Supporting Information

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

*Environmental Science: Water Research and Technology*

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Number of Pages: 10; Number of Figures: 3; Number of Tables: 3
Table S1: Physicochemical properties of the collectors

<table>
<thead>
<tr>
<th>Properties</th>
<th>Biochar</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Contact Angle ($^\circ$)</td>
<td>106.5±1.5</td>
<td>Completely wetting</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Roughness (nm)</th>
<th>Stylus Force (mg)</th>
<th>Biochar</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ra</td>
<td>Rq</td>
<td>Ra</td>
</tr>
<tr>
<td>1</td>
<td>41.4±22.6</td>
<td>75.5±41.6</td>
<td>0*</td>
</tr>
<tr>
<td>3</td>
<td>92.4±71.9</td>
<td>111.1±81.9</td>
<td>0*</td>
</tr>
<tr>
<td>8</td>
<td>0*</td>
<td>0*</td>
<td>3510.8±365.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specific Surface Area (m$^2$/gm)</th>
<th>Biochar</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>104.64±7.80</td>
<td>0.20±0.04</td>
</tr>
</tbody>
</table>

*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 $6.5 \times 10^{-21}$ for the bacteria and $3.64 \times 10^{-21}$ for the bacteriophage.
DLVO modeling details. DLVO forces were calculated using adapted Wiese and Healy expression for a sphere–flat plate system. Following equations are used to make the computation:

\[ V_{EDL} = 2\pi n_{\infty} K T \frac{\phi_p^2 + \phi_e^2}{2} \left[ \left( \frac{2\phi_p \phi_e}{\phi_p^2 + \phi_e^2} \ln \frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)} \right) + \ln(1 - \exp(-2\kappa h)) \right] \]

\[ \Phi_i = \frac{Ze\psi_i}{4KT} \]

\[ \kappa = \left( \frac{e^2 \sum n_{i,\infty} z_i^2}{\varepsilon \varepsilon_0 KT} \right)^{0.5} \]

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

\[ V_{Tot} = V_{EDL} + V_{vdW} \]

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

\( n_{\infty} \) = 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 x10^-23 m^2 kg s^-2 K^-1

\( T \) = Temperature= 298 K

\( \psi_p \) = Average Zeta potential of biochar particles in stormwater = -19.6 mV

\( \psi_c \) = 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.
\[ \epsilon_0 = \text{Permittivity of vacuum} = 8.854 \times 10^{-12} \text{ F/m} \]
\[ \epsilon = \text{Relative permittivity of water} = 80 \]
\[ h = \text{Separation distance} = 1-30 \text{ nm (dependent variable)} \]
\[ A_{123} = \text{Hamaker constant} = 6.5 \times 10^{-21} \text{ J for collector-water-bacteria}^4 \text{ and } 3.64 \times 10^{-21} \text{ for collector-water- MS2}^5 \]

**Collector efficiency and deposition rate constant calculation:**

\[ \eta_0 = 2.4 A_s^{0.333} N_R^{-0.081} N_{pe}^{-0.715} N_{vdw}^{0.052} + 0.55 A_s N_R^{1.675} N_A^{0.125} \]
\[ + 0.22 N_R^{-0.24} N_G^{1.11} N_{vdw}^{0.053} \]

\[ K_d = \frac{3(1 - f)V \alpha \eta_0}{2f d_c} \]

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:

\[ f = \text{porosity of the column} = 0.39 \text{ for sand columns and 0.44 for biochar columns} \]
\[ d_c = \text{diameter of the collectors} = 0.718 \text{ for sand; and a range of diameter (0.5 to 0.04 mm) for biochar} \]
\[ \text{to account for various percentage as revealed by the sieve analysis: the deposition coefficient was calculated as a weighted average of the deposition coefficient for each size range of biochar particles.} \]
\[ V = \text{flow velocity} = 3.53 \times 10^{-21} \text{ m/s} \]
\[ \mu = \text{dynamic Viscosity of stormwater, } \mu = 0.001 \text{ Pa.S} \]
\[ \rho = \text{relative density of the microbial cells, assumed 1.105 for bacteria}^7 \text{ and 1.38 for MS2}^8 \]
\[ \alpha = \text{attachment efficiency= assumed 1 for all microbes} \]
**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

\[ LRV = \frac{fL K_d}{2.303V} \]

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

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Column Porosity</th>
<th>Column Length (m)</th>
<th>Deposition Rate Constant (1/s)</th>
<th>LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph</td>
<td>0.44</td>
<td>0.045</td>
<td>0.001328</td>
<td>-0.32341</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.44</td>
<td>0.045</td>
<td>0.001726</td>
<td>-0.42048</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0.44</td>
<td>0.045</td>
<td>0.001749</td>
<td>-0.42604</td>
</tr>
<tr>
<td>Virus</td>
<td>0.44</td>
<td>0.045</td>
<td>0.016546</td>
<td>-4.03062</td>
</tr>
</tbody>
</table>

Table S3: LRV in 10.5 cm sand biofilter

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Column Porosity</th>
<th>Column Length (m)</th>
<th>Deposition Rate Constant (1/s)</th>
<th>LRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph</td>
<td>0.39</td>
<td>0.105</td>
<td>0.000459</td>
<td>-0.23102</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.39</td>
<td>0.105</td>
<td>0.000519</td>
<td>-0.26173</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0.39</td>
<td>0.105</td>
<td>0.000529</td>
<td>-0.26639</td>
</tr>
<tr>
<td>Virus</td>
<td>0.39</td>
<td>0.105</td>
<td>0.005286</td>
<td>-2.66312</td>
</tr>
</tbody>
</table>
References:


