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Effect of weathering on mobilization of biochar particles and bacterial removal in a stormwater biofilter

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ABSTRACT

To improve bacterial removal, a traditional stormwater biofilter can be augmented with biochar, but it is unknown whether bacterial removal remains consistent as the biochar weathers during intermittent exposure to stormwater under dry-wet and freeze-thaw cycles. To examine the effect of weathering on bacterial removal capacity of biochar, we subjected biochar-augmented sand filters (or simplified biofilters) to multiple freeze-thaw or dry-wet cycles for a month and then compared their bacterial removal capacity with the removal capacity of unweathered biofilters. To isolate the effect of physical and chemical weathering processes from that of biological processes, the biofilters were operated without any developed biofilm. Biochar particles were mobilized during intermittent infiltration of stormwater, but the mobilization depended on temperature and antecedent conditions. During stormwater infiltration without intermediate drying, exposure to natural organic matter (NOM) in the stormwater decreased the bacterial removal capacity of biochar, partly due to exhaustion of attachment sites by NOM adsorption. In contrast, exposure to the same amount of NOM during stormwater infiltration with intermediate drying resulted in higher bacterial removal. This result suggests that dry-wet cycles may enhance recovery of the previously exhausted attachment sites, possibly due to diffusion of NOM from biochar surfaces into intraparticle pores during intermediate drying periods. Overall, these results indicate that physical weathering has net positive effect on bacterial removal by biochar-augmented biofilters.

1. Introduction

Urbanization and the resulting changes in the natural landscape are severely affecting the water quantity and quality of surface waters and groundwater (US NRC, 2009). Impermeable surfaces in urban areas limit natural infiltration of stormwater and increase overland flow, which in turn increases flooding, land erosion, and contamination of water resources that receive stormwater (US EPA, 2009). To manage stormwater, bioinfiltration systems or biofilters have been increasingly included in the urban development plan. A biofilter is typically designed by replacing a section of native soil with a mixture of sand and compost (US EPA, 2000). Stormwater infiltrates the biofilter to augment shallow groundwater and slow overland flows. Biofilters can also be designed to remove contaminants from stormwater (Grebel et al., 2013; LeFevre et al., 2015). However, some contaminants including fecal indicator bacteria are not efficiently removed in biofilters (US EPA, 2009). Furthermore, biofilters may become sources of fecal indicator bacteria as the organisms can be mobilized from biofilter media during intermittent infiltration of stormwater (Mohanty et al., 2013).

To enhance bacterial removal, bioinfiltration media can be augmented with geomedia such as iron filings, iron oxide coated sand, zeolite, activated carbon, and biochar (Grebel et al., 2013). Among these geomedia, biochar—a low cost material derived by pyrolysis of biomass waste—is particular attractive because it can simultaneously remove a variety of contaminants (Ahmad et al., 2014). Several recent studies showed that biochar could remove fecal indicator bacteria from simulated groundwater (Abit et al., 2012, 2014; Bolster and Abit, 2012) and stormwater (Mohanty and Boehm, 2014; Mohanty et al., 2014a). However, these studies used fresh (i.e., previously unused) biochar, whose properties changed little since production. In contrast, biochar properties in

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the field are expected to change substantially due to interactions with air, water, soil minerals, and the biological community (Spokas, 2013). These physical, chemical, and biological processes are collectively referred as weathering. In nature, freeze-thaw and dry-wet cycles contribute to physical weathering (Naisse et al., 2014); oxidation, hydrolysis, and absorption of natural organic matter (NOM) or other aqueous chemicals contribute to chemical weathering (Nguyen et al., 2010; Uchimiya et al., 2010; Zimmerman, 2010); biofilm formation and chemical reactions mediated by living organisms contribute to biological weathering (Luo et al., 2013). All these processes may occur within stormwater biofilters. In particular, NOM in stormwater can compete with bacteria and exhaust attachment sites on geomedia including iron oxide-coated sand (Abudalo et al., 2010; Mohanty et al., 2013) and biochar (Mohanty et al., 2014a). However, these short-term (few hours) studies did not account for changes in geomedia properties during weathering.

