Technical Note

Biochar Selection for *Escherichia coli* Removal in Stormwater Biofilters

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Abstract: Biochar's capacity to remove pathogens from stormwater can vary by orders of magnitude, which makes it challenging for stormwater managers to select specific biochar from suppliers. In this study, the removal of *Escherichia coli* (*E. coli*) in model biofilters packed with sand and biochar from four suppliers was tested and correlation equations were developed that link short-term and long-term bacterial removal capacities of biochar with its commonly reported properties: surface area, carbon content, ash content, and volatile organic carbon content. The *E. coli* removal capacity of biochar was positively correlated with its surface area and carbon content and negatively correlated with ash content and volatile organic matter. Despite the presence of nutrients in stormwater, *E. coli* in pore water in biofilter did not grow between infiltration events, indicating biochar may continue to remove pathogens after rainfall. Overall, the results could help the selection of biochar from suppliers for the treatment of stormwater and inform the suppliers to tailor biochar production conditions to enrich specific biochar properties. **DOI: 10.1061/(ASCE)EE.1943-7870.0001843.**

Introduction

Pathogens and fecal indicator bacteria (FIB) are among the most difficult pollutants to remove from stormwater, making them the leading cause of total maximum daily load (TMDL) violations in many urban areas (USEPA 2002). Traditional amendments used in stormwater treatment systems, such as biofilters, have limited capacity to remove indicator bacteria (Hathaway et al. 2009). Biochar, a carbon amendment produced by pyrolysis of waste biomass, has been shown to improve contaminant removal (Lau et al. 2017; Mohanty et al. 2018; Sun et al. 2020). Biochar can be produced at any location, thereby making it widely available for use by stormwater managers (Xie et al. 2015). However, biochar properties can vary widely based on preparation conditions and feedstock types (Xiao et al. 2018). This makes it challenging for the stormwater manager to select specific biochar from suppliers.

It is generally recommended to use wood-based biochar prepared at a high pyrolysis temperature (Abit et al. 2012; Bolster and Abit 2012) without removing fine biochar (Guan et al. 2020; Mohanty and Boehm 2014; Sasidharan et al. 2016). Despite these constraints, bacterial removal, bacterial removal by biochar can vary widely (Boehm et al. 2020), indicating the competing effects of different properties, including carbon content, ash content (AC), volatile carbon content, and surface area (SA) (Manya 2012). This adds uncertainty in predicting the performance of biochar-amended biofilters (Boehm et al. 2020). This study aims to develop an empirical model to predict *Escherichia coli* (*E. coli*) removal capacity of biochar based on commonly reported bulk biochar properties. The model can be used by stormwater managers to select biochar from the suppliers for the treatment of stormwater.

Experimental Methods

Experimental Design and Operation

Synthetic stormwater was created in deionized water mixed with the following salts: 0.75 mM $CaCl_2$, 0.075 mM $MgCl_2$, 0.33 mM Na_2SO_4, 1 mM $NaHCO_3$, 0.072 mM $NaNO_3$, 0.072 mM NH_4Cl, and 0.016 mM Na_2HPO_4 (Mohanty and Boehm 2014). This limits the influence of the fluctuating composition of natural stormwater on the measurement and comparison of the removal capacity of four types of biochar.

The biofilter medium for each biofilter consisted of a mixture of coarse Ottawa sand (0.6–0.85 mm) and a biochar from one of the following suppliers: Terra Char (BioEnergy Innovations Global, Americas Solutions LLC, Columbia, Missouri), Agricultural Carbons (National Carbon Technologies, Oakdale, Minnesota), NAKED Char (American BioChar, Niles, Michigan), and Rogue Biochar (Oregon Biochar Solutions, White City, Oregon). Each biochar was characterized by SA, carbon content, AC, volatile carbon, and elemental composition (Table 1). Prior to packing, large biochar particles (>2.0 mm) were removed by sieving to minimize preferential flow through the filters. Sand and biochar (30% v/v) were mixed manually and packed in polypropylene columns with 2.54 cm in diameter and 30 cm in height (Mohanty and Boehm 2014).

