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Escherichia coli Removal in Biochar-Augmented Biofilter: Effect of Infiltration Rate, Initial Bacterial Concentration, Biochar Particle Size, and Presence of Compost

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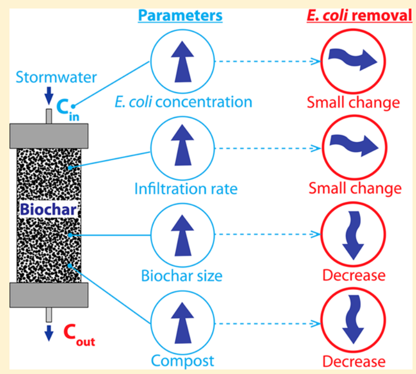
Ⓢ *Supporting Information*

ABSTRACT: Bioretention systems and biofilters are used in low impact development to passively treat urban stormwater. However, these engineered natural systems are not efficient at removing fecal indicator bacteria, the contaminants responsible for a majority of surface water impairments. The present study investigates the efficacy of biochar-augmented model sand biofilters for *Escherichia coli* removal under a variety of stormwater bacterial concentrations and infiltration rates. Additionally, we test the role of biochar particle size and "presence of compost on model" biofilter performance. Our results show that *E. coli* removal in a biochar-augmented sand biofilter is ∼96% and is not greatly affected by increases in stormwater infiltration rates and influent bacterial concentrations, particularly within the ranges expected in field. Removal of fine (<125 μm) biochar particles from the biochar-sand biofilter decreased the removal capacity from 95% to 62%, indicating biochar size is important. Addition of compost to biochar−sand biofilters not only lowered *E. coli* removal capacity but also increased the mobilization of deposited bacteria during intermittent infiltration. This result is attributed to exhaustion of attachment sites on biochar by the dissolved organic carbon leached from compost. Overall, our study indicates that biochar has potential to remove bacteria from stormwater under a wide range of field conditions, but for biochar to be effective, the size should be small and biochar should be applied without compost. Although the results aid in the optimization of biofilter design, further studies are needed to examine biochar potential in the field over an entire rainy season.



■ INTRODUCTION

Urban areas are rapidly expanding to accommodate growing populations. Urban development creates impervious surfaces which not only reduce natural infiltration of stormwater into soil[1] limiting groundwater recharge but also increase the amount and velocity of stormwater flow over land.[2] This causes flooding, erodes and degrades land,[3] and conveys contaminants from the developed lands to surface waters.[4] Because an additional 2.9 billion people are predicted to live in urban areas by 2050,[5] these problems are expected to worsen with time.[6]

The biofilter or bioretention system (hereafter referred to collectively as biofilter) is an example of a green infrastructure or low impact development (LID) that facilitates the infiltration of stormwater into the ground and reduces the velocity of overland stormwater flows in urban areas. It is traditionally designed by replacing a native block of soil with a mixture of sand (60−70%) and compost (30−40%) to increase the hydraulic conductivity of the block, thereby allowing rapid infiltration of stormwater either into the ground or an underdrain system connected to surface waters.[7] In some cases, plants are grown on top of biofilter to assist in pollutant removal and maintain hydraulic conductivity of filter media.[8] While a conventional biofilter increases groundwater recharge and minimizes flooding, it may not effectively remove all stormwater contaminants,[9] consequently causing contamination of receiving waters.[4] In particular, fecal indicator bacteria[9] (FIB) are difficult to remove in biofilters partly because the conventional biofilter media (mixture of sand and compost) have limited removal capacity.[10] To improve FIB removal, conventional biofilter media can be augmented with various engineered geomedia.[11] Among different types of geomedia, biochar—a carbonaceous geomedia produced by pyrolysis of biomass[12]—has been shown to have strong potential to remove FIB from stormwater.[13]

For biochar to be effective, it must consistently remove bacteria in a wide range of field conditions. For instance, stormwater infiltration rate may vary based on rainfall intensity and stormwater catchment area.[7] With an increase in infiltration rate, bacterial attachment on geomedia could decrease.[14,15] To date, the effect of infiltration rate on bacterial removal capacity of biochar has not been evaluated. Furthermore, the influent bacterial concentration in stormwater could vary by orders of magnitude based on the land use and

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season as well as antecedent conditions.[16,17] Yet, it is not clear if bacterial removal by geomedia depends on bacterial concentration in stormwater. While two studies in sand showed that the removal increased with increases in influent concentration,[15,18] another reported an opposite effect.[19] Additionally, the minimum bacterial concentration used in these previous studies was ~10^7 CFU mL^{-1}, which is 3 orders of magnitude higher than the maximum bacterial concentration expected in stormwater.[20] Several field studies showed that percentage removal was lower when bacterial concentration in influent stormwater was smaller.[21] Thus, further study is needed to evaluate the bacterial removal capacity of biochar under environmentally relevant concentrations.

