Supporting Information for:

Forces Governing the Transport of Pathogenic and Nonpathogenic *Escherichia Coli* in Nitrogen and Magnesium Doped Biochar Amended Sand Columns

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S1. ***Model bacterial cultures***

Escherichia coli O157:H7, ATCC 35150 and nonpathogenic *E. coli* k12, ATCC 10798 *E. coli* were activated by growing for 12 hours at 37^0C on a shaker rotating at 150 rpm in Luria−Bertani (LB) medium. Following incubation, 1% volume of the activated culture was transferred into 20 mL of LB medium and grown at the conditions above until the late exponential phase of growth and then harvested at 4 hours when the absorbance at 600 nm reached 0.7±0.05. The specific growth constants were found to be 1.40 hr^{-1} and 1.31 hr^{-1} while the doubling times were found to be 21.5 and 22.9 minutes for *E. coli* O157:H7 and *E. coli* k12, respectively. Our data indicated that the two strains are comparable in their growth kinetics. After growth, bacterial suspensions were centrifuged at 5100*g* for 10 min. Cell pellets were collected and washed three times with deionized water (DIW). Bacterial pellets were then resuspended in DIW to a concentration of 0.12 optical density at 600 nm. Since the ionic strengths of solutions in environmental systems varies considerably depending on the source of water [1], all experiments were conducted in DIW, representing the extremely low end of the ionic strength found in sand.

S2. Biochar's Preparation

Avicel cellulose was used for char production. The Nitrogen-Magnesium co-doped biochar was produced using ammonia through a one-step ammonization process at 4 different temperatures (400, 500, 600, and 700^0C).

Ammonization took place in a quartz tube furnace reactor (Thermo Scientific, NC, USA) with 50 mm outside diameter, 44 mm inside diameter and a 1000 mm length. Prior to pyrolysis, cellulose was impregnated with $MgCl_2$•$6H_2O$ solution at a solid: liquid ratio of 1:4 (g/mL) and stirred for 6 hours. The magnesium chloride solution was prepared by dissolving 11 g of

$MgCl_2 \cdot 6H_2O$ in 100 mL of deionized water (DIW). Then the mixture was dried at 90°C for 48 hr. After Mg-containing cellulose preparation, the Mg-containing cellulose was kept in the tubular furnace in contact with ammonia for 30 minutes at 25^0C. The temperature was then increased from 25^0C to the desired set temperature at a heating rate of 10^0C min^{-1} under ammonia environment. When the final temperature was reached, the sample was allowed to reside in the reactor for 30 minutes. Flow rate of 500 mL min^{-1} for ammonia was employed. The final biochar produced was cooled down to 25^0C before storage and characterization[2].

S3. Biochar's Characterization

S3.1 Biochar surface area and micropore volume measurements and analyses

Carbon dioxide (CO_2) adsorption isotherms were determined at 273 K on micromeritics TriStar II PLUS Surface Area and Porosity Analyzer at WSU (Norcross, GA, USA). Before each analysis, biochar samples were degassed at 473 K for 18 hours under a vacuum of 0.05—0.1 mbar. The degassing temperature was chosen based on the production temperature of the biochar to avoid sample degradation during preparation. CO_2 adsorption isotherms were measured between the partial pressure range of $p/p_o=10^{-5}$ to p/p_o =0.03 using 75 set equilibration points. Surface area and micropore volumes were estimated for CO_2 adsorption using the Dubin-Radushkevich (DR) equation[3, 4].

S3.2 Proximate analyses

Moisture, fixed carbon, volatiles, and ash content of biochars were measured using a thermo-gravimetric analyzer (TGA) SDTA851e (Mettler Toledo, US) as described elsewhere [2]. Briefly, the moisture content was determined as the weight loss after the biochar was heated in a crucible from 25 to 120^0C and held at this temperature for 3 minutes under a Nitrogen gas environment at a flow rate of 50 mL/min. Then the biochar was heated from 120 to 950^0C under

a Nitrogen gas environment to determine the volatile content of the biochar. Then, it was held for 5 minutes at 950^0C and later cooled down to 450^0C. Ash content was determined after heating the biochar from 450^0C to 600^0C under oxygen gas flow (50 mL/min).