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Table 1. Preparation condition and properties of four types of biochar used in this study

Parameters	Terra Char	Agricultural Carbons	NAKED Char	Rogue Biochar
Vendor	BioEnergy Innovations Global, Energy Americas Solutions LLC, Columbia, Missouri	National Carbon Technologies, Oakdale, Minnesota	American BioChar, Niles, Michigan	Oregon Biochar Solutions, White City, Oregon
Feedstock	Oak hardwood sawdust	Wood-based	southern yellow pine species	80% softwood, 15% hardwood, and 5% nutshells
Pyrolysis temperature (℃)	540	>550	550–990	>900
Surface area (m^2 g^{-1})	207	339	283	475
S (%)	0.003	0.002	0.005	0.041
C (%)	70.16	85.03	80.96	84.66
H (%)	1.89	2.77	0.59	0.83
N (%)	0.62	0.31	0.53	0.81
O (%)	9.36	7.78	5.67	5.43
Polarity index, $(O + N)/C$	0.142	0.095	0.077	0.074
Ash (%)	17.97	4.11	12.24	8.23
Volatile matter (%)	18.55	12.19	6.66	7.86
Fixed carbon (%)	63.48	83.7	81.1	83.91

After packing of the biofilters, synthetic stormwater without *E. coli* was injected for 24 h at 2 $\mathrm{mL\,min^{-1}}$ (e.g., hydraulic loading rate of 139.9 $\mathrm{gpd\,ft^{-2}}$) using a peristaltic pump (Masterflex L/S Digital Drive, Cole Parmer, Vernon Hills, Illinois) to condition the filter media. To maintain an upward flow direction, stormwater was injected at the bottom of the biofilter, and samples were collected on top. The upward direction ensured a complete saturation of the columns, enabling a comparison of the maximum removal capacity of the biochar without an interference from preferential flow (Mohanty et al. 2013). To measure the pore volume (PV) of the biofilter, e.g., the volume of the empty space in the filter media, the weight difference between wet biofilter and dry biofilter was measured (Table S1). The average PV of all biofilters was 50.3 ± 2.8 mL, and it is not statistically different (p-value > 0.05) between biofilters.

Estimation of Removal Capacity of Biochar

Stormwater containing *E. coli* was injected through the columns in an upward direction at 2.0 $\mathrm{mL\,min^{-1}}$ using a peristaltic pump, and effluent sample fractions were collected using 15-mL centrifuge tubes. Stormwater containing *E. coli* was created by growing *E. coli* in lysogeny broth (LB) solution for 16–24 h at 37°C and 120 rpm, followed by a triple wash procedure with phosphate buffer solution to remove the LB solution. The washed *E. coli* solution was used to spike the synthetic stormwater to achieve a concentration of nearly 10^5 $\mathrm{CFU\,mL^{-1}}$. Upward flow is necessary to compare the maximum removal capacity of biochar in order to identify the best biochar—the objective of this study. In a field application, where flow is downward, the removal capacity can be lower for all biochars. The removal capacity of the biofilter was calculated as $-\log_{10}(C_e/C_i)$, where C_e and C_i represent the concentration of *E. coli* in the effluent and influent, respectively. The clean-bed removal capacity (triplicated columns) was estimated by comparing the effluent concentration after the injection of approximately 5 PV of *E. coli*–contaminated stormwater. The long-term removal capacity of the biofilters can be significantly different from the clean-bed removal capacity because of an exhaustion of the attachment sites on biochar by *E. coli* and other competing agents in stormwater. The long-term removal capacity of biofilters (duplicated columns) was estimated after the injection of 57 + PV of contaminated stormwater in 10 intermittent rainfall events. In each infiltration event, 8 ± 1 PV of *E. coli*–contaminated stormwater was injected for 4 h followed by 24–96 h of flow interruption. 57 + PV was chosen as an indicator of long-term removal capacity because this rainfall quantity is equivalent to 9.1 years of *E. coli* loading to a biofilter located in Los Angeles (38.1 cm of rainfall per year), assuming a catchment area of 10 acres and an average *E. coli* concentration of 10 $\mathrm{CFU\,mL^{-1}}$ in the stormwater (Tables S2 and S3). The long-term removal capacity was calculated as the average of the last three rainfall events, which occurred at 57, 66, and 75 PV.

In between infiltration events, *E. coli* trapped in the pore water inside the biofilter can grow or die, thereby increasing or decreasing the effluent concentration. The growth–die-off index (GDI) was introduced; it is the negative log of the ratio of *E. coli* concentration before (C_b) and after (C_a) a flow interruption and is used to quantitatively determine the fate of *E. coli* trapped in biofilters during pause between infiltration events. Positive and negative GDIs indicate respectively a net-growth and die-off of *E. coli* between infiltration events.