Physical and chemical properties of biochar could vary based on feedstock properties, pyrolysis conditions, and post-treatment processes,[12] and these could subsequently change bacterial removal capacity of biochar by orders of magnitude.[11,22] Our recent work[13] showed that the variation in removal capacity between different types of biochar could be attributed to a difference in biochar chemical properties such as volatile organic matter and polarity. Because previous studies on biochar compared different types of biochar, the variability within the same biochar is not known. In particular, it is not clear how the particle size of a biochar affects its bacterial removal capacity. Because biochar size is related to its surface area and consequently the attachment sites on biochar,[22] biochar particle size may be a critical factor for good removal of bacteria.

Previous studies on biochar[13,23] did not examine the bacterial removal capacity of biochar in the presence of compost. Generally, compost is added to sand biofilters because it provides micronutrients, increases cation exchange capacity of sand, retains water—all these support the growth of plants on top of biofilter—and removes some contaminants including suspended particles and petroleum hydrocarbons.[24] However, addition of compost to a biofilter may have disadvantages. For instance, compost leaches dissolved organic carbon (DOC),[25] which has been shown to facilitate the mobilization of heavy metals,[26] herbicides,[27] and bacteria[25,28] from biofilter geomedia. Additionally, compost may leach nutrients such as nitrate and phosphate,[29] which could not only impair water quality of receiving waters[9] but also enhance the mobilization of bacteria from sand.[30] Bacterial removal capacity of biochar may decrease in the presence of compost, and this may affect the performance of biofilter.

The current study aims to examine the effect of stormwater infiltration rate, influent bacterial concentration, biochar particle size, and the presence of compost on bacterial removal in a biochar-amended biofilter. To achieve this, we used a commercially available biochar[13] and subjected it to intermittent infiltration of artificial stormwater containing *Escherichia coli* as a model fecal indicator, varying the geomedia mixture in the biofilter. Bacterial removal capacity of biochar was tested while varying influent *E. coli* concentration by 5 orders of magnitude and stormwater infiltration rate by a factor of 6. Our results demonstrated that the biochar amendment improved the bacterial removal capacity of a model biofilter under a wide range of environmental conditions, but the removal depended on the biochar particle size and the presence of compost.

EXPERIMENTAL METHODS

Geomedia Preparation. All geomedia were prepared following a method outlined elsewhere,[13] with an exception: geomedia were not sterilized prior to column experiments. Coarse Ottawa sand (0.6−0.85 mm, Fisher Scientific) was acid-washed and rinsed in deionized water.[31] A commercial biochar (Sonoma Compost Company, CA) was chosen for the study as it can be supplied for large-scale field application. The biochar was produced by a fast pyrolysis process. Softwood with a considerable amount of bark was fed to a pyrolysis chamber, where the wood was pyrolyzed within 1 to 3 s at a temperature range between 815 and 1315 °C. Because of the short residence time (1−3 s), most of the wood likely experienced the lower end of the set temperature range. The biochar particles were subsequently crushed and sieved to a size smaller than 1 mm. In order to examine the effect of the fine biochar particles on bacterial removal, a portion of the crushed biochar was further sieved to remove particles smaller than 125 μm, which constitutes 25% (by weight) of the crushed biochar. The biochar was characterized for detailed properties in our previous study.[13] Briefly, the biochar consists of 79% carbon, 12% ash, and 16% volatile matter.

Compost (American Soil & Stone, CA) was sieved to a size similar to the size of sand (0.6−0.85 mm) and washed in deionized water until the decanted water appeared clear. We sieved the compost to remove fine particles because they clogged the column in our preliminary experiments. All geomedia were dried at 110 °C overnight and stored at 4 °C prior to column experiments.

