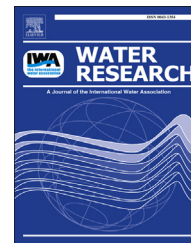


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Efficacy of biochar to remove *Escherichia coli* from stormwater under steady and intermittent flow

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ARTICLE INFO

Article history:

Received 7 January 2014

Received in revised form

29 April 2014

Accepted 19 May 2014

Available online 28 May 2014

Keywords:

Water treatment

Low impact development

Geomedia

Fecal indicator bacteria

Organic carbon

Biochar property

ABSTRACT

Biofilters, designed to facilitate the infiltration of stormwater into soil, are generally ineffective in removing bacteria from stormwater, thereby causing pollution of groundwater and receiving surface waters. The bacterial removal capacity of biofilters has been shown to be lower in the presence of natural organic matter (NOM) and during intermittent infiltration of stormwater. To improve the removal of fecal indicator bacteria (*Escherichia coli*) under these conditions, we amended sand with 5% (by weight) biochar, a carbonaceous geomedia produced by pyrolysis of biomass, and investigated the removal and remobilization of *E. coli*. Three types of biochar were used to evaluate the role of biochar properties on the removal. Compared to sand, biochar not only retained up to 3 orders of magnitude more *E. coli*, but also prevented their mobilization during successive intermittent flows. In the presence of NOM, the removal capacity of biochar was lower, but remained higher than sand alone. The improved retention with the biochar amendment is attributed to an increase in the attachment of *E. coli* at the primary minimum and to an increase in the water-holding capacity of biochar-amended sand, which renders driving forces such as moving air–water interfaces less effective in detaching bacteria from grain surfaces. Biochars with lower volatile matter and polarity appear to be more effective in removing bacteria from stormwater. Overall, our results suggest that a biochar amendment to biofilter media has the potential to effectively remove bacteria from stormwater.

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1. Introduction

As urban areas expand to accommodate growing populations, impervious surfaces replace the natural landscape and impede groundwater infiltration. The result is an overall

increase in the net volume and flow rate of stormwater over land (Davis and McCuen, 2005). Consequently, stormwater floods and erodes the urban landscape, and conveys contaminants from the land surface to streams, lakes, and other water bodies (US EPA, 2002).

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<http://dx.doi.org/10.1016/j.watres.2014.05.026>

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To mitigate the adverse impacts of stormwater, city planners are increasingly incorporating green infrastructure or low impact development (LID) into their development projects. Common LID includes the bioretention or biofilter system (US EPA, 2000): a shallow planted area where a native block of soil is replaced with a mixture of sand and compost to promote rapid infiltration of stormwater into the ground. After passing through the infiltration system, water enters the local surficial aquifer. In a biofilter, the filtered water is conveyed back to a surface stormwater conveyance via an underdrain. Henceforth, these two systems will be collectively referred to as biofilters. A review of biofilter performance across the US shows low and inconsistent removal of fecal indicator bacteria (FIB) (Leisenring et al., 2012), key stormwater contaminants responsible for impairment of many of the nation's impaired surface waters (US EPA, 2002). A recent lab-scale study showed that even when fecal bacteria are sequestered by media within a model biofilter, they can be detached from the media and mobilized during intermittent infiltration of stormwater (Mohanty et al., 2013). As a result, biofilters may possibly act as sources of fecal bacteria to infiltrating stormwater. Improvements in biofilter design are needed to address these issues.

To improve the removal of FIB, biofilters have been augmented with various engineered geomedia (Pitt and Clark, 2010), but the performance of these geomedia, particularly those with positive surface charge, is substantially lower in the presence of natural organic matter (NOM) (Torkelson et al., 2012; Mohanty et al., 2013). Moreover, most of the engineered geomedia are selective in removing certain group of contaminants, thereby making them ineffective in treating stormwater that may contain many types of contaminants (Pitt and Clark, 2010). On the other hand, biochar, a carbonaceous geomedia produced by pyrolysis of biomass (Manya, 2012), has potential to remove FIB and NOM in addition to the myriad of contaminants that may be present in stormwater. Previous laboratory column experiments have shown that biochar can effectively remove heavy metals (Park et al., 2011), organic contaminants (Chen et al., 2008), NOM (Kasozi et al., 2010), and nutrients including phosphate (Yao et al., 2011) seeded into deionized water. Two studies have showed that biochar-amended sandy soil increased the removal of a model fecal indicator bacterium, *Escherichia coli*, suspended in deionized water relative to unamended soil (Abit et al., 2012; Bolster and Abit, 2012). While these studies showed that biochar could remove *E. coli* from seeded deionized water, it is unclear whether the biochar can effectively remove bacteria from a more complex matrix such as stormwater that contains nutrients and natural organic matter (NOM). Moreover, the ability of biochar to sequester attached bacteria during intermittent flow has not been previously investigated.

