Engineering Solutions to Improve the Removal of Fecal Indicator Bacteria by Bioinfiltration Systems during Intermittent Flow of Stormwater

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ABSTRACT: Bioinfiltration systems facilitate the infiltration of urban stormwater into soil and reduce high flow events and flooding. Stormwater carries a myriad of pollutants including fecal indicator bacteria (FIB). Significant knowledge gaps exist about the ability of bioinfiltration systems to remove and retain FIB. The present study investigates the ability of model, simplified bioinfiltration systems containing quartz sand and iron oxide-coated quartz sand (IOCS) to remove two FIB (Enterococcus faecalis and Escherichia coli) suspended in synthetic stormwater with and without natural organic matter (NOM) as well as the potential for accumulated FIB to be remobilized during intermittent flow. The experiments were conducted in two phases: (1) the saturated columns packed with either sand or IOCS were contaminated by injecting stormwater with bacteria followed by injection of sterile stormwater and (2) the contaminated columns were subjected to intermittent infiltration of sterile stormwater preceded by a pause during which columns were either kept saturated or drained by gravity. During intermittent flow, fewer bacteria were released from the saturated column compared to the column drained by gravity: 12% of attached E. coli and 3% of attached Ent. faecalis were mobilized from the drained sand column compared to 3% of attached E. coli and 2% attached Ent. faecalis mobilized from the saturated sand column. Dry and wet cycles introduce moving air–water interfaces that can scour bacteria from grain surfaces. During intermittent flows, less than 0.2% of attached bacteria were mobilized from IOCS, which bound both bacteria irreversibly in the absence of NOM. Addition of NOM, however, increased bacterial mobilization from IOCS: 50% of attached E. coli and 8% of attached Ent. faecalis were released from IOCS columns during draining and rewetting. Results indicate that using geomedia such as IOCS that promote irreversible attachment of bacteria, and maintaining saturated condition, could minimize the mobilization of previous attached bacteria from bioinfiltration systems, although NOM may significantly decrease these benefits.

INTRODUCTION

Urbanization increases impervious surface coverage, which in turn reduces natural infiltration of stormwater into soil. As a result, urban regions experience a net reduction in groundwater recharge, increased flooding, and contamination of surface waters receiving overland flow of stormwater.2 Stormwater contains a myriad of contaminants including suspended solids, nutrients, heavy metals, hydrocarbons, and pathogens.2 The US Environmental Protection Agency estimated that stormwater from urban areas, which represent just 3% of the US land area, impairs of 13% of river segments, 18% of lakes, and 32% of estuaries surveyed.3 Thus, there is an urgent need to improve management of urban stormwater.

Low impact development is gaining popularity as a means for slowing urban stormwater flows. Bioinfiltration in particular is...
attractive for urban areas because it does not significantly alter the landscape and also maintains aesthetics. It is typically implemented by grading surfaces that drain stormwater to trenches with plants growing atop a mixture of sand, soil, and compost. Water percolates through the mixture to infiltrate the groundwater or discharge to a drain. Traditionally, biofiltration systems are designed to decrease the surface runoff of stormwater, but the work has primarily been empirical. Additional work is needed to obtain a fundamental understanding of the contaminant removal process under relevant conditions to inform the improved design of biofiltration systems.

Most of the prior studies that examined contaminant removal by biofiltration systems focused on abiotic contaminants. Fecal indicator bacteria (FIB) are recognized as the main contaminants that prevent the downstream potable and nonpotable use of stormwater due to their strict regulation. FIB are linked to increase risk of waterborne illness when consumed during recreational exposures and dietary ingestion; however, there are also a number of issues associated with their use for assessing water quality as reviewed by Boehm et al. Only a handful of studies have evaluated FIB removal by biofiltration systems. Most of these studies are focused on evaluating the removal capacity of geomedia during steady infiltration of stormwater. However, the flow regime during the wet season is intermittent in nature, in which wet periods are followed by dry periods. The change in flow may mobilize previously attached bacteria in the biofiltration system. It is therefore essential to better understand how the intermittent nature of flow through biofiltration systems affects their ability to remove contaminants.