Conditions within stormwater biofilters are dynamic because of intermittent infiltration of stormwater and the ensuing no-flow period when pore water dries or freezes. Variations in water regime in biofilters and climatic conditions such as temperature, antecedent drying or freezing duration, and humidity may physically and chemically alter biochar properties (Nguyen and Lehmann, 2009). These dynamic conditions could also enhance erosion of biochar particles by mobilizing small or colloidal biochar particles from biofilters. For instance, dry-wet cycles facilitate the mobilization of colloids from sand and soil (Cheng and Saiers, 2009; Mohanty et al., 2015b; Zhuang et al., 2007). During dry-wet cycles, colloids can be mobilized by several processes: 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 (DeNovio et al., 2004). During freeze-thaw cycles, expanding ice can create preferential flow paths in soil and enhance mobilization of colloids (Mohanty et al., 2014b). The same processes could mobilize and leach small biochar particles from the biofilters. Because small biochar particles typically adsorb more bacteria than the bulk biochar and contribute greatly to high bacterial removal in bulk biochar (Mohanty and Boehm, 2014), erosion or progressive mobilization of the biochar particles may decrease the overall bacterial removal capacity of biofilters. Despite evidence of uninhibited transport of biochar particles through sand (Wang et al., 2013b,c), no study to date has examined the detachment and mobilization of biochar particles during intermittent stormwater infiltration.

Only a few studies have examined the effect of biochar weathering on contaminant removal, and all those studies focused on chemical contaminants. Trigo et al. (2014) weathered biochar in the field for one and two years, and observed that the surface area, porosity, and aromaticity of biochar increased with time, which in turn enhanced the attachment of some herbicides. Uchimiya et al. (2010) showed that biochar weathered in the presence of NOM could either enhance or decrease removal of heavy metals, depending on the properties of heavy metals. Lou et al. (2013) weathered biochar in the presence of humic acid and found that humic acid decreased adsorption of pentachlorophenol. Incubating biochar at 60 and 110 °C for two months, Hale et al. (2011) showed that weathering could decrease the sorption of pyrene, possible because of oxidation of surface functional groups. Unlike dissolved chemicals that could absorb into intraparticle pores in biochar, bacteria mostly attach on outer surfaces of biochar (Luo et al., 2013). Consequently, biochar weathering may have a different impact on bacterial removal than on chemical removal. No study to date has examined the effect of biochar weathering on removal of bacteria from stormwater.

We examined the effect of physical and chemical weathering mediated by dry-wet and freeze-thaw cycles on bacterial removal capacity of biochar-augmented model biofilters. We hypothesized that intermediate dry-wet or freeze-thaw cycles would mobilize biochar from biofilters and affect the bacterial removal capacity of biofiters. Columns packed with a mixture of sand and biochar were subjected to 9 wetting cycles, each followed by a 70-h no flow period when the biofilters were subjected to either drying at 4, 22, or 37 °C or freeze-thaw cycle (freezing at −15 °C followed by thawing at 22 °C). Using fixed duration no-flow periods permitted us to test the hypotheses and to compare the effect of different treatment conditions on change in performance of biofilters during weathering. The biochar mobilized during intermittent flow was quantified. Comparing bacterial removal capacities of biochar-augmented biofilters weathered with or without dry-wet or freeze-thaw cycles, we showed that these weathering conditions affect the mobilization of biochar particles and bacterial removal from stormwater.

2. Materials and methods

2.1. 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 adding 10 mg C L^{-1} of Suwannee River NOM (International Humic Substances Society, MN, USA). This recipe provides an average concentration of major ions in urban stormwater (Grebel et al., 2013). It should be noted that the concentration of NOM in urban stormwater is extremely variable (Grebel et al., 2013), and the concentration used in this study is within the typical range measured in field conditions (Mermillod-Blondin et al., 2015). The pH of stormwater was adjusted to 7.1 ± 0.2 by adding small quantity of 1 M HCl or 1 M NaOH. The stormwater was sterilized by filtering through 0.2 μm pore-size membrane filters (Fisher Scientific, USA) and stored at 4, 22, or 37 °C for 4–6 h prior to experiments so that stormwater temperatures match the experimental conditions.

2.2. Bacterial suspension

We used a kanamycin-resistant strain of the Gram negative bacterium *Escherichia coli* (NCM 4236) as a model organism (Inwood et al., 2009). The detailed method to prepare the *E. coli* suspension in stormwater is described elsewhere (Mohanty and Boehm, 2014). Briefly, *E. coli* were cultured to stationary phase, centrifuged to remove growth media, and suspended in the synthetic stormwater to achieve an initial concentration of 10^5 colony forming units per mL (CFU mL^{-1}).

2.3. Geomedia preparation

Coarse Ottawa sand (0.6–0.85 mm, Fisher Scientific) was washed in deionized water, dried at 104 °C and stored at 4 °C before use. A commercial biochar (Sonoma Compost Company, CA) was used in this study. The biochar was produced by a fast pyrolysis process: softwood with bark was pyrolyzed within 1–3 s at a temperature range between 815 and 1315 °C. The biochar was dried overnight at 104 °C and then crushed and sieved to a size smaller than 1 mm. Detailed properties of the biochar including surface area, elemental composition, polarity index, volatile material, and ash content were measured and reported in our previous study (Mohanty et al., 2014a). Micropore volume was determined from N_2-adsorption data using the t-method (Lippens and De Boer, 1965).

2.4. Biofilter design

The model biofilter was designed using a polyvinyle chloride (PVC) pipe (2.5 cm internal diameter) glued to end fittings. Including the space in the end fittings and pipe, the effective column length was 17 cm. A screen mesh (75-μm opening) was inserted at both ends to hold the packed geomedia within the column. A mixture of sand and biochar (95% and 5% by weight, respectively) was packed in columns following a dry-packing method (Mohanty et al., 2014a). The model biofilter is designed to examine the impact of physical and chemical weathering conditions on bacterial removal by biochar-augmented sand, which can replace traditional biofilter geomedia (mixture of sand and compost) to improve bacterial removal (Mohanty and Boehm, 2014).

2.5. Weathering experiments

Experiments were conducted in three phases: conditioning, weathering, and bacterial injection (Table 1). During the conditioning phase, 1 L of deionized water was injected at 24 cm h^{-1} from the bottom of columns to remove any suspended particles from pore water. Then 80 mL (~2 PV) of synthetic stormwater was injected to displace deionized water from the pores. Although upward flow against gravity may increase deposition of colloids on geomedia compared to downward flow (Chrysikopoulos and Syngouna, 2014), upward flow was necessary to displace air from pores within the column during intermittent stormwater infiltration and to minimize preferential flow (Mohanty et al., 2013). Preferential flow typically causes under-utilization of geomedia (Blecken et al., 2009); thus, presence of preferential flow would have added further uncertainty to the measurement of bacterial removal in biofilters under different weathering conditions. The pore volume (PV) was estimated by subtracting the dry column weight from the saturated column weight. The estimated PV (~40 mL) could be slightly lower than the actual PV because some air may be trapped within biochar pores.

To weather the geomedia within the biofilter, packed columns were subjected to 9 wetting cycles, each wetting cycle followed by a no-flow period when biofilters were subjected to either freeze-thaw or drying treatment at 4, 22 or 37 °C. Triplicate columns were used for each type of treatment. During a wetting event, 2 PV of sterile stormwater (filtered through 0.2 μm pore-size membrane filters) stored at 4, 22 or 37 °C were injected upward at 24 cm h^{-1}, and the effluents were collected at 0.5 PV fractions and analyzed for suspended biochar. Then the columns were overturned to maintain the water flow direction relative to the media and drained by gravity for 30 min, which resulted a drainage of less than 0.1 PV of pore water. After gravitational drainage, the columns were subjected to either freeze-thaw or drying treatment for 70 h. For the freeze-thaw treatment, triplicate columns were kept at −15 °C for 36 h to completely freeze the pore water and at 22 °C for next 34 h to thaw the pore water. For drying treatment, triplicate columns were incubated at 4, 22 or 37 °C for 70 h. Based on our preliminary study, these treatment durations are long enough to initiate drying or completely freeze and thaw pore water in the columns. The columns were overturned again before the next wetting cycle. For the columns subjected to dry-wet cycles, stormwater stored at 4, 22 or 37 °C (same as drying temperature) was used during the wetting cycles. For the columns subjected to freeze-thaw cycles, stormwater stored at 22 °C (same as the thawing temperature) was used during the wetting cycles. The weathering treatments lasted for a month.

To estimate bacterial removal capacity of the weathered biochar-augmented biofilters, 4 PV of the stormwater seeded with *E. coli* (~10^5 CFU mL^{-1}) were injected at 24 cm h^{-1} and effluents were collected at 0.5 PV fractions. The removal capacity of weathered biofilter was estimated based on the relative concentration of *E. coli* during the breakthrough plateau, which typically occurred after injection of 1.5 PV of contaminated stormwater (Mohanty and Boehm, 2014). To examine the effect of intermediate drying on bacterial removal, control experiments were conducted where the same volume of stormwater as used during weathering treatments (18 PV) stored at 4, 22 or 37 °C was continuously infiltrated through additional fresh or unweathered biofilters and bacterial removal capacity was estimated thereafter; the control experiments lasted approximately 8 h and were conducted in triplicate. The control experiment at 22 °C was also used as a control for freeze-thaw treatment.

To examine the effect of NOM loading on the bacterial removal capacity of the biochar, we measured bacterial removal by a biofilter pre-exposed to only 2 PV of stormwater (or 1/10 th of NOM exposure during the control experiment) and compared that with the result of the control experiment at 22 °C.

We estimated the bacterial removal capacities of biofilters without pre-exposure to stormwater-laden NOM as follows. After conditioning the biofilters by injecting 1 L of DI water, 4 PV of bacteria-laden stormwater was injected and effluents were analyzed at 0.5 PV fractions to estimate the initial bacterial removal capacity.

It should be noted that for the control and weathering experiments, we used biofilters without pre-exposure to bacteria and did not measure their initial bacterial removal capacity as doing so would have required the addition of bacteria to all columns, thereby initiating biological weathering. This would have made it difficult to distinguish and compare the effect of physico-chemical weathering from that of biological weathering.

2.6. Sample analysis

During the weathering phase of the experiments, we measured

Table 1
Experimental phases and the underlying purpose for each phase.

Phase	Treatment condition	Purpose
Conditioning	Infiltration of 1 L of deionized water at 24 cm h^{-1}	To equilibrate moisture content and flow rate, and to remove suspended particles from pore water.
	Infiltration of 2 PV (80 mL) of synthetic stormwater at 24 cm h^{-1}	To displace deionized water from pores.
Weathering[a]	Infiltration (at 24 cm h^{-1}) of 2 PV of synthetic stormwater following a 70-h drying or freeze-thaw cycle.	To physically and chemically weather biochar.
	Freeze-thaw cycle: freezing at −15 °C for 36 h followed by thawing at 22 °C for 34 h.	
	Drying cycle: drying at 4, 22 or 37 °C for 70 h.	
Bacterial removal	Infiltration (at 24 cm h^{-1}) of 4 PV of synthetic stormwater seeded with *E. coli*	To estimate bacterial removal capacity of biofilters.

[a] Weathering cycle was repeated 9 times with total cumulative infiltration of 18 PV of stormwater in a month. For the control experiment, 18 PV of stormwater incubated at 4, 22 or 37 °C was continuously injected at 24 cm h^{-1} without intermediate drying or freeze-thaw treatment.

the turbidity of the column effluent in 0.5 PV fractions using absorbance at 890 nm (Hach spectrophotometer, DR 2800). Turbidity was used as a surrogate measurement for suspended biochar particle concentration eluted from sand columns (Wang et al., 2013b). At 890 nm, the color of the stormwater (from NOM) has minimum interference with the turbidity measurement (data not shown). The turbidity was converted to biochar particle concentration using a calibration curve. To prepare calibration standards, a known quantity of biochar particles (<75 μm in diameter) collected by dry-sieving the bulk biochar, was suspended in synthetic stormwater and the suspension was serially diluted with the synthetic stormwater.

We assumed that biochar alone contributed to the effluent turbidity, and sand colloids had no or limited contribution. This is because sand was thoroughly cleaned prior to the experiment and the dissolution of amorphous silica colloids or the process that typically generates colloids from sand grains requires a more severe treatment (e.g., shaking for 2 h in 0.002 N NaOH, as described by Lenhart and Saiers (2002)) than the conditions in our experiment. A previous study confirmed that colloids mobilization from sand is negligible (Lenhart and Saiers, 2003). In contrast to sand, biochar is relatively soft and porous, and composed of aggregated particles which are more susceptible to physical erosion (Lehmann and Joseph, 2009).

In order to test for possible bacterial contamination during the month-long weathering experiment, we measured bacterial concentration in effluents collected during the 9 wetting cycles using Luria–Bertani (LB) agar (Difco, Miller, Fisher Scientific). Effluents collected during a wetting cycle were mixed and enumerated using spread plate technique.

During the injection of kanamycin-resistant *E. coli*, we used LB agar mixed with kanamycin at 25 μg mL^{-1} to measure effluent bacterial concentration (Mohanty and Boehm, 2014). Bacterial concentration in 0.5 PV effluent fractions was quantified by combination of a spread plate technique and membrane filtration. 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. To measure *E. coli* concentration below the detection limit of the spread plate technique, 1–10 mL of effluent was filtered through a membrane (0.45 μm pore size, sterile cellulose filter, Millipore) and enumerated on the agar plate.

2.7. Data analysis

Removal capacities of all columns were calculated based on the relative concentration of *E. coli* during the breakthrough plateau. To identify statistically significant differences between results from different experiments, 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 $p < 0.05$.

3. Results

3.1. Mobilization of biochar particles

Biochar particles were mobilized during wetting cycles following drying or freeze-thaw treatment (Fig. 1). During a wetting cycle, the biochar particle concentration peaked initially at the beginning of the cycle, but then dropped rapidly as infiltration continued. The peak height decreased during successive wetting cycles, and this decrease was more pronounced at 22 and 37 °C than at 4 °C. After 5 dry-wet cycles, the peak concentrations for the biofilters at 4, 22 and 37 °C dropped to respectively 46%, 80% and 97% of the initial peak concentration observed during the first infiltration event. After the same number of treatment cycles, the peak concentration for the biofilters subjected to freeze-thaw cycles dropped to 60% of its initial peak. During the control experiment where biofilters were exposed to the same volume of stormwater as the weathering experiments with no drying or freezing, the turbidity of the effluent was similar to the turbidity of the influent. The effluent biochar concentrations in the control experiments were at or below the method detection limit (Fig. 1).

Over the course of the weathering treatments, the total mass of biochar mobilized from the biofilters subjected to freeze-thaw cycles and dry-wet cycles at 4, 22, and 37 °C (average and one standard deviation from triplicate biofilters) were 11.1 ± 4.1, 32 ± 7.7, 7.8 ± 0.2, and 8.0 ± 1.1 mg, respectively (Fig. 2). Between all weathering treatment conditions, dry-wet cycles at 4 °C mobilized significantly ($p < 0.001$) more biochar particles. The total mass of biochar mobilized during the other three treatment conditions was not statistically different ($p > 0.85$). During weathering experiments, we did not observe CFU counts in the effluent, which suggests that the filters had limited or no biofilm formation.

3.2. Effect of weathering conditions on bacterial removal capacity of biochar

Bacterial removal depended on the volume of stormwater that passed through the biofilters prior to the injection of bacteria-laden stormwater. The volume of stormwater controlled the extent of NOM exposure on biochar in the biofilters. The initial bacterial removal capacity of biofilters that were not exposed to NOM in stormwater was above 5 $\log_{10}$ units (the method detection limit). However, the removal capacity decreased following exposure to NOM in stormwater during control and weathering experiments. Bacterial removal capacity of the biofilter that was previously exposed to 2 PV of synthetic stormwater at 22 °C via continuous infiltration was 4.2 $\log_{10}$ units. When the biofilter was exposed to 20 PV of synthetic stormwater also via continuous infiltration at the same temperature (control experiments), bacterial removal decreased to 1.7 $\log_{10}$ units (Fig. 3).

Among the control or weathering experiments, bacterial removal did not change ($p > 0.05$) with an increase in stormwater temperature (Fig. 3). Compared to the control experiment at 4 °C, biofilters weathered by dry-wet cycles at the same temperature removed more bacteria ($p < 0.05$). Biofilters weathered by dry-wet cycles at 22 and 37 °C, however, did not removed significantly ($p > 0.05$) more bacteria than the control biofilters at the same temperature. Biofilters weathered by freeze-thaw cycles or dry-wet cycles exhibited similar bacterial removal capacity (or difference in removal was statistically insignificant).

4. Discussion

4.1. Mobilization of biochar particles during intermittent flow

The mobilization pattern of biochar particles from the model biofilters during dry-wet and freeze-thaw treatments was similar to colloid mobilization observed in sand or soil in other studies (Cheng and Saiers, 2009; Mohanty et al., 2015a; Zhuang et al., 2007). Colloid mobilization during dry-wet cycles has been attributed to colloid release by moving air–water interfaces, thin-water film expansion, reconnection of stagnant-water zones with bulk-water flow, and increase in shear forces (DeNovio et al., 2004). All these processes could contribute to mobilization of biochar particles in our experiments. Additionally, owing to its lower density than other particles such as clays and sand, biochar may preferentially erode from the packing material (Rumpel et al., 2006)

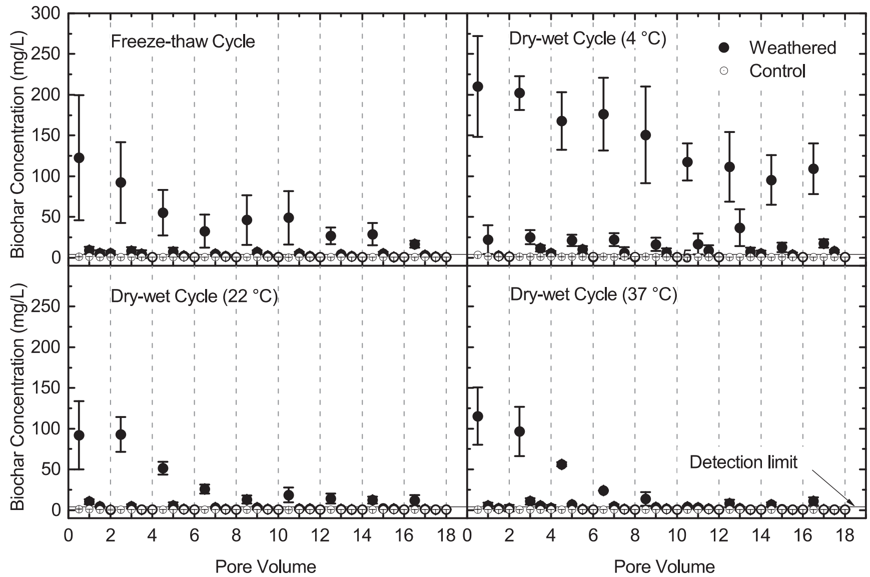

Fig. 1. Mobilization of biochar particles during steady infiltration of stormwater (control) and during intermittent infiltration of stormwater following drying or freeze-thaw treatments (weathered). The control for the freeze-thaw treatment is same as the control at 22 °C. Vertical dash lines indicate the start of periodic drying or freeze-thaw treatment. Error bars indicate one standard deviation of values obtained from triplicate experiments. Biochar particle concentration was estimated from turbidity measurements using a calibration curve between particle concentration and turbidity.

and then move through the porous media (Wang et al., 2013a). Because the duration (a month) of the weathering experiments was significantly longer than the duration (8 h) of the control experiments, it could be argued that time-dependent mobilization or diffusion (Schelde et al., 2002) of biochar particles from grain surface to pore water could have contributed to higher concentration of biochar in the effluents from weathered biofilters compared to the concentration in the effluent from the control biofilters. If diffusion, which increases with temperature, is a major contributing factor to biochar mobilization, then biochar mobilization at 4 °C should have been lower than the mobilization at 22 or 37 °C. However, biochar mobilization was highest at 4 °C, suggesting diffusion was not the cause of higher biochar mobilization during weathering.

The peak concentration of biochar particles in the effluent decreased during successive dry-wet cycles, which suggests that the pool of mobile biochar particles available for mobilization depleted over time. The decrease in peak concentration depended on temperature maintained during the weathering experiments. By the last dry-wet cycle at 22 and 37 °C, the peak concentration was low, similar to the influent turbidity. During dry-wet cycles at 4 °C, the peaks decreased slowly with successive dry-wet cycles, but remained consistently higher than influent turbidity even during the last cycle. The total mass mobilized during dry-wet cycles at 4 °C was also greater than the total mass mobilized during dry-wet cycles at 22 and 37 °C. Although the wetting event temperature was the same (22 °C) for the freeze-thaw cycles and dry-wet cycles at 22 °C, the particle mobilization peaks were higher after the freeze-thaw cycles than the dry-wet cycles. This could be a result of

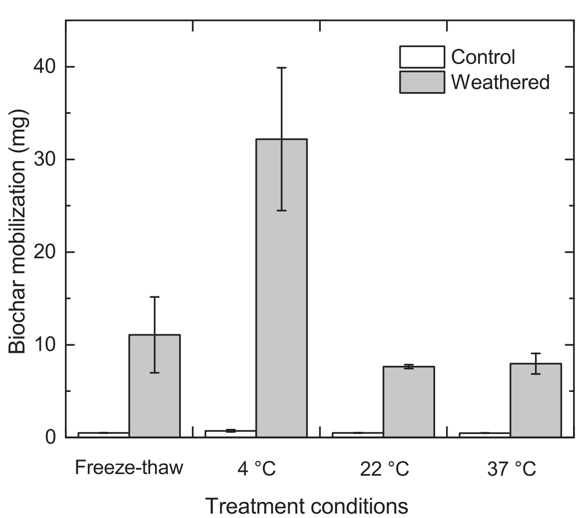

Fig. 2. Total mass of biochar mobilized from control and weathered biofilters. The control for the freeze-thaw treatment is same as the control at 22 °C. Error bars indicate one standard deviation of values from triplicate column experiments.

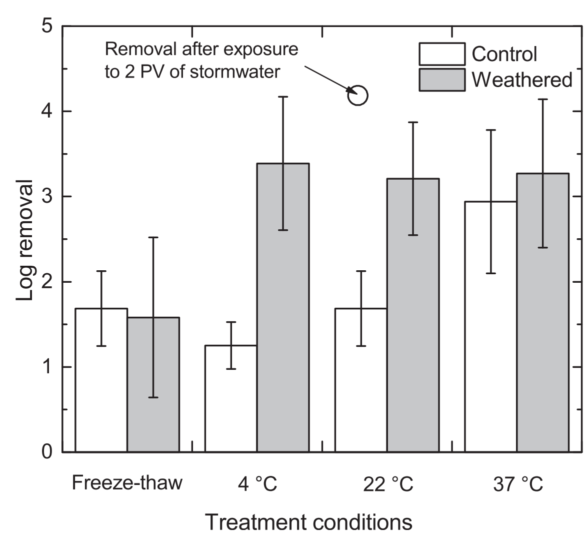

Fig. 3. Bacterial removal by control and weathered biofilters. The control for the freeze-thaw treatment is same as the control at 22 °C. The open circle represents the bacterial removal by the biofilter pre-exposed to 2 PV of stormwater at 22 °C. Error bars indicate one standard deviation of values obtained from triplicate column experiments. The initial bacterial removal capacity of all biofilters (prior to the exposure to stormwater or NOM) is above 5 $\log_{10}$ units (the method detection limit).