S3.3 Fourier Transform Infrared (FTIR) Spectroscopy of biochars

FTIR analysis was conducted to study the functional groups available on the biochars and to confirm that the biochars were doped with Magnesium and Nitrogen. FTIR spectra were obtained using a Shimadzu IRPrestige 21 spectrometer equipped with MIRacle single reflection ATR Ge probe according to protocols discussed elsewhere[1].

S4. Physiochemical Characteristics of Sand

Table S1: Physiochemical characteristics of sand

Characteristic	
Bulk density (g cm^{-3})	1.564
porosity (vol/vol)	0.33
pH (in H_2O)	7.5
Mean particle diameter (μm)	411
Sphericity (%)	0.73

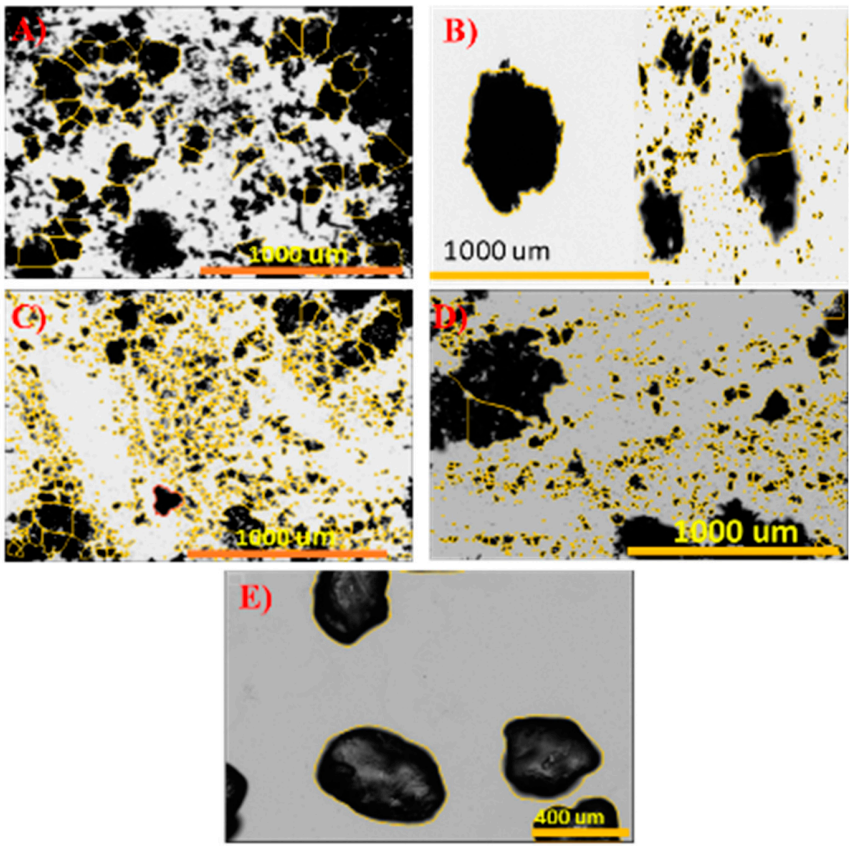

Figure S1: Processed images of biochar different types pyrolyzed at A-D) 400, 500, 600 and 700°C, respectively and E) sand.

S5. Contact Angles Measurements and Modeling

Contact angles of sand, biochar and bacteria were measured using the sessile drop method with a drop shape analyzer DSA100 (KRŰSS Hamburg Germany). The measurements were performed at room temperature and ambient humidity. Three different liquids that vary in dielectric constants (ε) were used. These were DIW-ε=78, formamide-ε=84, and glycerol-ε=47.2. To determine the contact angles of sand or biochar, double-sided tape was placed on a smooth microscope glass slide. Clean dry sand or biochar was then sprinkled on slide, and the slide was placed with the tape side down. A 100g weight was placed on the slide for about 5 seconds. The slide was then gently shaken to remove any loose granules. This process was repeated once more to make sure homogenous coverage of sand or biochar on the slide was

achieved. Five contact angles were then measured using each of the 3 liquids. Measurements were repeated in triplicates, and the average contact angle was reported.

