Supporting Information

Role of Microbial Cell Properties on Bacterial Pathogen and Coliphage Removal in Biochar-modified Biofilters

Environmental Science: Water Research and Technology

A. R. M. NABIUL AFROOZ[1,2,3], ANA K PITOL[4,5], DIANNA KITT[2,6], ALEXANDRIA B. BOEHM*[1,2]

[1]Department of Civil and Environmental Engineering, 473 Via Ortega, Stanford University, Stanford, California 94305

[2] Engineering Research Center (ERC) for Re-inventing the Nation's Urban Water Infrastructure (ReNUWIt), USA

[3] Southern California Coastal Water Research Project (SCCWRP), Costa Mesa, California 92626

[4] Eawag, Swiss Federal Research Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

[5] Laboratory of Environmental Chemistry, School of Architecture, Civil, and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), CH 1015 Lausanne, Switzerland

[6] Department of Civil and Environmental Engineering, University of Illinois, 205 N. Mathews, Urbana Illinois, 61801

* Corresponding author: Alexandria B. Boehm, Email: aboehm@stanford.edu, Phone: 650 724 9128

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Table S1: Physicochemical properties of the collectors [1]

Properties	Biochar			Sand	
Water Contact Angle (°)	106.5±1.5			Completely wetting	
Roughness (nm)	Stylus Force (mg)	R_a	R_q	R_a	R_q
	1	41.4±22.6	75.5±41.6	0*	0*
	3	92.4±71.9	111.1±81.9	0*	0*
	8	0*	0*	3510.8±365.8	3706.6±391.4
Specific Surface Area (m^2/gm)	104.64±7.80			0.20±0.04	

*indicates indeterminable data either because of too low (sand) or too high (biochar) stylus force.