Colloids are transported by advection through soil and are deposited on the grain surface by settling, interception, and diffusion as predicted by colloid filtration theory. Attachment of colloids occurs when total repulsive forces between colloid and grain surface are less than the total attractive forces. However, a fraction of attached colloids can detach during intermittent flow due to scouring by moving air–water interfaces, release by thin-film expansion, reconnection of stagnant-water zones with bulk-water flow, and increase in shear forces. The collective process of detachment and transport of colloids is referred to as mobilization henceforth. Similar to inorganic colloids, pathogens or biocolloids can be mobilized during intermittent flow. Redman et al. showed that weakly attached bacteria or bacteria attached at a secondary minimum could be mobilized by an infiltration front. Bacteria, however, may also be attached to grains at the primary minimum where they may be strongly bound. Such attachment between bacteria and grains is expected if the grains have a positive charge, for example when iron oxides are present. It is not clear whether an infiltration front is capable of mobilizing bacteria attached at the primary minimum. If not, then the use of geomedia that promote strong attachment could be used to minimize the mobilization of bacteria from a biofiltration system during intermittent flow.

The aim of this study is to examine the effect of intermittent flow on the removal and mobilization of FIB from synthetic stormwater with and without natural organic matter (NOM) in a model, simplified biofiltration system. We hypothesized that bacteria that are reversibly attached to geomedia are more susceptible to remobilization during intermittent flow. Thus, use of geomedia such as positively charged metal oxides that promote irreversible attachment of bacteria could minimize bacterial mobilization during intermittent flow. We used Enterococcus faecalis and Escherichia coli as model bacteria and quartz sand and iron-oxide coated quartz sand (IOCS) as model geomedia to test the hypothesis.

## MATERIALS AND METHODS

### Preparation of Sands

To remove surface impurities including metal oxides and organic carbon, coarse Ottawa sand (0.6–0.85 mm, Fisher Scientific) was treated with strong acid and mild base. Briefly, the sand was rinsed in 10 M hydrochloric acid for 24 h followed by rinsing in 0.01 M NaOH. Clean sand was washed several times in deionized water to remove residual acid or base and dried at 110 °C. Iron oxides were coated on clean sand following the method outlined by Benjamín et al. (details in the Supporting Information). The iron-oxide coated sand (IOCS) produced by this method has previously been shown to contain 3.2% iron. The sand and IOCS were autoclaved (121 °C, 100 kPa, 15 min) and stored in sterile containers prior to use in the column experiments.

### Synthetic Stormwater

Synthetic stormwater was prepared using deionized water and salts to achieve the following concentrations: 5.1 mM of NaCl, 0.75 mM of CaCl2, 0.075 mM of MgCl2, 0.33 mM of Na2SO4, 1 mM of NaHCO3, 0.072 mM of NaNO3, 0.072 mM of NH4Cl, and 0.016 mM of Na2HPO4. The ionic strength of the stormwater was 20 mM. The pH was adjusted to between 6.9 and 7.2 using 0.1 N NaOH or 0.1 N HCl, and then, the solution was autoclaved. This recipe provides an average concentration of major ions in urban stormwater, though the concentration of the ions in stormwater is highly variable and could exceed the average concentration by orders of magnitude. Depending on the experiment, Suwannee river NOM (International Humic Substances Society, MN, USA) was added to the stormwater at 20 mg C L−1.

### Bacteria Preparation

**Escherichia coli K12** (ATCC 10798) and **Enterococcus faecalis** VS83 (ATCC 700282) were selected as model bacteria to assess the behavior of FIB that are ubiquitous in stormwater and are important causes of water quality violations. E. coli K12 is a motile, Gram negative bacterium and **Ent. faecalis** is a nonmotile, Gram positive bacterium. Their persistence and transport have been evaluated previously in stormwater. Both were cultured and suspended in the synthetic stormwater to achieve a concentration of 0.8–1.8 × 109 colony forming units (CFU)/mL (method details in Supporting Information). The bacterial suspension in stormwater was kept at 4 °C for 16–18 h for bacteria to adapt to stormwater prior to its use in the column experiments.

### Biofiltration Experiment

**Sand, IOCS, or 50% (w/w) mixtures of sand and IOCS were wet-packed in glass chromatography columns (Kontes, 15 cm length, 2.5 cm diameter). The porosity of the packed media was gravimetrically estimated to be 0.39 ± 0.01, and the pore volume (PV) was estimated to be 29.5 ± 0.5 mL (average ± standard deviation of replicated columns). Following the packing, deionized water (~20 PV) was flushed upward through packed media to remove loosely bound particles. Next, 5 PV of dx.doi.org/10.1021/es051368i Environ. Sci. Technol. 2013, 47, 10791–10798
synthetic sterile stormwater was used to chemically equilibrate sand.