This study aims to evaluate the efficacy of biochar to remove FIB under complex conditions that typically occur during natural infiltration of stormwater. We hypothesized that augmenting sand media with biochar would increase bacterial removal from a synthetic stormwater, and decrease the mobilization of attached bacteria during intermittent flows relative to sand media alone. To test these hypotheses, we used three types of biochar and a model fecal bacterium: *E. coli*.

2. Experimental methods

2.1. Preparation of sands and biochar

To remove surface impurities, coarse Ottawa sand (0.6–0.85 mm, Fisher Scientific) was treated with 12 M hydrochloric acid and then washed in deionized water until the pH of water became neutral (Lenhart and Siers, 2002). Three types of biochar made from wood-chips were used: a commercially available biochar obtained from Sonoma Compost Company, CA (referred as Sonoma biochar, henceforth), and two steam-activated biochars produced in the laboratory via pyrolysis of wood chips at 350 and 700 °C, which are referred respectively as low temperature (LT) biochar, and high temperature (HT) biochar henceforth. The sand and biochars were dried at 110 °C overnight, autoclaved (121 °C, 100 kPa, 15 min), and stored in sterile containers prior to use in the column experiments.

2.2. Characterization of biochars

The physical and chemical properties of biochar including surface area, elemental composition (i.e., C, H, O, N, and S), volatile matter, total carbon content, and ash content were analyzed using methods described elsewhere (Novak et al., 2009). Briefly, surface area was estimated by adsorption of nitrogen gas using an automated surface area analyzer (Micromeritics Gemini 2360, GA, USA). The percentage of volatile material, ash content, elemental composition (C, H, O, N, and S) of oven-dried biochar samples were estimated using ASTM D 3172 and 3176 standard methods (ASTM, 2006). We calculated H/C, O/C, and (O + N)/C ratios, which have been used to quantify the content of polar functional groups or polarity index (Chen et al., 2008).

2.3. Synthetic stormwater

Synthetic stormwater was prepared by dissolving 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 in deionized water and then sterilizing the solution using an autoclave (121 °C, 100 kPa, 45 min). This recipe provides an average concentration of major ions in urban stormwater (Grebel et al., 2013). Suwannee River NOM (International Humic Substances Society, MN, USA) was added at 20 mg C L⁻¹ for use in some experiments, as described below. The ionic strength of the stormwater was 4.7 mM. The pH was adjusted to 7.0 ± 0.1 using 1 M HCl or 1 M NaOH. Dissolved organic carbon (DOC) was measured in stormwater with and without NOM using a TOC analyzer (TOC-5000A, Shimadzu Co., Japan).

2.4. Bacteria solution preparation

E. coli K12 (ATCC 10798), a motile, Gram-negative bacterium was prepared following methods outlined elsewhere (Mohanty et al., 2013). Briefly, *E. coli* were cultured to stationary phase, centrifuged to remove growth media, and suspended in the synthetic stormwater to achieve a

concentration of $1.2\text{--}1.7 \times 10^6$ colony forming units (CFU)/mL. The *E. coli* suspension was kept at 4°C for 16–18 h for *E. coli* to adapt to stormwater prior to its use in column experiments.

2.5. Biofilter experiment

Sand and a mixture of sand and each type of biochar (5% w/w) were dry-packed in glass chromatography columns (Kontes, 15 cm length, 2.5 cm diameter). Dry-packing was chosen over wet-packing as biochar floats during wet-packing, preventing uniform distribution. After packing, biochar occupied ~22% of the space inside the column, which was calculated by subtracting the volume (ratio of sand weight to bulk density of sand) occupied by the sands in the biochar and sand mixture from the total column volume. Hereafter, these columns are referred to as 'sand' and 'biochar' columns even though the biochar column also contains sand (95% by weight or 78% by media volume). To condition the geomedia, 1 L of deionized water (~33 pore volume) followed by 150 mL (~5 PV) of synthetic sterile stormwater (either with or without NOM, depending on experiment) were flushed upward through packed geomedia. Upward flows displace air from most pores between geomedia grains within column. The relative saturation of each column was estimated by measuring the weight of the column before and after packing with dry and saturated geomedia at different stages of experiments. The pore volume was estimated by subtracting the weight of dry-packed column from completely saturated column.

The column experiments were conducted in two phases: (1) attachment phase and (2) mobilization phase. During the attachment phase, 90 mL (3 PV) of the stormwater-bacterial suspension were injected at 0.1 cm min^{-1} ($\sim 0.5\text{ mL min}^{-1}$) through each column from the bottom (upward flow). Subsequently, 90 mL (3 PV) of sterile stormwater was injected to remove bacteria from pore water. Any bacteria remaining in the column were assumed to be attached at interfaces within the column. The next phase, the mobilization phase, examined if attached bacteria can be detached during intermittent flow. During this phase, the pump was stopped for 0.5 h, and the columns were overturned and pore water was drained by gravity. The column was overturned to maintain the water flow direction relative to the media during draining as would occur in the field. Following the pause, the drained column was overturned again, to maintain the flow direction, and 60–80 mL of sterile synthetic stormwater was pumped upward through the column. These draining and wetting steps were repeated twice. The experiments took approximately 12 h. We used upward flow in order to minimize preferential flow, which could affect the net removal capacity of geomedia during intermittent flow (Mohanty et al., 2013).

Infiltration experiments were repeated with sand and LT biochar, increasing the pause interval to 21 h to investigate if longer intervals between infiltrations would have any impact on bacteria remobilization. The experiments continued over 3 days after the injection of *E. coli*. A total of forty column experiments were conducted to examine the effect of biochar types, pause duration, and NOM on the attachment and mobilization of *E. coli*. Experiments involving a 0.5-h pause used 24 columns – eight combinations of geomedia (four types) and stormwater (with and without NOM) each run in

triplicate. Experiments involving a 21 h-pause used a total of 16 columns where four replicate columns tested two types of geomedia (sand and LT biochar) and stormwater with and without NOM.

2.6. Sample collection and measurements

Column effluent was collected in 10-mL fractions using an automated fraction collector (Model CF1, Spectrum Chromatography). The bacterial concentration in the effluent was quantified by spread plating techniques and reported as colony forming unit (CFU) per mL of effluent. Each sample was enumerated in duplicate at three decimal dilutions using tryptic soy agar (TSA, Difco, Fisher Scientific). Concentrations calculated using plates with between 30 and 300 CFU were averaged to obtain a concentration. This technique of measuring *E. coli* directly rather than using a surrogate measurement, like turbidity, was chosen as culture-based measures of *E. coli* are used to assess water impairment in practice.

The persistence of *E. coli* in the stormwater feed solution (with and without NOM) was tested by incubating the feed solutions inoculated with *E. coli* in triplicate (15 mL centrifuge tube) at room temperature ($\sim 23^\circ\text{C}$) for 4 days. To examine the persistence of *E. coli* in the presence of biochar and sand, we repeated the incubation tests adding biochar and sand to the feed solution also in triplicate (Supplementary Material). The concentration of *E. coli* was monitored daily using the same technique as for the effluent samples – spread plating on TSA. In addition, the experiments without geomedia were repeated to estimate cell counts using a hemocytometer and microscope (method details in Supplementary Material).

2.7. Data analysis

The log-removal of *E. coli* from stormwater during the attachment phase was calculated and is referred to as removal capacity. The number of attached bacteria in the column was calculated using a mass balance, assuming bacteria behave conservatively in the column.

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

3. Results

3.1. Characterization of biochars

The physical and chemical properties of the three biochars varied (Table 1). Sonoma biochar had approximately five times more surface area than the other two biochars. Dry biochar was composed of fixed carbon (72–81%), ash (12.2–15.4%), and volatile matter (6–16%). The ash contents of all biochars were similar, but the fixed carbon and volatile matter varied among biochars. Sonoma biochar contained 1.8 and 2.7 times more volatile matter than LT and HT biochar, respectively. The fixed

Table 1 – Physical and chemical properties^a of biochars.

Biochar ID	Surface area ^b	Volatile matter	Elemental composition (%, oven-dry wt. basis)					Atomic ratios			
	m ² g ⁻¹	(%)	Ash	C	H	O	N	S	H/C	O/C	(O + N)/C
Sonoma	326.2 ± 5.9	16.03	12.22	78.88	0.69	7.77	0.4	0.043	0.009	0.098	0.104
LT	65.9 ± 1.2	8.73	15.36	80.67	0.71	2.23	1.02	0.009	0.009	0.028	0.040
HT	64.9 ± 6.5	5.95	12.74	84.38	0.59	1.49	0.78	0.019	0.007	0.018	0.027

^a Chemical properties were measured by a single estimate from a biochar sample.

^b Surface area was measured in triplicate.

carbon fraction of HT biochar (81%) was higher than Sonoma biochar (72%) and LT biochars (76%). The (O + N)/C ratio or polarity index was greater in Sonoma biochar compared to other two biochars.

3.2. Attachment of *E. coli* on sand and biochar

During application of bacteria-laden stormwater without NOM, *E. coli* concentrations in biochar column effluent were ~2 orders of magnitude smaller than concentrations in the effluent of the sand columns ($p < 0.05$, Fig. 1 or Figure S1). The LT biochar column removed significantly more *E. coli* than Sonoma biochar (mean difference = 0.7 log, $p < 0.05$), but there was no other significant difference between removal capacities between other biochars. The presence of NOM in stormwater significantly lowered the removal capacity of the biochars relative to experiments without NOM; the removal capacities reduced by 2.4, 2.5, and 1.2 logs for LT, HT, and Sonoma biochars, respectively ($p < 0.05$). NOM had no impact ($p = 0.22$) on the removal capacity of sand. Removal capacities of the LT, HT, and Sonoma biochars in the presence of NOM were significantly higher than those in sand by 1.0, 0.6, and 0.9 log units ($p < 0.05$) (Table 2).

3.3. Detachment of *E. coli* during intermittent flows

During each pause, 48–53% of pore water was drained by gravity from the sand columns, whereas only 8–18% drained from biochar-amended sand columns. After rewetting, nearly 95% of pores were refilled with infiltrating stormwater in all columns.

During intermittent flow separated by 0.5-h pauses, attached bacteria were mobilized from the sand and biochar columns (Table 2). The concentration of *E. coli* in the column effluent during the intermittent flow events varied from below detection limit to as high as 2×10^6 CFU/mL. The concentration of *E. coli* was typically high at the start of infiltration and decreased as infiltration continued. The fraction of attached bacteria mobilized from the biochar columns was significantly lower than that from sand columns ($p < 0.05$). Combining data from experiments with and without NOM, two intermittent flow events mobilized on average 19% of attached *E. coli* from sand columns, but mobilized only 1%, 1%, and 3% of attached *E. coli* from LT biochar, HT biochar, and Sonoma biochar, respectively. Without NOM, intermittent flows mobilized 14.7% of *E. coli* attached to sand columns, which further increased to 24.3% in the presence of NOM, but the difference was not statistically significant ($p = 0.14$). Intermittent flows

released 0.001–0.2% of *E. coli* attached to biochar without NOM. In the presence of NOM, mobilization from biochar columns was significantly higher than in the case without NOM (2–5% higher, $p < 0.05$). The fraction mobilized during intermittent flow did not significantly vary with biochar types ($p > 0.87$). In all experiments with 0.5-h pause, the total fraction mobilized during the second infiltration mobilized 58% (on average) fewer *E. coli* than the first infiltration (Fig. 1).

3.4. Effect of interval between intermittent flows on the detachment of *E. coli*

A 21-h pause did not change the moisture content of the columns compared to the 0.5-h pause. However, the longer pause affected the mobilization of *E. coli* from sand columns (Table 2). Without NOM, an increase in pause duration from 0.5 h to 21 h caused a net decrease in the fraction of *E. coli* mobilized from sand from 15% to 8% ($p < 0.05$, Fig. 2). With NOM, the longer pause duration increased the total fraction of *E. coli* mobilized from sand during intermittent flows from 24% to 83% ($p < 0.05$). The longer pause duration decreased the mobilization of *E. coli* from biochar, but not significantly ($p > 0.05$).

The mobilization of *E. coli* in successive intermittent flows depends on the media type (biochar or sand) and NOM concentration. During the 21 h pause experiments in sand columns, the second intermittent flow mobilized 27% fewer *E. coli* than first intermittent flow without NOM, but mobilized 69% more *E. coli* in presence of 20 mg C L⁻¹ NOM. In biochar columns, however, the second intermittent flow mobilized 65% fewer *E. coli* than the first intermittent flow irrespective of NOM concentration in the stormwater.

3.5. Persistence of bacteria in stormwater matrix

Monitoring of influent stormwater seeded with *E. coli* suggested their growth over 4 days (Fig. 3 and S2). The growth rate (average ± standard deviation) of *E. coli* in stormwater with NOM was 1.2 (±0.3) d⁻¹, which decreased to 0.5 (±0.2) d⁻¹ in stormwater without NOM. The DOC content of synthetic stormwater with and without NOM was 1.5 and 21.2 mg/L.

We repeated the growth experiment adding biochar or sand to the stormwater. In the presence of sand, bacterial concentrations in the aqueous phase increased over five days, but the concentration of bacteria in the presence of biochar decreased below the detection limit within one day and remained undetected through out the experiment (Figure S3).