Synthetic Stormwater. Synthetic stormwater was prepared by dissolving 0.75 mM of $CaCl_2$, 0.075 mM of $MgCl_2$, 0.33 mM of Na_2SO_4, 1 mM of $NaHCO_3$, 0.072 mM of $NaNO_3$, 0.072 mM of NH_4Cl, and 0.016 mM of Na_2HPO_4 in deionized water and then sterilized using an autoclave (121 °C, 100 kPa, 45 min). Suwannee River natural organic matter (International Humic Substances Society, MN, USA) was added to the solution at 10 mg C L^{-1}. The pH was adjusted to 7.1 ± 0.2 using 1 M HCl or 1 M NaOH. The ionic strength of the stormwater was 4.7 mM. This recipe provides an average concentration of major ions in urban stormwater.[20]

Bacterial Suspension. A kanamycin-resistant strain of the Gram-negative bacterium *Escherichia coli* (NCM 4236) was used as a model indicator.[32] *E. coli* were suspended in stormwater following a method outlined elsewhere.[33] Briefly, *E. coli* were cultured to stationary phase, centrifuged to remove growth media, and suspended in the synthetic stormwater to achieve different initial concentrations within 10^3 to 10^7 colony forming units (CFU) mL^{-1}. For *E. coli* to adjust to stormwater prior to the column experiments, the *E. coli* suspension was kept at 4 °C for 12−14 h.

Biofilter Design. The model biofilter consists of a glass chromatography column (Kontes, 15 cm length, 2.5 cm diameter) with Teflon fittings at both ends. Each fitting has a built-in mesh (20-μm pore opening) to prevent geomedia particles from washing out of the column during stormwater infiltration. Dry geomedia were packed in columns following a method outlined in a previous study.[13] Dry-packing was chosen over wet-packing because both biochar and compost initially float in water or settle at a slower rate than sand when added simultaneously to water column during wet-packing procedure preventing uniform packing.

Four types of columns were used with different configurations of geomedia. These consisted of a (1) sand column (100% sand), (2) compost column (mixture of 70% sand and 30% compost, by volume), (3) biochar column (mixture of 70% sand and 30% biochar), and (4) biochar-compost column (mixture of 70% sand, 15% biochar, and 15% compost). To examine the effect of biochar particle size on bacterial removal capacity, an additional biochar column was packed with biochar without fine (<125 μm) biochar particles. Bacterial removal capacities of all these columns were measured at an infiltration rate of 12 cm h^{-1} (or 1 mL min^{-1}) and at an influent *E. coli* concentration of 10^5 CFU mL^{-1}.

To examine the effect of the infiltration rate and influent bacterial concentrations on bacterial removal capacity of biochar, biochar columns (with fine biochar particles) were tested at four additional infiltration rates, keeping influent *E. coli* concentration constant at 10^5 CFU mL^{-1}, and at four additional influent bacterial concentrations, keeping infiltration rate constant at 12 cm h^{-1}.

To examine the effect of compost on bacterial removal capacity of biochar, compost was added to a mixture of sand and biochar in two configurations: mixed and layered. In the mixed configuration, a uniform mixture of sand (70%), compost (15%), and biochar (15%) was packed in a column; whereas, in the layered configuration, sand mixed with compost or biochar (30% by volume) was packed in two separate layers, with the compost layer near the inlet and the biochar layer in the half of the column adjacent to the outlet. Columns with both configurations had the same mass of biochar, compost, and sand but differed by how geomedia were distributed.

Biofilter Experiments. All experiments were conducted in triplicate. Prior to all experiments, packed columns were conditioned as follows. First, 1 L or nearly 33 pore volume (PV) of deionized water was injected at 12 cm h^{-1} from the bottom of the column to remove colloidal particles and to leach DOC from compost. Then, 150 mL (~5 PV) of synthetic stormwater without *E. coli* were injected at the same rate to equilibrate geomedia with the stormwater constituents. We used upward flow throughout the experiment in order to displace air from pores within the column and minimize preferential flow.[33] The pore volume (~30 mL) was estimated by subtracting the weight of the dry column from the fully saturated column. Although, upward flow is effective at displacing air from columns, it may not remove all air, particularly air trapped within biochar pores. Thus, the pore volume estimated in our study could be slightly lower than the actual pore volume of the packed column. Furthermore, any remaining air could influence bacterial attachment and detachment within the biofilter. However, air is also likely to be present in any field application of biochar.

With the exceptions of infiltration rate and influent bacterial concentration, the detailed methods for column experiments were described in previous studies.[13,33] Briefly, to examine removal capacity of the columns, 90 mL (3 PV) of the stormwater with *E. coli* (~10^5 CFU mL^{-1}) were injected followed by injection of 90 mL (3 PV) of stormwater without *E. coli* at 12 cm h^{-1}. To test for the mobilization potential of the bacteria deposited within column, the contaminated columns were subjected to two intermittent infiltration events. During each event, the pump was stopped for 0.5 h, and the columns were overturned to maintain the water flow direction relative to the media during gravitational draining.[13,33] Following the pause, the drained column was overturned again to maintain the flow direction, and 60–70 mL (2–2.3 PVs) of sterile synthetic stormwater was pumped upward through the column at 12 cm h^{-1}.

To examine the effect of infiltration rate on *E. coli* removal in the biochar columns, 90 mL (~3 PV) of bacteria-laden (~10^5 CFU mL^{-1}) stormwater was injected through preconditioned biochar columns at four additional infiltration rates: 24, 36, 48, and 72 cm h^{-1} (or 2, 3, 4, and 6 mL min^{-1}, respectively), which respectively correspond to average residence times of 15, 10, 7.5, and 5 min. To evaluate the kinetics of *E. coli* removal in biochar, the flow was stopped for 5 h (without gravity drainage), and then an additional 50 mL (1.6 PVs) of stormwater with *E. coli* was injected. A change in relative concentration of effluent after flow-interruption was used as an indicator to evaluate bacterial removal kinetics.[34]

To examine the effect of influent bacterial concentration on *E. coli* removal, biochar columns were infiltrated (at 12 cm min^{-1}) with 90 mL (~3 PV) of the stormwater containing *E. coli* at the following concentrations: 10^3, 10^4, 10^5, 10^6, and 10^7 CFU mL^{-1}.

Sample Analysis. Using an automated fraction collector (Model CF1, Spectrum Chromatography), water samples were collected from columns in 10 mL fractions. In these fractions, bacterial concentration was quantified by a spread plating technique. We used Luria–Bertani Agar (Difco, Miller, Fisher Scientific) mixed with kanamycin (25 μg mL^{-1}). By injecting stormwater without *E. coli* in a preliminary study and analyzing its effluent, we observed an absence of CFUs on the agar plates, suggesting the medium is selective to injected *E. coli*. We chose culture-based measurement because this method has a lower detection limit than other surrogate measurements based on turbidity, and it is used to assess water impairment in practice. The concentration was reported as CFU per mL of effluent. Each sample was enumerated in duplicate at three decimal dilutions, and the concentration was calculated using plates with between 30 and 300 CFU. Where the expected *E. coli* concentration was too low for plate counts, the samples was analyzed by membrane filtration: one mL of effluent was filtered through a membrane (0.45 μm pores, sterile cellulose filter with 47 mm diameter, Millipore) and enumerated on the agar plate.

Data Analysis. Removal capacities of all columns were calculated from the relative concentration of *E. coli* during the breakthrough plateau (typically within 1.5 to 3 PV). In the experiments with intermittent flow, the number of attached bacteria in the column was calculated using a mass balance, assuming no growth or death of bacteria in the column during experiment. Because intermittent flow lasted a maximum of 3 h following the injection of bacteria, any bacteria accumulated due to growth within biofilter are likely insignificant compared to total bacteria deposited.[13] In all experiments, we monitored the concentration of *E. coli* in the stormwater feed solution at the start and the end of the experiment (maximum 6-h duration), to examine if *E. coli* had grown during the experimental period. *E. coli* concentration did not increase during the experimental period.

To identify statistically significant differences between the bacterial removal capacities and the fraction of attached bacteria mobilized from columns under different experimental conditions, one-way analysis of variance (ANOVA) was performed with Tukey's post hoc test. All statistical analyses were performed using SPSS Statistics (v.20, IBM, NY, USA). Differences were considered significant at $p < 0.05$.

RESULTS

Effect of Biochar Size on *E. coli* Attachment and Remobilization. We compared the results from sand columns with that from biochar columns with or without fines in order to determine whether addition of biochar improved bacterial removal during *E. coli* injection and the following intermittent infiltration events. During application of bacteria-laden stormwater, effluent *E. coli* concentrations from biochar columns were nearly 1 order of magnitude smaller than that from the sand columns (Figure 1). The sand columns removed 35 ± 6% (average ± one standard deviation of triplicate columns) of injected *E. coli*, whereas addition of 30% (by volume) biochar significantly ($p < 0.001$) increased the removal to 95 ± 1% (Table 1). However, removal of fine (<125 μm) biochar particles lowered the removal in the biochar column to 62 ± 5%, which is still significantly greater ($p < 0.001$) than that of the sand column.

Table 1. Percentage of Applied *E. coli* Deposited in Columns Packed with Different Combinations of Geomedia and the Percentage of the Deposited *E. coli* Mobilized during Two Intermittent Flows[e]

geomedia[a]	percentage of injected bacteria deposited[b]	percentage of deposited bacteria mobilized[c]
A. sand	34.6 ± 6.3	22.0 ± 2.9[d]
B. sand (70%) + compost (30%)	15.4 ± 3.3	61.0 ± 13.2
C. sand (70%) + biochar (30%)	95.2 ± 1.0	2.2 ± 1.1
D. sand (70%) + biochar (30%) without fines (<125 μm) particles	61.8 ± 4.9[d]	10.1 ± 2.3
E. mixture of sand (70%), biochar (15%), and compost (15%)	51.0 ± 5.8[d]	38.0 ± 11.5[d]
F. layer of geomedia B followed by a layer of geomedia C	52.7 ± 5.8[d]	39.3 ± 3.1[d]

[a]The percentage in the mixture of geomedia are based on volume. [b]Percentage deposited before intermittent flow; this was calculated from the ratio of total bacteria deposited to total bacteria injected. Total bacteria deposited were estimated by subtracting total bacteria eluted from total bacteria injected before intermittent flow. [c]Percentage calculated from the ratio of total *E. coli* mobilized during two intermittent flows to total *E. coli* deposited in columns before intermittent flows. [d]Values within the same column are statistically similar ($p > 0.05$). [e]The value indicates the average (± one standard deviation) of the result from triplicate experiments.

In these experiments, intermittent flow mobilized *E. coli* previously deposited on biochar and sand (Table 1). During intermittent flow, the effluent *E. coli* concentration was typically high at the start of infiltration and decreased as the infiltration continued. The total amount of *E. coli* mobilized from biochar columns was significantly ($p < 0.001$) lower than that from sand columns: 22 ± 3% (average ± one standard deviation of triplicate columns) of deposited *E. coli* were mobilized from sand columns compared with only 2 ± 1% from biochar columns and 10 ± 2% from biochar columns without fine biochar particles.

Effect of Infiltration Rate on *E. coli* Removal. Under the different flow conditions, removal was high in the biochar columns, varying between 91% and 96%, on average (Figure 2). An ANOVA indicated that removal was significantly ($p < 0.05$) affected by infiltration rate, and this was due to the ∼5% difference between removal at the infiltration rate of 72 cm h^{-1} and the removal at other rates (post hoc Tukey test, $p < 0.05$). After the breakthrough plateau of experiments with infiltration rate 24, 36, 48, and 72 cm h^{-1}, a 5-h flow interruption reduced the effluent *E. coli* concentration to nearly 20% of its concentration during the breakthrough plateau (Figure S1).

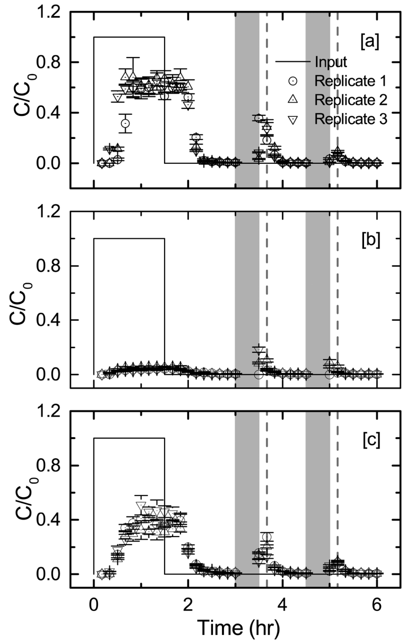

Figure 1. Transport and mobilization of *E. coli* through columns packed with (a) sand, (b) mixture of sand and biochar, and (c) mixture of sand and biochar where biochar particles smaller than 125 μm were removed. The *E. coli* concentration was 1.3 (±0.4) × 10^5 CFU/mL in the injected stormwater, and the stormwater infiltration rate was 12 cm h^{-1}. The gray area indicates the 0.5 h pause during which the column was drained, and the dashed lines indicate the timing of the first samples after the pause. The error bar indicates one standard deviation of measurements.

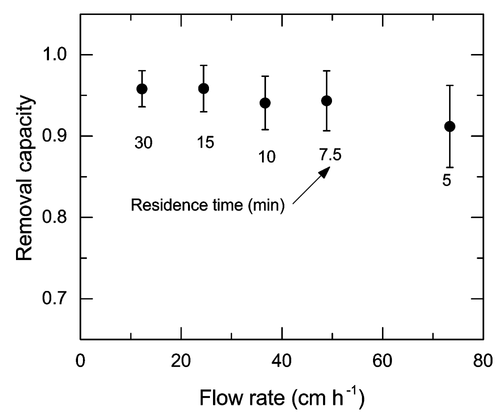

Figure 2. Effect of stormwater flow or infiltration rate on *E. coli* removal in biochar columns. The error bar indicates one standard deviation of triplicate experiments. The influent concentration was approximately 1.3 (±0.4) × 10^5 CFU mL^{-1}.

Effect of Influent Concentration on *E. coli* Removal. *E. coli* removal in the biochar columns did not change ($p > 0.2$) with increases in influent bacterial concentration to as high as 10^6 CFU mL^{-1} (Figure 3). Within this concentration range, the average removal was 97%. When the influent concentration was ∼10^7 CFU mL^{-1}, the removal decreased significantly ($p < 0.006$) to 91%.

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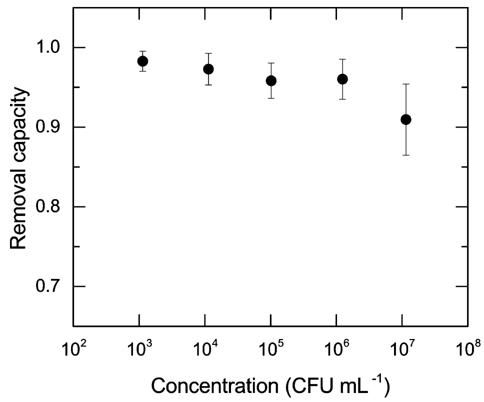

Figure 3. Effect of influent *E. coli* concentration on their removal in biochar column. The stormwater infiltration rate was 12 cm h^{-1}. The error bar indicates one standard deviation of triplicate experiments.

Effect of Compost on Removal and Remobilization of *E. coli*. Addition of compost not only decreased ($p < 0.004$) the deposition of *E. coli* in sand and biochar columns but also increased ($p < 0.001$) the remobilization of deposited *E. coli* during intermittent flow (Table 1, Figure S2). During injection, 15 ± 3% (average ± one standard deviation of triplicate columns) of injected *E. coli* were deposited in the compost column; and 61 ± 13% (average ± one standard deviation of triplicate columns) of the deposited *E. coli* were mobilized during two intermittent infiltration events (Table 1). Replacing half of the compost (by volume) with biochar significantly ($p < 0.001$) increased *E. coli* deposition during bacterial injection to 51 ± 6% and significantly ($p < 0.025$) decreased the bacterial mobilization to 38 ± 11% during intermittent flow. During intermittent flow, the bacterial concentration peaks in the biochar-augmented compost columns were higher than those in the compost columns, but the peaks accounted for a smaller fraction of the deposited *E. coli* compared with the compost columns. Removal and remobilization in these columns were not significantly ($p > 0.99$) different from the columns where the same amount of compost and biochar were packed in two separate layers.

DISCUSSION

Effect of Biochar on Removal and Remobilization of *E. coli*. Compared with sand columns, biochar-augmented sand columns not only removed more *E. coli* but also decreased the mobilization of attached *E. coli* during intermittent flow. These results are in agreement with a previous study, in which different types of biochar were used to examine the effect of DOC (20 mg L^{-1}) on removal and remobilization of *E. coli* in a model biofilter.[13] The detailed reasons for high bacterial removal in the biochar column were discussed in the previous study.[13] Briefly, according to the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory,[35] under unfavorable conditions (i.e., negative surface charges of geomedia and bacteria surface), bacteria most likely attach at the secondary minimum on sand,[36] whereas they may attach at the primary minimum on biochar because of an overall increase in attractive forces, such as hydrophobic and steric interactions. Additionally, the rough surface and irregular shape of biochar can promote bacterial attachment by straining.[37] Biochar increased the pH of pore water to ∼9 (in contrast to pH 7.5 for sand column and 7.1 for compost column, data not shown); the electrostatic repulsion is expected to be greater in biochar than sand. Because bacterial retention in the biochar columns was greater than that in the sand columns, the DLVO theory cannot explain the increase in deposition of bacteria in the biochar columns. This result is similar to the previous studies, which showed that non-DLVO forces including hydrophobic attraction, steric interaction, and straining could potentially enhance the bacterial attachment on biochar.[23,38]