After conditioning the geomedia with synthetic stormwater, the column experiments were conducted in two phases: (1) attachment phase under saturated conditions and (2) remobilization phase when the column was either drained or remained saturated before application of sterile stormwater. During the attachment phase, 3 PV of bacterial suspension followed by 3 PV of sterile synthetic stormwater were injected at 0.1 cm min\(^{-1}\) through the columns from the bottom (upward flow). The contaminated columns were subjected to two intermittent flow events to mimic the intermittency of actual rain and flooding events. The pump was stopped for 30 min. One of the columns was kept saturated; the other column was overturned, and pore water was drained by gravity. The column was overturned to maintain the water flow direction relative to the sand during draining. The residual water in the drained column was gravimetrically estimated to be 0.45–0.5 PV. Following the 30 min pause, the drained column was overturned again, to maintain the flow direction, and 2 PV of sterile synthetic stormwater was pumped upward through the 2 columns. The pump was stopped again for 30 min, and the process was repeated. Throughout the attachment and remobilization phases, sterile synthetic stormwater was introduced to the columns at a flow velocity of 0.1 cm min\(^{-1}\). The effluent was collected in 10 mL (0.3 PV) fractions using an automated fraction collector (model CF1, Spectrum Chromatography). During the pauses, samples were not collected from the undrained columns, and samples were collected as water drained from the drained columns. The bacterial concentrations in the influent and effluent were quantified by spread plating techniques and reported as colony forming unit (CFU) per mL of effluent. Each sample was enumerated at three decimal dilutions with duplicate plates per dilution. The CFU counts on duplicate plates from the dilution with between 30 and 300 CFU were averaged to calculate the concentration. Standard deviations of the concentrations of influent and effluent samples were calculated from the replicate plate counts; the standard deviation of \( C/ C_0 \) was determined by propagating the error for \( C \) and \( C_0 \). Twenty-four column experiments (Table 1) were conducted with \( E. \) coli and \( Ent. \) faecalis separately using varying fractions of coated sand (0, 50%, and 100%) and NOM (0 and 20 mg C L\(^{-1}\)). Experiments using quartz sand were conducted in duplicate.

### RESULTS

**Mobilization of Bacteria in Uncoated Sand.** \( E. \) coli and \( Ent. \) faecalis retention and subsequent remobilization through sand during two intermittent flow events are reported in Figure 1. Results from the duplicate experiments agreed well. The breakthrough concentration varied between organisms: the average relative breakthrough of \( Ent. \) faecalis (~40%) was slightly higher than that of \( E. \) coli (~30%) indicating more \( E. \) coli were attached to sand than \( Ent. \) faecalis (Table 2). Intermittent flows mobilized 2.8–13.2% of previously attached bacteria from sand, though the amount of bacteria mobilized during the second intermittent flow decreased compared to that mobilized in the first intermittent flow. Fewer bacteria were mobilized from saturated columns than from columns that were allowed to drain during the pause period. A greater fraction of attached \( E. \) coli compared to \( Ent. \) faecalis were mobilized from drained columns.

**Effect of Coated Sand on Remobilization of Bacteria during Intermittent Flow.** IOCS removed more bacteria than sand during injection of bacteria-laden stormwater (Figure 2). IOCS removed 99% of injected \( E. \) coli and 97% of injected \( Ent. \) faecalis (Table 2). Decreasing the fraction of IOCS to 50% did not decrease removal of either bacterium. IOCS also minimized detachment of bacteria during intermittent flow. Less than 0.03% of attached bacteria were remobilized from 100% IOCS when the column was allowed to drain between infiltration events. Less than 0.25% of attached bacteria were mobilized from the column packed with 50% IOCS. It is interesting to note that the fraction of bacteria mobilized from drained columns was similar to that mobilized from undrained columns when the columns were packed with IOCS suggesting

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**Table 1. Experimental Conditions to Evaluate the Effect of Saturation and Iron Oxide Coated Sand (IOCS) on Mobilization of \( Ent. \) faecalis and \( E. \) coli during Intermittent Flow Condition**