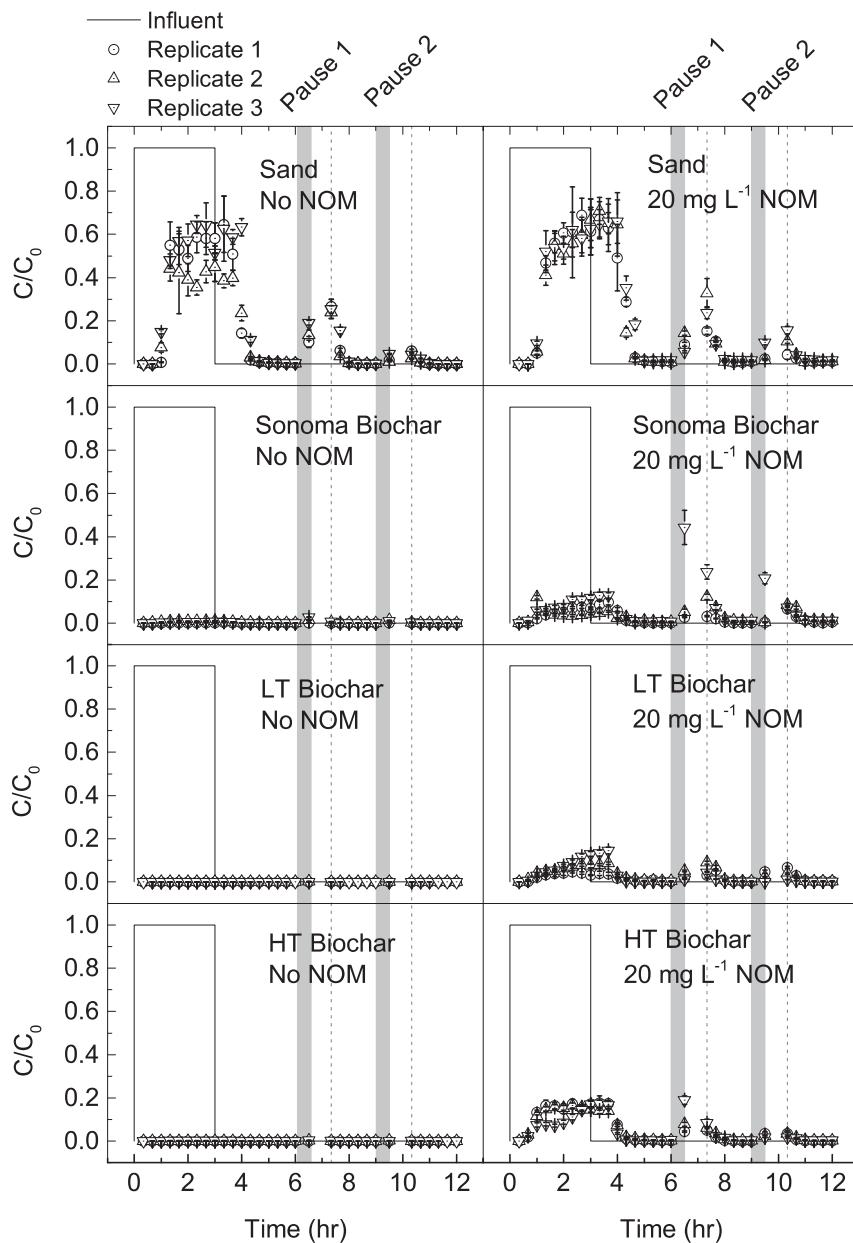


Fig. 1 – Transport and mobilization of *E. coli* through sand and biochar columns with and without NOM. The influent concentration was approximately $1.3 (\pm 0.2) \times 10^6$ CFU/mL. The gray area indicates the 0.5 h pause during which the column was drained, and the dashed lines indicate the timing of the first samples after the pause. The error bar indicates one standard deviation of measurements. The figure in log-scale is provided in the supplementary materials ([Figure S1](#)).

4. Discussion

4.1. Effect of biochar amendment on attachment of *E. coli*

Biochar-augmented sand columns removed 1 to 3 orders of magnitude more *E. coli* compared to sand during saturated flow. This indicates that even in a complex stormwater matrix, biochar has strong potential to remove waterborne bacteria. The increase in bacterial attachment may be due to the

overall increase in attachment sites in the biochar columns. Biochar is highly porous relative to sand, thus the surface area of biochar is at least 5 orders of magnitude larger than sand; sand has a typical surface area of $0.01 \text{ m}^2 \text{ g}^{-1}$. Addition of 5% biochar by weight increased the net surface area available for adsorption by a factor of 360 in columns amended with LT or HT biochar and a factor of 1790 in the columns amended with Sonoma biochar. However, the actual surface area available for bacterial attachment could be smaller because the estimated values include the internal surface area of pores within biochar that may not be accessible to bacteria.

Table 2 – Log removal of applied *E. coli* in columns packed with sand and different types of biochar during injection of contaminated stormwater, and the percentage of attached bacteria mobilized during two intermittent flows with 0.5-h and 21-h intervals.