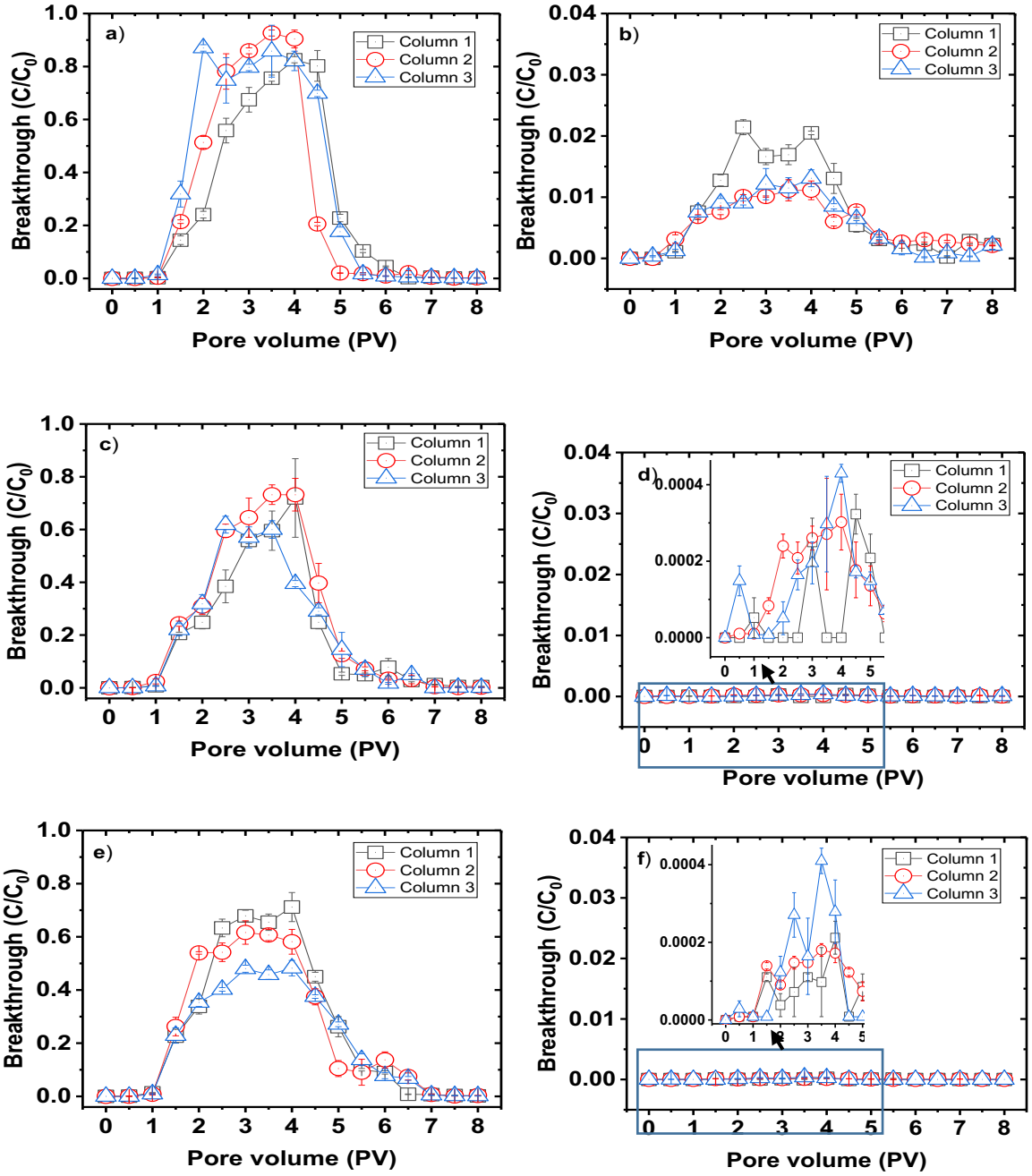

Fig S1. Bacterial transport in 15 x 2.5 cm (sand or biochar-amended sand packed) laboratory biofilter columns. a) *E. coli* transport in sand; b) *E. coli* transport in biochar-amended sand; c) *Salmonella* transport in sand; d) *Salmonella* transport in biochar-amended sand; e) *Staphylococcus* transport in sand; f) *Staphylococcus* transport in biochar-amended sand. Breakthrough curves for *Salmonella* (Figure d) and *Staphylococcus* (Figure f) are small compared to *E. coli* (Figure b):

hence the breakthrough curves for *Salmonella* and *Staphylococcus* are enlarged and shown as insets. Error bars represent standard deviation between replicate technical measurements (n = 2). All experiments were conducted at room temperature.

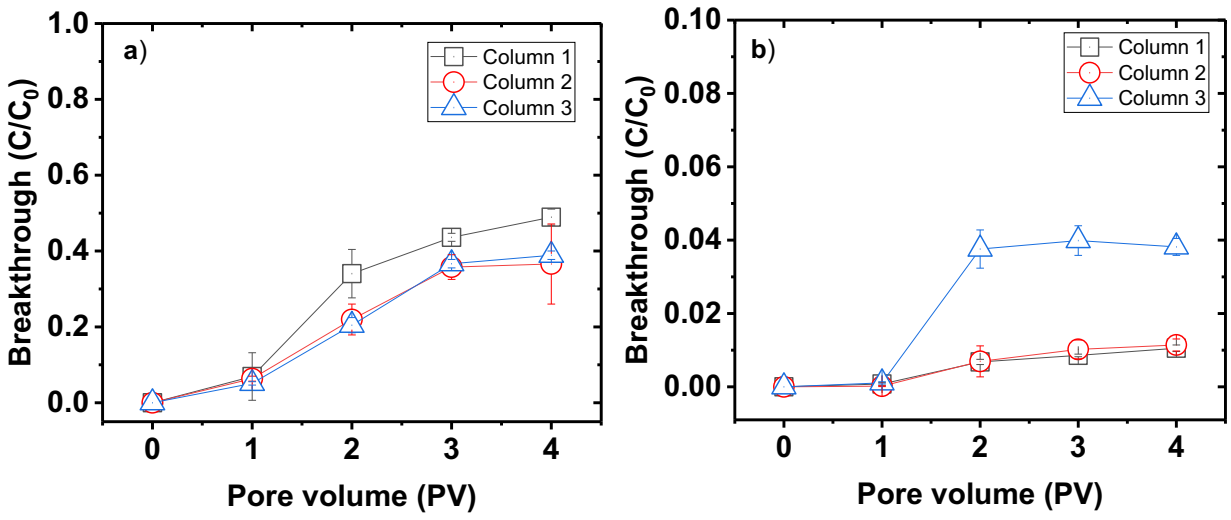

Fig S2. MS2 coliphage transport in 15x2.5 cm (sand or biochar-amended sand packed) laboratory biofilter columns. a) MS2 transport in sand; b) MS2 transport in biochar-amended sand. Due to resource limitations, only half the breakthrough curves were analyzed. Error bars represent standard deviation between replicate technical measurements (n = 2). All experiments were conducted at room temperature.