<table>
<thead>
<tr>
<th>bacteria</th>
<th>geomedia</th>
<th>NOM (mg C/L)</th>
<th>intermittent flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E. ) coli K12</td>
<td>sand</td>
<td>0(^{0}), 20</td>
<td>drained, undrained</td>
</tr>
<tr>
<td>50% sand + 50% IOCS</td>
<td>0</td>
<td>drained, undrained</td>
<td></td>
</tr>
<tr>
<td>100% IOCS</td>
<td>0, 20</td>
<td>drained, undrained</td>
<td></td>
</tr>
<tr>
<td>( Ent. ) faecalis V583</td>
<td>sand</td>
<td>0(^{0}), 20</td>
<td>drained, undrained</td>
</tr>
<tr>
<td>50% sand + 50% IOCS</td>
<td>0</td>
<td>drained, undrained</td>
<td></td>
</tr>
<tr>
<td>100% IOCS</td>
<td>0, 20</td>
<td>drained, undrained</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)A total of 24 column experiments were conducted. \(^{b}\)Experiments were conducted in duplicate.
dry–wet cycles had no impact on detachment of bacteria from IOCS.

**Effect of NOM on the Remobilization of Bacteria.** The removal capacity of sand and IOCS decreased in the presence of 20 mg C L⁻¹ NOM (Figure 3). The removal capacity of sand (average ± standard deviation of replicate columns) decreased from 70% (±1.1%) to 66.5% (±0.9%) for *E. coli* and from 60.1% (±4%) to 37.5% (±5.2%) for *Ent. faecalis* in presence of NOM, whereas the removal capacity of IOCS decreased from 99.1% (±0.1%) to 48.5% (±7.8%) for *E. coli* and from 97.5% (±0.6%) to 71.2% (±7.4%) for *Ent. Faecalis* (Table 2). Thus, removal of *E. coli* was actually lower by IOCS than sand in the presence of NOM, whereas removal of *Ent. faecalis* was still higher by IOCS than sand in the presence of NOM. During intermittent flow, NOM in stormwater increased the detachment of both organisms relative to the experiments without NOM; an exception was *E. coli* in the drained sand column. The influence of NOM on bacterial mobilization depended on the media and bacterial type: the advancing wetting front mobilized more *Ent. faecalis* but less *E. coli* from sand than IOCS.

**DISCUSSION**

**Effect of Geomedia Types on Removal of Bacteria.** IOCS removed a greater fraction of bacteria from stormwater containing 20 mg C L⁻¹ of NOM. The gray shades indicate 30 min pauses when the column was either undrained (circle) or drained (triangle). The error bar indicates one standard deviation. The bacterial concentrations are also presented in log scale in the Supporting Information (Figure S3).

Table 2. Fraction of Applied *E. coli* and *Ent. faecalis* Retained in Columns Packed with Different Fractions of Sand and IOCS before and after Intermittent Flows and the Percentage of Attached Bacteria Mobilized during Intermittent Flow

<table>
<thead>
<tr>
<th>IOCS (%)</th>
<th>NOM mg C/L</th>
<th>bacteria retained (%) before* (and after†) intermittent flow</th>
<th>bacteria mobilized during intermittent flow‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
<td><em>Ent. faecalis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>undrained</td>
<td>drained†</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>70 (69)</td>
<td>68 (59)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>70 (69)</td>
<td>71 (64)</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>98 (98)</td>
<td>98 (98)</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>67 (58)</td>
<td>66 (57)</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>43 (29)</td>
<td>54 (29)</td>
</tr>
</tbody>
</table>

*100 times ratio of total bacteria attached to total bacteria injected. Note that, before the intermittent flow, the “drained” and “undrained” columns were replicates. †100 times ratio of total bacteria retained after intermittent flows to total bacteria injected. ‡100 times ratio of total bacteria eluted during two intermittent flows and total amount of bacteria attached before intermittent flows. The column was saturated before intermittent flow and drained during intermittent flows. *Ratio of percentage of bacteria mobilized from drained column and undrained column during intermittent flow.
sand and bacteria) is characterized by a deep attractive well or primary minimum at a small separation distance, a maximum energy barrier, and a shallow attractive well or secondary minimum that is extended to larger distance. Under unfavorable conditions (electrostatic repulsion), bacteria can attach to the secondary minimum. In our study, sand removed a greater fraction of bacteria than that described in reported studies. This is likely a result of the high ionic strength (20 mM) of our stormwater compared to the aqueous matrices used in previous studies. At an ionic strength of 20 mM, bacteria reportedly attach in the secondary minimum and can detach during infiltration of lower ionic strength water.