biochar fragmentation by expanding of ice crystal during freezing (Carcaillet, 2001). A lower mobilization of biochar at the high temperature may be attributed to several processes. Based on colloid-filtration theory, attachment efficiency of colloids and nanoparticles increases with an increase in temperature (Zhang et al., 2012). Consequently, at higher temperatures, mobilized biochar particles are more likely to deposit on stationary grain surfaces during their transport through the column. Furthermore, at higher temperatures (22° and 37 °C), evaporation could disconnect saturated pores and trap colloids at stagnant air–water interfaces (Abdel-Fattah and El-Genk, 1998). However, little is known about mobilization mechanism of biochar particles in porous media (Kookana et al., 2011) and further study is needed to explain temperature-dependent migration of biochar particles observed here.

Mobilization of biochar particles from biochar-amended biofilters could have unintended consequences. For instance, mobilized biochar particles could reintroduce the contaminants sequestered on biochar within biofilters and increase colloid-enhanced transport of the contaminants within biofilters (Massoudieh and Ginn, 2008). Furthermore, mobilized biochar particles may clog the biofilter in the long term, similar to how fine sediments have been shown to clog biofilters at the lab scale (Kandra et al., 2015).

4.2. Effect of stormwater temperature on bacterial removal capacity of biochar

In nature, the temperature of stormwater varies, which could affect the performance of the biofilter (Jones and Hunt, 2009; Roseen et al., 2009). Temperature of stormwater during winter could be close to 0 °C, whereas the temperature of stormwater during summer could exceed 40 °C as the stormwater flows over heated asphalt (Van Buren et al., 2000). To examine how the bacterial removal capacity of biochar changed with temperatures that may be typical in winter, spring, and summer, we injected bacteria-laden stormwater at 4, 22, and 37 °C, respectively, through biochar-augmented biofilters. Results of our control and weathering experiments show that bacterial attachment did not change ($p > 0.5$) with an increase in stormwater temperature. This result conflicts with the findings of previous studies conducted with different geomedia, which showed that bacterial attachment on polystyrene (Fletcher, 1977) and sand (Kim and Walker, 2009) increased with an increase in temperature. Based on colloid filtration theory, the single collector efficiency increases with temperature (Harvey and Garabedian, 1991) and an increase in temperature from 4 to 22 °C would increase single-collector efficiency by 140%. However, for the same temperature increase in our study, bacterial removal did not increase significantly, indicating colloid-filtration theory alone can not explain the observed bacterial attachment in biochar-amended biofilters. Bacteria can attach on geomedia by processes including physical straining (Bradford et al., 2006) and hydrophobic attachment (van Loosdrecht et al., 1987), which are less sensitive to temperature, and these processes are shown to be relevant for bacterial attachment on biochar (Abit et al., 2012; Mohanty and Boehm, 2014; Mohanty et al., 2014a). Thus, we surmise that straining and hydrophobic attachment could be the major contributing factor for temperature-independent bacterial attachment in our study.

4.3. Effect of weathering on bacterial removal capacity of biochar

Biochar within a biofilter weathers due to its interaction with stormwater constituents (e.g., NOM) and other biofilter media including sand, soil, and compost. The weathering processes could alter biochar properties and consequently its bacterial removal capacity. In our study, bacterial removal capacity of the biofilters at 22 °C decreased from 4.2 to 1.7 $\log_{10}$ units when the volume of stormwater exposure prior to bacterial injection increased from 2 PV to 20 PV. The increase in stormwater volume resulted in 10 times increase in NOM exposure to biochar. Furthermore, bacterial removal capacity of control and weathered biofilters decreased relative to the initial bacterial removal capacity (without pre-exposure to stormwater or NOM) at all temperatures. We attribute this decrease in bacterial removal to adsorption of NOM, which can exhaust bacterial attachment sites on biochar and sand (Mohanty et al., 2014a). We assumed that contribution of sand to bacterial removal is negligible compared to biochar (5% by weight) within the biofilters. In our previous study (Mohanty et al., 2014a), we estimated that bacterial removal by sand columns at 22 °C with and without NOM were 0.21 and 0.29 $\log_{10}$ units, respectively, which are orders of magnitude lower than the removal by the mixture of sand and biochar in this study. Thus, a decrease in bacterial removal of over 2 $\log_{10}$ units in the presence of NOM in the present study is attributed to changes in biochar properties, not sand. Our results corroborate the findings of previous studies (Kasozi et al., 2010; Uchimiya et al., 2010), which demonstrate that NOM adsorption on biochar can decrease the removal of chemical contaminants.