Geomedia	Log removal ^a		Bacteria mobilized (%) ^b			
	w/o NOM	w/NOM	0.5-h Pause		21-h Pause	
			w/o NOM	w/NOM	w/o NOM	w/NOM
Sand	0.29 ± 0.09	0.21 ± 0.01	14.7 ± 5.5	24.3 ± 7.2	8.0 ± 1.3	77.6 ± 17.3
LT biochar	3.62 ± 0.27	1.18 ± 0.22	0.01 ± 0.01	2.29 ± 1.08	0.003 ± 0.002	0.074 ± 0.032
HT biochar	3.28 ± 0.52	0.83 ± 0.05	0.02 ± 0.02	2.56 ± 0.40	–	–
Sonoma biochar	2.32 ± 0.32	1.16 ± 0.20	0.11 ± 0.09	5.17 ± 2.69	–	–

^a Log removal of *E. coli* during their injection; log removal is calculated by taking negative logarithm of the ratio of effluent and influent concentration.

^b 100 times ratio of total *E. coli* eluted during two intermittent flows and total *E. coli* attached before intermittent flows.

Another explanation for the effectiveness of biochar on the removal of *E. coli* from stormwater is that *E. coli* may bind more efficiently to biochar than sand, possibly due to an increase in overall attractive forces between the bacteria surface and grain surfaces. According to Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Malte, 1999), an *E. coli* cell may experience a combination of attractive van der Waal forces and repulsive electrostatic forces when it comes into close proximity to grains. Under unfavorable attachment conditions (where the bacterium and grain are of the same net charge), most *E. coli* are likely to attach to sand or biochar at the secondary minimum (Redman et al., 2004), although some fraction may attach at the primary minimum near rough grain surfaces and wedge pore spaces (Torkzaban et al., 2008). Non-DLVO forces from hydrophobic and steric interactions may also influence the attachment of bacteria on sand and biochar (Chen and Walker, 2012). For attachment to occur, *E. coli* must overcome electrostatic repulsion between negatively charged surfaces of *E. coli*, biochar, and sand (Table S1), which increases with increasing pH of solution (Hayashi et al., 2001). Because biochar increases the pH of pore water (Novak et al., 2009), the electrostatic repulsion is expected to be greater in biochar than sand. (The pH of effluent from our biochar columns was ~9 while from the sand columns it was ~7.5, data not shown). However, bacterial retention in the biochar

columns was greater than in the sand columns, suggesting attachment on biochar may occur as a result of the non-DLVO forces including hydrophobic attraction. Hydrophobic attraction is expected to be much greater between bacteria and biochar than bacteria and sand due to the high organic carbon content of biochar (Abit et al., 2012). Addition of 5% biochar to the sand column increased the net fixed organic carbon by 4%. Thus, biochar may retain *E. coli* at the primary minimum due to the increased hydrophobic interactions (van Loosdrecht et al., 1987). Previous column studies demonstrated an increase in *E. coli* retention upon biochar addition to a sandy soil, and retention occurred primarily near the inlet of the columns, suggesting straining at intra- or inter-pores could also contribute to *E. coli* removal (Abit et al., 2012; Bolster and Abit, 2012).

The attachment of other indicator organisms and pathogens to biochar could differ from *E. coli* because of a difference in their surface characteristics (Abit et al., 2014). For instance, Abit et al. (2012) observed that removal of different *E. coli* isolates by biochar could vary. Gamesano and Logan (1998)

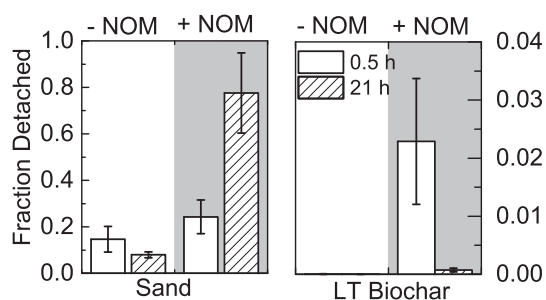


Fig. 2 – Fraction of attached *E. coli* mobilized from sand (left) and LT biochar columns (right) in stormwater with and without NOM during two intermittent flows. The gray background represents results from experiment with NOM. The error bar indicates one standard deviation of results obtained from four replicate column experiments. Note that the scale of y-axis is magnified for LT biochar.