Re-Entrainment of Bacteria during Intermittent Flow. During intermittent flow, the fraction of bacteria mobilized from the saturated sand columns was lower than the fraction of bacteria mobilized from the sand columns from which the pore water was drained during the pause periods. Thus, the introduction of an air phase during draining increased bacterial mobilization. This result agrees with the outcomes of previous studies done with inorganic colloids and bacteria. Shear forces induced by flowing water and colloidal dispersion from grain surfaces can mobilize bacteria from sand during transient flow in both saturated and unsaturated sand columns. In unsaturated columns, additional processes including thin film expansion and air–water interface scouring can mobilize bacteria during intermittent flow. Thus, changes in water-film thickness and the air–water interface appear to be important mechanisms for bacterial mobilization during intermittent flow in the sand columns. In IACS columns, only a small fraction of attached bacteria were mobilized during intermittent flow, even with the introduction of the air–water interface. This result supports the hypothesis that bacteria attached at the primary minimum are less likely to be remobilized by moving air–water interfaces during intermittent flow. Thus, maintaining saturated conditions and use of geomedia promoting attachment of bacteria at the primary minimum could minimize bacterial mobilization in bioinfiltration systems during intermittent flow conditions. Although saturated conditions appear beneficial for minimizing bacterial mobilization by intermittent stormwater flow, they may induce anoxic conditions. These, in turn, may affect the fate and transport of FIB and need further consideration.

Effect of Bacterial Species on Their Removal and Remobilization. Sand removed more E. coli than Ent. faecalis. This is consistent with the work of Chen and Walker who observed 89% removal of E. coli compared with 15% removal of Ent. faecalis in laboratory column studies (aqueous phase had 1 mM NaCl and no NOM), though the difference in the observed removal in their studies decreased with an increase in ionic strength. The result could be explained by the nature of attachment sites each species prefers and the type of interaction that exists between bacteria and sand surface. Generally, Ent. faecalis is more hydrophobic and has a more negative surface charge compared to E. coli. In addition to electrostatic forces, non DLVO interactions including hydrophobic and steric interaction can remove both species from the aqueous phase. Interaction energy calculations suggest that the hydrophobic interaction is important for attachment of Ent. faecalis to sand whereas steric interaction is important for E. coli. A greater fraction of attached E. coli was susceptible to remobilization during intermittent flow compared with Ent. faecalis. This indicates that Ent. faecalis was relatively strongly bound to sand, likely due primarily to hydrophobic interactions, whereas E. coli was more weakly bound likely due to steric interactions. Previous work indicates motility has an effect on bacterial attachment to porous media, so it possible that the nonmotile E. coli will exhibit different attachment behavior than the motile E. coli strain used herein.

IACS removed a similar fraction of E. coli and Ent. faecalis from stormwater in the absence of NOM. Assuming a perfect attachment efficiency of negatively charged bacteria on positively charged IACS, we attribute a similarity in removal fractions of both bacterial species to a similarity in their deposition rates on attachment sites. In contrast to our results, Truesdail et al. found that Gram-positive bacteria deposited at a higher rate than Gram-negative bacteria on geomedia (Al-coated sand) with net positive charge whereas Gram-negative bacteria deposited at a higher rate than DLVO calculations predicted on geomedia that had net negative charge (sand). They attributed this discrepancy to the effect of non-DLVO parameters such as surface charge distribution, surface roughness, and bacterial hydrophobicity. Importantly, their experimental design was different from ours; they used batch experiments with limited quantities of coated sand so that the attachment sites might have been exhausted during the experiment. The effluent concentrations in our study were fairly constant during the attachment phase, indicating that the attachment sites on the grain surface were not exhausted during the experiment.

Effect of NOM on Bacterial Removal and Remobilization. Addition of NOM to the stormwater reduced the removal of both bacteria by sand, though the reduction was greater for Ent. faecalis compared with E. coli. The influence of NOM on attachment of bacteria could be explained by five mechanisms (Supporting Information, Figure S4): (1) alteration of geomedia surface charge by adsorption of NOM, (2) competition between NOM and bacteria for attachment sites, (3) blocking (also referred as steric hindrance) of attachment sites from bacteria due to the adsorbed NOM blanket, (4) modification of surface hydrophobicity of bacteria, and (5) dissolution of iron oxide. NOM likely reduced bacterial attachment in the sand columns because NOM competed with bacteria for attachment sites. In the IACS columns, alteration of the grain surface charge may have also contributed to the reduction in attachment. NOM may modify the interaction energy of attachment between bacteria and grains. Alteration of grain surface charge and the presence of an NOM blanket on attachment sites could force bacterial attachment to occur in the secondary minimum of the NOM-modified grain. This in turn would increase the potential of bacterial release during intermittent flow. This may explain why a greater fraction of attached bacteria were mobilized during intermittent flow when NOM was present in stormwater. An additional explanation for the increased transport of bacteria from IACS during intermittent flow in the presence of NOM is dissolution of the iron-oxide. Fe(III)-oxide, which is stable at pH 7, can form complexes with NOM that reduces its stability and results in its dissolution. Although we did not measure Fe concentrations in the column effluent, we observed reddish colored effluent from the IACS column when NOM was present in stormwater but not when NOM was absent in stormwater. This suggests that NOM might have caused iron dissolution following the flow pauses. Further work is needed to explore this possibility more fully.