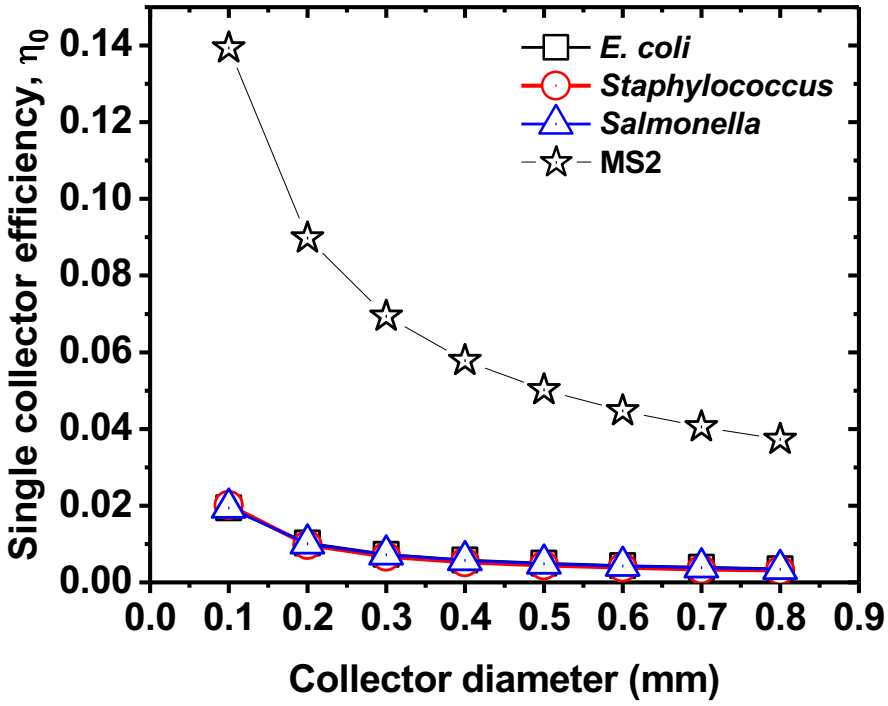

Figure S3: Theoretical total single collector contact efficiency of the collectors for the bacteria and bacteriophage used in this study. Total contact efficiency is a summation of the efficiencies obtained from Brownian diffusion, interception, and sedimentation. Individual contact efficiencies were calculated using the correlation equation described in equation 2. The average hydrodynamic diameter values of the microbial particles were used for calculating the collector efficiency. The Hamaker Constants were assumed to be 6.5 x 10^{-21} for the bacteria and 3.64x 10^{-21} for the bacteriophage.

DLVO modeling details. DLVO forces were calculated using adapted Wiese and-Healy expression for a sphere–flat plate system [2, 3]. Following equations are used to make the computation:

$$V_{EDL} = 2\pi a n_{\infty} KT \frac{{\Phi_p}^2 + {\Phi_e}^2}{2} \left[\left(\frac{2\Phi_p \Phi_c}{{\Phi_p}^2 + {\Phi_c}^2} * \ln\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right) + \ln(1-\exp(-2\kappa h))\right]$$

$$\Phi_i = \frac{Ze\Psi_i}{4KT}$$

$$\kappa = \left(\frac{e^2 \sum n_{i,\infty} {z_i}^2}{\epsilon\epsilon_0 KT}\right)^{0.5}$$

$$V_{VdW} = -\frac{A_{123}}{6}\left[\frac{a}{h} + \frac{a}{h+2a} + \ln\left(\frac{h}{h+2a}\right)\right]$$

$$V_{Tot} = V_{EDL} + V_{VdW}$$

Where,

V_{EDL}= Electrical double layer repulsive energy

V_{VdW}= Van-der-Waals attractive energy

V_{Tot}= DLVO energy barrier

a= Radii of microbial cells= 742 nm for *E. coli;* 944 nm for *Staphylococcus;* 770 nm for *Salmonella* and 35.5 nm mV for MS2.

e= Negative charge of an electron = $1.6*10^{-19}$ C

$n_{i,\infty}$= Equivalent concentration of electrolyte i

z_i= Valence of electrolyte i

n_{∞}= Bulk electrolyte density of the stormwater= 4.7 mM equivalent (using the recipe of the synthetic stormwater)

Z = 1 (using the recipe of the synthetic stormwater for a 4.7 mM ionic strength)

K= Boltzmann constant = 1.38×10^{-23} m^2 kg s^{-2} K^{-1}

T= Temperature= 298 K

Ψ_p=Average Zeta potential of biochar particles in stormwater = -19.6 mV

Ψ_c= Zeta potential of microbial cells in stormwater = -23.4 mV for *E. coli;* -21.2 mV for *Staphylococcus;* -11.7 for *Salmonella* and -13.7 mV for MS2.