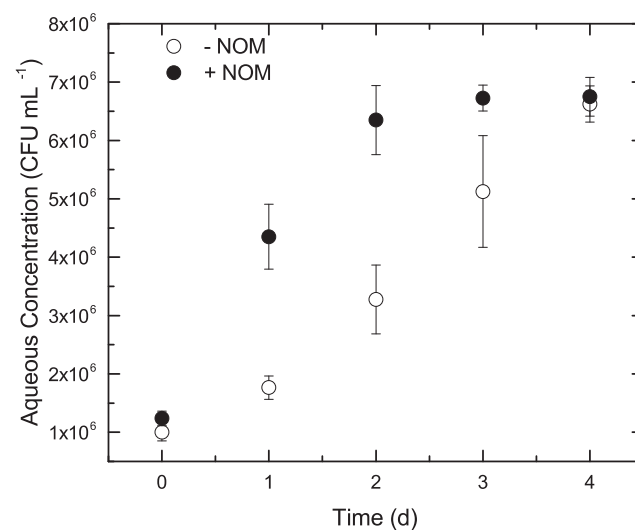


Fig. 3 – Growth of *E. coli* in stormwater at room temperature (23 °C) with and without 20 mg C L⁻¹ NOM. The error bars indicate one standard deviation of triplicate experiments.

found that motile bacteria, compared to non-motile bacteria, were less likely attached to sand in column experiments, particularly at low flow velocity and low ionic strength. Additional research should explore the ability of biochar to remove different health-relevant organisms including pathogenic bacteria and viruses.

The concentration of bacteria in the influent could also affect their net removal in columns. [Hazedaroglu et al. \(2009\)](#) observed that the removal capacity of *E. coli* in sand columns was not a function of influent concentration when it was below 10^7 CFU/mL, but the removal capacity decreased when the influent concentration was increased to 10^8 CFU/mL. The decrease in removal capacity at higher influent concentration was attributed to exhaustion of favorable attachment sites on sands. Because biochar has so many potential attachment sites, they may not be similarly exhausted. The influent concentrations used in our study are high relative to those expected in actual stormwater ([Grebel et al., 2013](#)). However, future work should explore more fully the effect influent concentration has on geomedia removal capacity.

4.2. Effect of biochar amendment on mobilization of *E. coli*

E. coli attached to interfaces in both biochar and sand columns were mobilized during intermittent flow; however, the mobilized fraction was smaller in biochar-amended columns compared to unamended columns. This suggests that a biochar amendment may minimize the mobilization of sequestered bacteria from a biofilter, thereby improving its overall removal efficiency. Bacteria mobilization during intermittent flow has been attributed to several processes that may occur during draining and rewetting: an increase in shear forces at the grain boundary ([DeNovio et al., 2004](#)), scouring by a propagating air–water interface ([Saiers et al., 2003](#)), and reduction of capillary forces on bacterial cell ([Crist et al., 2004](#)). The observed reduction of *E. coli* mobilization from the biochar columns may be explained by bacterial attachment at the primary minimum promoted by hydrophobic forces ([Abit et al., 2012](#)) and increased water-holding capacity or decreased intrusion of air during gravitational drainage. A stronger bacterial attachment between *E. coli* and biochar compared to sand could decrease the likelihood of bacterial mobilization from biochar surface. Furthermore, during gravity drainage, pores within and in between biochar particles could retain water by capillary pressure ([Abel et al., 2013](#)). A decrease in water drainage could reduce the movement of air–water interfaces in biochar columns, which could further decrease bacterial mobilization during intermittent flow ([DeNovio et al., 2004](#)).

4.3. Effect of NOM on attachment and detachment of *E. coli*

NOM is present in natural waters and may be particularly high and variable in stormwater. Thus, it is important to understand the effect of NOM on bacteria removal. The removal of *E. coli* from stormwater in the sand and biochar columns was lower in the presence of NOM. The lower removal is attributed to competition of NOM for attachment sites and an increase in

electrostatic repulsion between grain and cell surfaces after adsorption of NOM ([Foppen et al., 2008](#)). In previous studies ([Foppen et al., 2006](#); [Mohanty et al., 2013](#)), NOM reduced the removal capacity of a mixture of sand and iron-oxide coated sand to a value similar to that of unamended sand, thereby eliminating all benefits of geomedia amendment. However, in this study under similar conditions, removal capacities of biochar-amended columns were 0.6–1 log higher than the capacity of unamended columns in the presence of NOM. Biochar amendment also reduced the remobilization of attached *E. coli* during intermittent flows of stormwater with NOM. Under similar conditions, more than 50% of attached *E. coli* were mobilized from iron oxide coated sand ([Mohanty et al., 2013](#)). Collectively, results of these studies indicate that carbonaceous geomedia such as biochar may be more effective than geomedia with positive surface charge in removing bacteria from NOM-laden stormwater.