Because hydrophobic interactions are important for the attachment of Ent. faecalis to sand, NOM, which is
hydrophobic, may effectively compete with *Ent. faecalis* for the same attachment sites. This reasoning could explain the greater reduction in attachment of *Ent. faecalis* than *E. coli* in the presence of NOM in our sand columns. The greater fraction of *Ent. faecalis* than *E. coli* released from sand columns in the presence of NOM during intermittent flow is consistent with more *Ent. faecalis* being weakly attached than in the case of no NOM owing to the presence of an NOM blanket on the grains.

NOM reduced the removal of *E. coli* to a greater degree than *Ent. faecalis* in IOCS columns. Reasons for this may include: (1) the surface charge of *Ent. faecalis* is more negative than that of *E. coli* so a greater amount of *Ent. faecalis* can be electrostatically attracted to unoccupied sites than that of *E. coli*, (2) *Ent. faecalis* attaches to the NOM blanket by hydrophobic interactions more readily than *E. coli*, and (3) *E. coli* is sterically hindered by attached NOM to a greater extent than *Ent. faecalis*. All these processes could lead to less attachment, and relatively weaker attachment of *E. coli* than *Ent. faecalis* on IOCS in the presence of NOM. *E. coli*, owing to a relatively weaker attachment in the presence of NOM, was more vulnerable to mobilization than *Ent. faecalis*.

**Environmental Implications.** The current study highlights the importance of intermittent flow on remobilization of attached bacteria from a simplified bioinfiltration system. We showed that the bioinfiltration systems could act as sources of FIB instead of sinks during intermittent infiltration of stormwater due to remobilization of FIB by air–water interfaces. Thus, previous studies that examined the performance of bioinfiltration systems under continuous application of stormwater may underestimate their net removal capacity. We demonstrated that maintaining saturated conditions between two infiltration events decreased the mobilization of bacteria during intermittent flow. Saturated conditions might be maintained in the systems by controlling the flow of runoff through them from stormwater capture basins or raising the outlet or underdrain,\(^\text{34}\) for example. The potential benefits of geomedia to improve bacterial removal and decrease their mobilization during intermittent flow were highly variable. In the presence of NOM, IOCS demonstrated higher removal and less mobilization of *Ent. faecalis* compared to sand, whereas the opposite was true for *E. coli*. Thus, the benefits of geomedia appear highly dependent on water quality and bacterial surface characteristics. Future work will assess the performance of other geomedia including biochar and iron filings. The latter may provide a benefit over IOCS as there may be a greater number of attachment sites compared to IOCS.

There are several limitations to the present study. It was designed specifically to test the effects of intermittent flow on indicator bacterial mobilization in short-term studies using small 1-D columns that were packed uniformly with media without any aging processes. The small size of the columns precludes analysis of column length-scale effects.\(^\text{32,35}\) The short duration of the experiments and their initial sterile conditions did not allow for the development of an active microbial community, which will occur in real systems and will likely modify attachment efficiencies and affect bacterial predation rates. For example, the removal of FIB was observed to increase dramatically over the first 12 months in a bioinfiltration system containing a mixture of soil, sand, and mulch.\(^\text{18}\) Therefore, future studies should evaluate effects of geomedia aging as well as inactivation and grazing\(^\text{17}\) on the removal of model organisms in the bioinfiltration systems. Additional research is also needed to understand the impacts of NOM, which may come from many different sources and whose concentrations may be highly variable in stormwater. Nonetheless, understanding the interactions of NOM with geomedia or bacteria in our simplified system provides a foundation for understanding more complex, realistic systems. Finally, the work here focused on FIB removal; additional work should be done using actual waterborne pathogens including viruses and protozoa.

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