During intermittent flow, bacteria are mobilized by several processes: an increase in shear forces at the grain boundary,[39] scouring by a propagating air−water interface,[40] and reduction of capillary forces on bacterial cell.[41] An observed decrease in *E. coli* mobilization in biochar column compared with sand column has been attributed to a net increase in strength of bacterial binding and decrease in the intrusion of air during draining.[13] Because the driving forces during intermittent flow are less effective in detaching bacteria from the primary minimum, bacteria mobilization is expected to be lower in biochar than sand column.[33] In our study, a flow pause lasted only 30 min during intermittent flow, whereas the flow pause in nature could last several hours to days, depending on rainfall conditions. During a longer pause, attached bacteria could either accumulate due to growth[13] or decay due to inactivation.[42] Thus, intermittent flow with a different duration of pause could mobilize different amount of bacteria from biofilter.

Effect of Biochar Particle Size. We demonstrated that removal of fine (<125 μm) biochar particles not only lowered the *E. coli* removal of biochar from 95% to 62% but also increased *E. coli* mobilization during intermittent flow by a factor of 5. This result is attributed to decrease in overall surface area of biochar after removal of fine biochar particles; coarse biochar particles have less surface area or attachment sites than fine biochar particles.[43] Because bacteria mostly attach to the outer surfaces of biochar particles or internal wall of pores larger than a bacterium size (∼1 μm), the surface area responsible for bacterial attachment could be much smaller than surface area estimated by N_2 adsorption isotherm which would include internal pores smaller than ∼1 μm. Additionally, fine biochar particles may increase the compactness of packing by filling the gaps between larger grains.[44] This could increase bacterial removal by straining.[45]

Effect of Infiltration Rate. According to the stormwater best management practice guide for biofilters by the U.S. Environmental Protection Agency,[46] a minimum residence time of stormwater in a biofilter should be 5 min. For pollution control, the recommended stormwater infiltration rate through a biofilter is between 13 and 30 cm h^{-1}, although the infiltration rate could exceed this range in some cases.[46] Within this range, the biochar column consistently removed more than 96% of injected bacteria, indicating an infiltration rate increase did not affect bacteria removal. An increase in infiltration rate is generally expected to decrease colloidal deposition on geomedia if removal depends on residence time[14] or if the hydrodynamic drag near the grain surface is large enough to prevent effective attachment of colloids on geomedia.[47] At the maximum infiltration rate (72 cm h^{-1}) in our study, which corresponds to 5 min of residence time, the removal in biochar columns was 91 ± 5%, suggesting that approximately one log removal occurred within 5 min. Because an increase in flow velocity did not substantially decrease bacterial removal in biochar, we surmise that a major fraction of bacteria were instantaneously attached to biochar and the hydrodynamic drag in high flow condition was not sufficient to detach the deposited bacteria from biochar. As a result, the performance of biochar-amended biofilter remained consistent at high infiltration rates. Because a high hydraulic conductivity of geomedia is desired to maximize stormwater infiltration, the ability of biochar to remove bacteria while allowing rapid infiltration of stormwater makes it an attractive biofilter amendment.

Kinetics of Bacterial Removal during Rainless Period. During a rainless period following a rainfall, the bacteria concentration in stagnant pore water within a biofilter could change due to growth or die off. If these bacteria are not removed from pore water during this period, they may contribute to downstream contamination in the following rainfall even if the stormwater influent contains no bacteria.[48] Thus, it is important to understand the fate of bacteria in biochar augmented columns during no-flow conditions. A decrease in concentration of *E. coli* during the 5-h flow interruption suggests that *E. coli* in pore water were removed by rate-limited processes[34] including inactivation. Biochar contains mineral ash and organic carbon, which may attach bacteria at different rates. In particular, attachment on purely hydrophobic carbon surfaces could be slower than on mineral ash.[49] This could cause a greater removal with longer residence time. Additionally, if the attachment is transport limited, due to diffusion of bacteria from pore water to biochar-water interface, bacteria concentration could also decrease during flow interruption.[34]