To determine the contact angles of bacteria, diiodomethane (ε= 5.32) was used instead of formamide. Prior to contact angle measurements the bacteria were prepared using techniques described elsewhere[5]. Briefly, one colony was inoculated in 20 mL of LB broth and allowed to incubate overnight at 37^0C while shaking at 150 rpm to activate growth. 200 µl of the overnight growth culture was transferred to 20 mL of fresh LB broth and incubated at 37^0C until it reached late exponential phase of growth in about 4 hours. The bacteria were then harvested and washed twice by centrifugation for 10 mins at 5100 *g* each round. The bacterial pellet was then resuspended in 20 mL of DIW and using a negative pressure filter. The bacterial solution was filtered on a silver membrane filter with a pore size diameter of 0.45 µm. To establish constant moisture content, the filters with bacteria on them were placed on the surface of a LB agar for one hour in an incubator at 37^0C. The contact angles were determined as described above. The surface tension components of sand, biochar or bacteria were determined according to the Young Dupre equation (equation S1)[6].

$$(1 + cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right) \quad \text{(S1)}$$

Where θ is the contact angle measured, γ_L is the total surface tension of the liquid, γ_i^{LW} is the Lifshitz-van der Waals or apolar surface tension component of condensed material, γ_i^{+} and γ_i^{-} are the electron acceptor and electron-donor parameters of the Lewis-acid base components of the surface tension of condensed material, and the subscripts "S" and "L" refer to the bacterial and liquid phases. The surface tensions of the probing liquids are known (Table S2)[7]. Once all liquid surface tensions were input in equation 1, the equation will have three unknowns ($\gamma_S^{LW}, \gamma_S^{+}$ and γ_S^{-}).

Once the surface tension components of the solid were computed, the Hamaker constant (H_{132}) for bacteria labelled 1 transferring in sand or biochar labelled 2 under water labelled 3 can be calculated using equations S2 and S3[6].

$$H_{132} \approx \left(\sqrt{A_{11}} - \sqrt{A_{33}}\right)\left(\sqrt{A_{22}} - \sqrt{A_{33}}\right) \qquad \text{(S2)}$$

$$A_{ii} = 24\pi H_0^2 \gamma_i^{LW} \qquad \text{(S3)}$$

Where H_0 is the closest separation distance taken as 0.157 nm.

Table S2. Summary of the surface tension components of the propping liquids (mJ/m^2)[7].

Liquid	$\boldsymbol{\gamma_L}$	$\boldsymbol{\gamma_L^{LW}}$	$\boldsymbol{\gamma_L^+}$	$\boldsymbol{\gamma_L^-}$
Formamide	58.2	39	2.28	39.6
Polar DIW	72.8	21.8	25.5	25.5
Glycerol	64.0	34.0	3.92	57.4
Diiodomethane	50.8	50.8	0	0

Table S3: Contact angles of biochar, sand, and *E. coli* strains and the surface tensions and Hamaker constants (H_{132}) for *E. coli*/(biochar/sand) interactions in water

	Mean contact angle (º)						
	Biochar pyrolysis temperature (^{0}C)					***E. coli***	
Solvent	**400**	**500**	**600**	**700**	**Sand**	**O157:H7**	**k12**
DIW	115	129	130	123	49	24	26
Glycerol	119	129	136	111	90	43	50
Formamide	92	96	103	120	32		
DIM	-	-	-	-	-	94	76
γ_s^{lw} (mJ/m^2)	108	135	115	3	395	11	20
γ_s^+(mJ/m^2)	36	51	48	14	161	7	2
γ_s^-(mJ/m^2)	0.4	0.8	0.1	1.1	39	66	67
γ_s^{AB}(mJ/m^2)	7	12	5	8	158	43	23
γ (mJ/m^2)	115	147	120	11	552	54	4
H_{132} (×10^{20} J)-*E.coli* O157:H7	-1.44	-1.74	-1.52	0.75	-3.8		
H_{132} (×10^{20} J)-E. coli k12	*-0.24*	*-0.29*	*-0.25*	*0.12*	*-0.60*		

S6. Interfacial free energy calculations

Surface thermodynamics explain the interactions between the bulk surfaces without knowing any molecular details. An interfacial energy (γ_{cs}) is needed in order for a bacterial cell (C) to interact with a substrate (sand or biochar) (S) in an aqueous environment (W). This amount of energy is needed to replace the cell water (γ_{cw}) and substrate water interfacial energies (γ_{sw}) with a cell-substrate interfacial energy. Using a free energy notation, γ_{cs} must be less than $(\gamma_{cw} + \gamma_{sw})$ in order for the bacteria to attach to sand or biochar. The macroscopic thermodynamic approach of calculating thermal energies assume that the cell membrane is smooth and homogeneous which is not totally true [8].