ϵ_0 = Permittivity of vacuum = $8.854*10^{-12}$ F/m

ϵ=Relative permittivity of water = 80

h = Separation distance = 1-30 nm (dependent variable)

A_{123}= Hamaker constant=$6.5x10^{-21}$ J for collector-water-bacteria[4] and $3.64\ x10^{-21}$ for collector-water- MS2 [5]

Collector efficiency and deposition rate constant calculation:

$$\eta_0 = 2.4{A_s}^{0.333}{N_R}^{-0.081}{N_{Pe}}^{-0.715}{N_{vdW}}^{0.052} + 0.55A_s{N_R}^{1.675}{N_A}^{0.125} + 0.22{N_R}^{-0.24}{N_G}^{1.11}{N_{vdW}}^{0.053}$$

$$K_d = \frac{3(1-f)V\alpha\eta_0}{2fd_c}$$

Values of A_s, N_R, N_{Pe}, N_{vdW}, N_A, and N_G were calculated using the equations described in the literature[6] and the following values for corresponding parameters:

f = porosity of the column = 0.39 for sand columns and 0.44 for biochar columns

d_c=diameter of the collectors= 0.718 for sand; and a range of diameter (0.5 to 0.04 mm) for biochar to account for various percentage as revealed by the sieve analysis: the deposition coefficient was calculated as a weighted average of the deposition coefficient for each size range of biochar particles.

V= flow velocity = $3.53x10^{-21}$ m/s

μ = dynamic Viscosity of stormwater, μ = 0.001 Pa.S

ρ = relative density of the microbial cells, assumed 1.105 for bacteria [7] and 1.38 for MS2 [8]

α= attachment efficiency= assumed 1 for all microbes

Theoretical log removal value (LRV) calculation:

To calculate the theoretical log removal for a 15-cm column packed with 70:30 mix of sand and biochar, we assumed 70 % length of the column (10.5 cm) is packed with sand and the rest is packed with biochar. We estimated the log removal in the sand portion and the biochar portion individually and added them to obtain the total LRV for the biochar-amended biofilters. The LRV was calculated using the following equation

$$LRV = \frac{fLK_d}{2.303V}$$

Where f is the porosity of the packed media, L is the length of the column, K_d the deposition rate constant, and V the flow velocity.

Table S2: LRV in 4.5 cm biochar biofilter

Microbe	Column Porosity	Column Length (m)	Deposition Rate Constant (1/s)	LRV
Staph	0.44	0.045	0.001328	-0.32341
E. coli	0.44	0.045	0.001726	-0.42048
Salmonella	0.44	0.045	0.001749	-0.42604
Virus	0.44	0.045	0.016546	-4.03062

Table S3: LRV in 10.5 cm sand biofilter

Bacteria	Column Porosity	Column Length (m)	Deposition Rate Constant (1/s)	LRV
Staph	0.39	0.105	0.000459	-0.23102
E. coli	0.39	0.105	0.000519	-0.26173
Salmonella	0.39	0.105	0.000529	-0.26639
Virus	0.39	0.105	0.005286	-2.66312

References:

1. A. N. Afrooz and A. B. Boehm, Escherichia coli removal in biochar-modified biofilters: effects of biofilm, *PLoS ONE*, 2016, **11**, e0167489.
2. S. Lin and M. R. Wiesner, Exact Analytical Expressions for the Potential of Electrical Double Layer Interactions for a Sphere−Plate System, *Langmuir*, 2010, **26**, 16638-16641.
3. S. E. Truesdail, J. Lukasik, S. R. Farrah, D. O. Shah and R. B. Dickinson, Analysis of Bacterial Deposition on Metal (Hydr)oxide-Coated Sand Filter Media, *Journal of colloid and interface science*, 1998, **203**, 369-378.
4. S. E. Truesdail, J. Lukasik, S. R. Farrah, D. O. Shah and R. B. Dickinson, Analysis of Bacterial Deposition on Metal (Hydr)oxide-Coated Sand Filter Media, *Journal of colloid and interface science*, 1998, **203**, 369-378.
5. S. Sasidharan, S. Torkzaban, S. A. Bradford, R. Kookana, D. Page and P. G. Cook, Transport and retention of bacteria and viruses in biochar-amended sand, *Science of the Total Environment*, 2016, **548**, 100-109.
6. N. Tufenkji and M. Elimelech, Correlation Equation for Predicting Single-Collector Efficiency in Physicochemical Filtration in Saturated Porous Media, *Environmental science & technology*, 2004, **38**, 529-536.
7. E. Martinez-Salas, J. Martin and M. Vicente, Relationship of Escherichia coli density to growth rate and cell age, *Journal of bacteriology*, 1981, **147**, 97-100.
8. D. A. Kuzmanovic, I. Elashvili, C. Wick, C. O'Connell and S. Krueger, Bacteriophage MS2, *Structure*, 2003, **11**, 1339-1348.