the titrator was 1×10^8 bacteria/mL, and the base used to titrate the bacterial solution was 0.1 N NaOH.

The hydrophobicity of the bacterial cells was measured by the microbial adhesion to hydrocarbon test (MATH test).[7] The relative hydrophobicity was determined by the percentage of 4 mL bacterial cells partitioned into 1 mL of n-dodecane (laboratory grade, Fisher Scientific) after vortexing for 2 min (AutoTouch Mixer Model 231, Fisher Scientific) and a 15-min room temperature incubation. The optical density of the water phase was determined by spectroscopy (Shimadzu Corp.), and the percentage of bacteria which passed to the oil phase was calculated.

The bacterial electrophoretic mobility (EPM) was measured by a ZetaPALS analyzer (Brookhaven Instruments Corporation, Holtsville, NY) in 1, 10, and 100 mM KCl with unadjusted pH (5.6-5.8). Bacterial solution was diluted to an optical density of $OD_{600} = 0.2$ to 0.225 before running through ZetaPALS. The zeta potential of the cells was then calculated by electrophoretic mobility values using the Smoluchowski equation.[8]

2.4 Surface Preparation and Characterization

Glass and quartz slides (9mm x 20mm) were used for the deposition and disinfection studies, respectively. Prior to coating or direct use in experiments they were cleaned by the subsequent method: slides were placed in a 2% (v/v) extran solution and sonicated for 15 min. The slides were then rinsed with deionized water and immediately placed in a 2% (v/v) RBS35 solution and sonicated for 15 min at 50ºC. Slides were again rinsed with deionized water and sat in Nochromix overnight. Slides were rinsed six times with deionized water and then stored in sterile Petri plates until usage.

For select experiments with organic matter, the quartz surfaces were cleaned with the procedure mentioned above, and subsequently were coated with Suwannee River humic acids (SRHA) according to a previously established flow through method.[9] In between flowing DI water and KCl solutions, 2 mL of poly-L-lysine (PLL)-free solution, 2 mL PLL, 2 mL PLL-free, and 2 mL SRHA solution were flowed through the system. PLL-free solution was comprised of 10 mM N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES) (Sigma-Aldrich, St. Louis, MO) and 100 mM KCl. PLL

solution was made by adding 0.1 g/L PLL into PLL free solution. SRHA solution was made by adding 28.3 mg of dry SRHA powder (International Humic Substance Society) into 52 mL DI water and shaken for 2 hrs. All solutions made were passed through 0.22 μm cellulose acetate membrane filters. When flowing through the PP flow cell, the PLL binds to the quartz. Subsequently, the SRHA solution was passed through the flow cell and bound to the PLL layer. Hence a layer of SRHA was formed on the quartz surface.

For iron oxide (α-Fe_2O_3) disinfection experiments, the clean glass slides were hand coated with 25 μL of the α-Fe_2O_3 solution for an even distribution. The slides were allowed to dry and were kept in the dark until experiments. In order to coat the surfaces with α-Fe_2O_3 for the PP experiments, an additional pretreatment of the glass was required. Following the cleaning procedure described above, the glass slides were treated with Piranha solution (the mixture of 30% H_2O_2 and 95.6% H_2SO_4 in 1:3 v/v ratio) to hydroxylate the glass surface, making the glass surface hydrophilic. The hydrophilic glass then was coated with α-Fe_2O_3 nanoparticles with a spin coating method at room temperature and 1500 rpm for 25 sec using a Laurell spin coater (WS-400A-6NPP/LITE) (Laurell Technologies Corp.; Johnstown, PA). The α-Fe_2O_3 solution used in the spin coating was made by the following method. α-Fe_2O_3 nanoparticles were made by adding 24 mL of 1 M ferric nitrate solution into 200 mL of boiling DI water at a steady speed of about 1 mL every 30 sec (finish adding 24 mL in 12 min).[10] The solution turned from light brown to dark brown to very dark brown, and then the reaction took place to turn the solution to dark red under boiling conditions. The solution was boiled on a hot plate to reduce the volume from 200 mL to 100 mL in about 30 min. After forming 100 mL of a

dense solution, followed by a cooling step, dark red precipitate formed with very small particle sizes (5-10 nm). Next, a 10-min 9000 rpm centrifugation was applied to the solution. After centrifugation the supernatant was decanted and 100 mL DI water added and vortexed to re-dissolve the α-Fe_2O_3 in DI water at a dilution of 1:10 v/v. Afterward, the coated glass samples were dried at room temperature.

Before each PP experiment, the α-Fe_2O_3-coated glass slide was first cleaned with DI and ethanol, and then installed in the flow cell. The cleaning process with DI water and ethanol did not substantially remove the α-Fe_2O_3 coating. To ensure the glass substrate was successfully coated with α-Fe_2O_3, the glass before and after the coating process was analyzed via a Cary® 50UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA) where the difference in absorbance was attributed to the presence of the α-Fe_2O_3 nanoparticle coating (Figure S1).

Table S1. O157:H7 Bacterial Transfer Rate Coefficients (k) for O157:H7 with Full or Partial EPS on Quartz, SRHA-, and α-Fe_2O_3-coated Surfaces.