Effect of Influent *E. coli* Concentration. *E. coli* concentration in stormwater can vary from below detection limit to as high as 10^4 CFU mL^{-1}.[20] Within this concentration range and at higher concentrations up to 10^6 CFU mL^{-1}, bacterial removal in the biochar augmented model biofilter column was not affected by influent *E. coli* concentration. At a concentration of $\sim 10^7$ CFU mL^{-1}, the removal decreased to 91%, indicating that some of the attachment sites could be exhausted at this extremely high but perhaps unrealistic concentration of *E. coli*. There is limited research examining the removal of bacteria at this wide concentration range. Haznedaroglu et al.[18] varied *E. coli* concentration by 2 orders of magnitude (5×10^6, 10^7, and 10^8 CFU mL^{-1}) in deionized water at ionic strength 10 mM and observed that the increased influent *E. coli* concentration decreased the removal in a sand column from 65% to nearly 45%. The decrease in removal was attributed to exhaustion of attachment sites on sands.

Effect of Compost. A conventional biofilter media contains up to 30% compost to enhance removal of stormwater contaminants and to support plant growth.[50] We showed that addition of compost to sand or biochar columns decreased the deposition of injected *E. coli* and increased mobilization of the deposited *E. coli*. A decrease in bacterial deposition in the presence of compost is attributed to leaching of DOC, which may have exhausted the attachment sites in sand and biochar (Figure S3). In our previous study,[13] we showed that an addition of 20 mg DOC L^{-1} (or ~1 mg of DOC per g of biochar) to artificial stormwater decreased *E. coli* removal capacity of Sonoma biochar by 2 orders of magnitude. Based on the concentration of DOC leached from compost during preconditioning of the column (details in Supporting Information), biochar in the compost-biochar column could have been exposed to ~36 times more DOC (~9.8 mg DOC per g of biochar) than biochar without compost. The total amount of DOC leached from compost could be much higher than the estimated value, because DOC is expected to leach continuously during successive infiltration events.[25,51] Thus, DOC from compost could render biochar ineffective for removal of fecal indicator bacteria.

Environmental Implications. This study demonstrated that biochar amendment improved the bacterial removal capacity of a sand biofilter under a wide range of environmental conditions, including variable stormwater infiltration rates and bacterial loading, although the results could vary with different types of bacteria or different isolates of the same bacteria.[23,38,52] In our study, particle size of biochar is found to be critical for bacterial removal from stormwater. Because bacterial removal in biochar decreased after removal of fine biochar particles (less than 125 μm in size), commercial biochar should be crushed to a small size in order to maximize their bacterial removal capacity. Additionally, we showed that the DOC leached from compost completely eliminates the benefit of biochar addition for removal of *E. coli*. Thus, biochar should not be mixed with compost in a biofilter if the goal is to reduce *E. coli* concentrations in stormwater. In future biofilter design, biochar could completely replace compost in biofilter media because, while removing more contaminants than compost, biochar could retain the benefits of compost (i.e., support plant growth[53]) and eliminate the negative impact of compost such as leaching of nitrate and phosphate,[54] heavy metals,[55] chemical,[56,57] and biological contaminants.[13,23] However, biochar should also be screened for any contaminants that may potentially leach from biochar.[58] Although biochar could cost nearly 10 to 20 times more than compost, biochar is expected to last much longer, potentially nearly a hundred years,[59] indicating biochar may be cost-effective in long-term with the added benefit of sequestering carbon under ground.[60]

The experiments conducted herein were short in duration and do not include potential effects of physicochemical and biological aging[61] which may including clogging from biofilm formation or presence of fine particles within the geomedia. We also did not include suspended solids in the artificial stormwater and thus did not evaluate the effect these suspended particles have on clogging. Future work that considers these issues is needed. We did not evaluate the effect of preferential flow, which may occur during downward flow of stormwater in field condition.[62] The present study also did not specifically evaluate the effect of bacterial growth[13] or die off, possibly due to inactivation by biochar.[42] In a previous study,[13] we observed growth of *E. coli* in the simulated stormwater over a period of 4 days but at a very low rate that would be negligible over the duration of the short-term experiments conducted here.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Effect of 5-h flow interruption on effluent concentration (Figure S1), transport and mobilization of *E. coli* through compost and compost mixed with biochar column (Figure S2), DOC leached from compost biofilters (Figure S3), and calculation to estimate the DOC leached per gram of compost. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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