4.4. Effect of flow interval durations on the detachment of *E. coli*

During a wet season, the duration between two consecutive storm events may vary from less than an hour to a few days or longer, which could affect the mobilization of bacteria sequestered in the filter media. In the sand columns without NOM, intermittent flows with 21-h interval mobilized fewer bacteria than intermittent flow with 0.5-h interval. In the sand columns with NOM, intermittent flows with longer interval mobilized more bacteria, indicating growth of *E. coli* or a changing condition within the column that render attached cells more susceptible to mobilization as the column ages in presence of NOM. It appears that the former process can at least partially explain the observed phenomenon. We found that *E. coli* appears to grow in the artificial stormwater matrix with and without NOM, and the growth rate in stormwater with NOM is approximately two times faster than in stormwater without NOM. Within 24 h of growth, the concentration of *E. coli* increased 4 fold in the presence of NOM. If *E. coli* in the column grow at the same rate as *E. coli* in the stormwater suspension, then a 21 h-pause would be long enough to replenish or regenerate *E. coli* in sand columns with NOM compared with the regeneration during 0.5 h-pause. Mobilization of *E. coli* from biochar columns, however, did not change significantly when the interval between intermittent flows was increased from 0.5 h to 21 h, which indicates that biochar could have sequestered bacteria that may have grown. The batch study confirmed that biochar either prevented the growth of *E. coli* or removed them from stormwater despite their growth. Further study is needed to examine the persistence of attached bacteria using a visualization technique ([Crist et al., 2004](#)). Nevertheless, the ability of biochar to potentially preventing the growing cells from passing through biofilter media renders it attractive for their use in biofilters.

4.5. Effect of biochar types on removal of *E. coli*

A previous study showed that bacterial removal capacity of different biochars varied by several orders of magnitude and the removal capacity depended on biochar preparation conditions including pyrolysis temperature and feedstock origin

(Abit et al., 2012). It is important to know which property of biochar correlates well to high bacterial removal so that a screening method to select the most effective biochar can be developed. Although Sonoma biochar had five times more surface area than LT and HT biochar, the removal capacity of Sonoma biochar was one order of magnitude less than the removal capacities of the other biochars. This indicates that increase in surface area alone does not explain the removal of bacteria by biochar. Comparing the properties of biochar with their removal capacity, it appears the biochars with the lowest polar surface or greatest hydrophobicity and lowest volatile matter had the highest *E. coli* removal capacities. Biochar pores smaller than bacteria size may not be available for bacterial attachment. Some of the larger pores can be blocked by the volatile matter, decreasing the attachment of bacteria (Chen et al., 2012). Biochar containing a higher O/C, N/C or (O + N)/C ratio is expected to be more interactive with polar compounds (Wang et al., 2007) or less interactive with hydrophobic surface such as bacteria surface (Kingshott et al., 2003). An increase in atomic ratio (O/C, N/C) also indicates increases in polar groups known to reduce bacterial attachment on geomedia (Kingshott et al., 2003). These surface properties, however, can change as biochar ages (Uchimiya et al., 2010). Moreover, a long-term exposure to environmental conditions is likely to lead to biofilm growth (Luo et al., 2013), which in turn could affect its capacity to remove bacteria.

5. Conclusions

This study demonstrated that amending sand with biochar improved the removal of bacteria from stormwater during intermittent infiltration of stormwater, thereby making it attractive for use in biofilters or bioretention systems. Because biochar is less expensive than other available engineered geomedia and can be produced locally from biowaste, a large scale application of biochar is economically viable (Lehmann and Joseph, 2009). The following are the major conclusions of this study:

- Biochar-amended sand removed more *E. coli* from stormwater than quartz sand.
- Intermittent infiltration of stormwater remobilized *E. coli* attached to sand and the mobilization was higher with an increase in NOM.
- Compared to sand, biochar had lower mobilization of *E. coli* during intermittent flows.
- An increase in interval duration between rainfalls had contrasting effect on *E. coli* mobilization in presence of NOM: while mobilization increased in sand columns, it did not change in biochar columns.
- The increase in overall removal of *E. coli* by biochar is attributed to stronger attachment of *E. coli* at biochar surfaces and higher water-holding capacity of biochar amended sand.
- Biochar with low volatile matter and polarity was most effective in removing *E. coli*.

Acknowledgments

This work is supported by Re-inventing Nations Urban Water Infrastructure (ReNUWIT), an Engineering Research Center (ERC) funded by the U.S. National Science Foundation (Grant# EEC-1028968). We thank Tom Miles (T.R. Miles, Technical Consultants Inc.) for donating the Sonoma biochar. We appreciate David W. Rutherford (US Geological Survey, Golden, CO) and Bridget Ulrich (Colorado School of Mines) for measuring surface area of biochars.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2014.05.026>.

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