Collector Surface	Ionic Strength (mM KCl)	EPS	k (m/s)
Quartz	100	Full	$2.54\times10^{-8}\pm2.95\times10^{-9}$
Quartz	100	Partial	$1.66\times10^{-8}\pm2.52\times10^{-9}$
Quartz	10	Full	$1.20\times10^{-8}\pm4.48\times10^{-9}$
Quartz	10	Partial	$2.71\times10^{-9}\pm1.29\times10^{-10}$
Quartz	1	Full	$1.58\times10^{-8}\pm3.95\times10^{-9}$
Quartz	1	Partial	ND[1]
Quartz SRHA	100	Full	$1.24\times10^{-8}\pm4.93\times10^{-10}$
Quartz SRHA	100	Partial	$7.75\times10^{-9}\pm1.24\times10^{-9}$
Quartz SRHA	10	Full	$1.19\times10^{-8}\pm2.61\times10^{-9}$
Quartz SRHA	10	Partial	$1.08\times10^{-9}\pm5.87\times10^{-10}$
Glass iron oxide	100	Full	$3.71\times10^{-8}\pm1.85\times10^{-9}$
Glass iron oxide	100	Partial	$1.75\times10^{-8}\pm3.18\times10^{-9}$
Glass iron oxide	10	Full	$2.76\times10^{-8}\pm8.51\times10^{-10}$
Glass iron oxide	10	Partial	$2.36\times10^{-8}\pm1.86\times10^{-9}$

[1]ND: No deposition detected.

Table S2. DLVO interaction energies between *E. coli* O157:H7 and collector surfaces

Energy Barrier Height[1] (*kT*)

IS (mM)	O157:H7 with full EPS			O157:H7 with partial EPS		
	quartz	SRHA	α-Fe_2O_3	quartz	SRHA	α-Fe_2O_3
1	5.6	ND	ND	9.77	ND	ND
10	NB[2]	NB	NB	NB	NB	NB
100	NB	NB	NB	NB	NB	NB

[1] Energy barrier heights calculated using DLVO theory.[11]   Hamaker constant used was 6.5×10^{-21} J.[12]
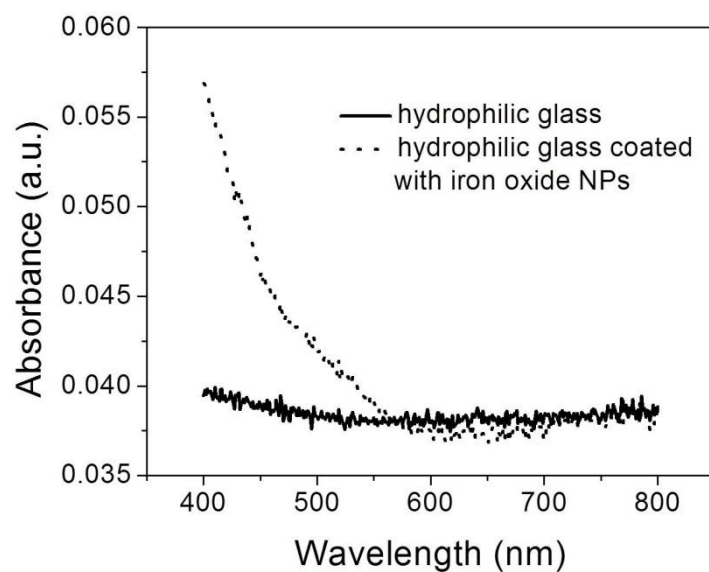[2] NB: no energy barrier.

Figure S1. Absorbance values of substrates from UV-visible spectroscopy with and without α-Fe$_2$O$_3$ coating on glass surface. The trend of increasing absorbance at lower wavelengths is indicative of the α-Fe$_2$O$_3$ nanoparticles coating the glass substrate.
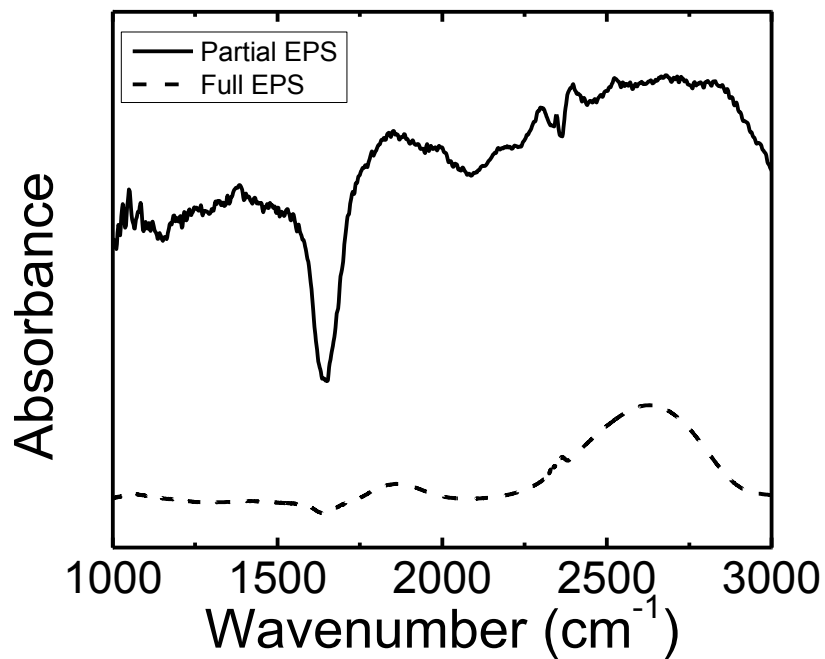
Figure S2. Fourier transform infrared spectroscopy (FT-IR) of supernatant from partial and full EPS in *E. coli* 0157: H7. Major functional group removed due to sonication was C=O stretching from proteins (1650 cm^{-1}).

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