$$\gamma_{cs} = \gamma_{cs}^{lw} + \gamma_{cs}^{AB} \quad \text{(S4)}$$

$$\gamma_{cs}^{lw} = 2\sqrt{\gamma_c^{lw}\gamma_s^{lw}} \quad \text{(S5)}$$

$$\gamma_{cs}^{AB} = 2\left(\sqrt{\gamma_c^+\gamma_c^-} + \sqrt{\gamma_s^+\gamma_s^-} - \sqrt{\gamma_c^-\gamma_s^+} - \sqrt{\gamma_c^+\gamma_s^-}\right) \quad \text{(S6)}$$

$$\gamma_{sw} = \gamma_{sw}^{lw} + \gamma_{sw}^{AB} \quad \text{(S7)}$$

$$\gamma_{sw}^{lw} = 2\sqrt{\gamma_s^{lw}\gamma_w^{lw}} \quad \text{(S8)}$$

$$\gamma_{sw}^{AB} = 2\left(\sqrt{\gamma_s^+\gamma_s^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_w^-\gamma_s^+} - \sqrt{\gamma_s^+\gamma_w^-}\right) \quad \text{(S9)}$$

$$\gamma_{cw} = \gamma_{cw}^{lw} + \gamma_{cw}^{AB} \quad \text{(S10)}$$

$$\gamma_{cw}^{lw} = 2\sqrt{\gamma_c^{lw}\gamma_w^{lw}} \quad \text{(S11)}$$

$$\gamma_{cw}^{AB} = 2\left(\sqrt{\gamma_c^+\gamma_c^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_w^-\gamma_c^+} - \sqrt{\gamma_c^+\gamma_w^-}\right) \quad \text{(S12)}$$

Where *AB* refers to the acid-base polar components of surface tension, *lw* is the apolar van der Waals component of surface tension.

S7. Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory of colloidal stability

The DLVO Theory is referred to as well as the double electric layer theory. Energy components can be repulsive or attractive based on the chemical structure, surrounding environment, and surface potentials. Most of the time, these forces are not discussed separately. Electrostatic energies (E_e) can be described using the following equation:

$$E_e = \frac{2\pi a_1 a_2 n K_B T(\Phi_1^2+\Phi_2^2)}{(a_1+a_2)\kappa^2}\left[\frac{2\Phi_1\Phi_2}{\Phi_1^2+\Phi_2^2} ln\left(\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right) + ln(1-\exp(-2\kappa h))\right] \quad \text{(S13)}$$

$$\Phi = \frac{ze\Psi}{K_B T} \quad \text{(S14)}$$

$$\kappa = \left(\frac{1}{\varepsilon_0 \varepsilon K_B T}\sum_{i=1}^{i=n} z_i^2 e_i^2 n\right)^{0.5} \quad \text{(S15)}$$

Where: a_1: The radius of sphere 1 (m) (bacteria), a_2: The radius of second sphere (m) (biochar or sand particle), n: Solution molarity (mol/m^3), K_B: Boltzmann constant (1.380649×10^{-23} J/K), T: Absolute temperature (K), z: Solution ion valance, e: Electron charge (-1.602 x 10^{-19} C), Φ_1 and Φ_2: Normalized surface potentials of spheres 1 and 2, ε_o: Relative permittivity of vacuum = 8.85×10^{-12} C^2/J.m, ε: Relative permittivity of solvent = 78 for water, ψ_1 and ψ_2: Surface potentials of spheres 1 and 2 (V) obtained from electrophoretic mobility measurements, κ: the Debby Hückel parameter (1/m), and h: separation distance (m).

According to equation S14, an increase in the electrolyte concentration results in a decrease in the Debby Hückel length and an accompanying reduction in the electrostatic energy. The strength of the columbic energy dissipates exponentially away from the surface. The reciprocal of the Debby Hückel length represents the center of mass of the diffuse counter ion cloud. The electrostatic energy or Columbic energy component of DLVO energies is mainly dependent on the

charge of the two surfaces, the separation distance between them and the geometry of the two interacting surfaces.

The van der Waals interaction energy (E_{LW}) between two dissimilar spheres can be calculated using a Hamaker expression, corrected for retardation effects [9]

$$E_{LW} = -\frac{H_{132}a_1a_2}{6h(a_1+a_2)} \quad \text{(S16)}$$

The negative sign indicates attractive forces.

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