In nature, stormwater infiltration is intermittent, and the time between consecutive rainfalls could be several days, when pore water may dry or freeze depending on the season. At a particular temperature, the average bacterial removal of biofilters weathered by dry-wet cycles was greater than the average bacterial removal of the control biofiters (steady flow), and the difference in results between control and weathering experiments was significant at low temperature (e.g., 4 °C). Thus, intermediate drying could be beneficial for biofilter performance. We attribute this result to regeneration of attachment sites that were exhausted due to adsorption of NOM, as explained below. During dry-wet cycles, the adsorbed NOM could diffuse into internal micro- and nano-pores (Kasozi et al., 2010; Lou et al., 2013). In our previous study (Mohanty et al., 2014a), we measured that the micropore volume of the biochar is 0.16 $cm^3\ g^{-1}$, which is 85% of the total pore volume of the biochar. The highly porous networks within biochar particles provide a sink for NOM that is initially adsorbed on the outer surface of biochar particles (Kasozi et al., 2010). Compared to the control experiments, which lasted only 8 h, the weathering experiments lasted for a month, thereby providing an extended period of time for the adsorbed NOM to diffuse into intraparticle pores (Ulrich et al., 2015). This process could have lowered the concentration of NOM on the outer surface of the particles—the likely location on biochar for bacterial attachment. Thus, the bacterial removal capacity of fresh biochar estimated in laboratory condition (Mohanty et al., 2014a) could be smaller than its actual potential in field conditions, particularly when dry-wet cycles are prevalent.

In contrast to dry-wet cycles, freeze-thaw cycles did not improve the bacterial removal capacity of biofilters relative to the biofilters that were exposed to same volume of stormwater without freeze-thaw cycle. This result suggests that freeze-thaw cycle did not promote the replenishment of attachment sites occupied by NOM. When water freezes, mineral salts (from stormwater and ash component in biochar) are excluded from ice crystals and precipitate on grain surfaces (Dietzel, 2005). These precipitates could block the intra-particle pores in biochar, thereby lowering the removal of NOM from biochar surfaces by diffusion.

Overall, our results show that weathering by dry-wet cycles could improve biochar-augmented biofilter performance, but weathering by freeze-thaw cycles may not provide the same benefit. Our use of fixed drying or freeze-thaw duration (70 h) allowed us to compare the effect of different weathering conditions on biochar mobilization and bacterial removal. Previous studies on weathering also used a fixed treatment duration: 24 h (Hale et al., 2011) or 66 h (Mohanty et al., 2014b). In nature, however, the weathering conditions such as drying and wetting duration, temperature, and humidity could vary, and the variation of these parameters could affect the treatment efficiency of stormwater biofilters (McCarthy et al., 2012). In particular, drying duration has been shown to affect colloid mobilization processes in soil (Majdalani et al., 2008; Mohanty et al., 2015b)—the same processes that mobilize biochar from biofilters. Therefore, further study should evaluate the bacterial removal capacity of biochar that is weathered in field in situ for extended periods of time. Furthermore, the bacterial removal estimated in this study could differ for other isolates of *E. coli* (Bolster et al., 2010) and bacterial species (Mohanty et al., 2013).

5. Conclusions

Using model biofilters and subjecting them to different weathering conditions over a month, we examined the effect of dry-wet and freeze-thaw cycles on the mobilization biochar particles from the biofilters and on bacterial removal capacity of the biofilters. The major conclusions of this study are:

- Dry-wet and freeze-thaw cycles increase mobilization of biochar particles from biofilters, and the mobilization appears to increase with decrease in stormwater temperature.
- Stormwater temperature did not affect bacterial removal in biochar-augmented biofilters.
- NOM in stormwater decreases bacterial removal capacity of biochar-augmented biofilters, partly due to exhaustion of attachment sites on biochar by adsorption of NOM.
- Freeze-thaw cycles did not change bacterial removal capacity of biofilters, but dry-wet cycles did.
- Biofilters exposed to NOM without intermediate drying cycles removed fewer bacteria than the biofilters exposed to the same amount of NOM with intermediate drying cycles.
- We attribute this increase in bacterial removal by intermediate drying cycles to replenishment of the NOM-exhausted sites, possibly due to diffusion of NOM occupying surface attachment sites to macro- and micro-pores inside biochar particles.

Overall, we showed that dry-wet cycles could be beneficial for bacterial removal, but freeze-thaw cycles may not provide a similar benefit. The results indicate that biochar, despite exposure to NOM in stormwater, has potential to consistently remove bacteria in biofilters, although field study is needed to examine how variable climatic conditions such as drying duration, temperature, and humidity affect bacterial removal capacity of biochar-augmented biofilters.

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