Analysis of Pearson's correlations was used to verify the correlation between biochar properties and *E. coli* removal capacities. ANOVA, principal component analysis (PCA), and partial least-squares (PLS) regression models were used to interpret the results and rank the properties that predict biochar capacity to remove fecal indicator bacteria.

Results and Discussion

Removal Capacity Varied by More Than an Order of Magnitude

The clean-bed removal of *E. coli* depended on the biochar types (Fig. 1) and varied by more than one order of magnitude between different biochars. For instance, the clean-bed removal capacity of biofilters packed with Rogue Biochar (log removal of 3.36 ± 0.70) or Agricultural Carbons (3.67 ± 0.72) was greater than biofilters packed with Terra Char (1.98 ± 0.38) or NAKED Char (1.90 ± 0.50). The long-term removal capacity was different from their clean-bed removal capacities based on biochar type (Table S4). After the exposure of 57 + PV of contaminated stormwater, which is equivalent to 9.1 years of *E. coli* loading in biofilters in Los Angeles (details in the Supporting Material), the removal capacities of Rogue Biochar and Terra Char increased ($p < 0.05$) by 71% and 62%, respectively, whereas the removal capacity of Agricultural Carbons decreased ($p < 0.05$) by 20%. No significant difference

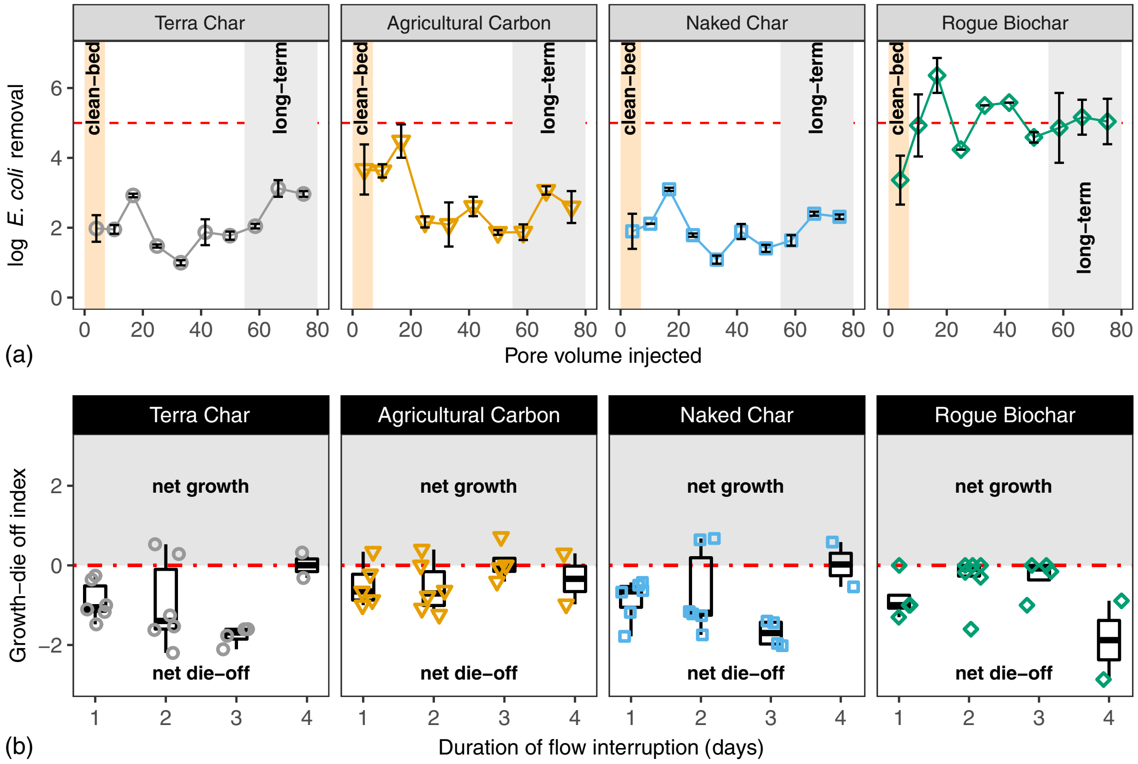


Fig. 1. (a) *E. coli* removal capacity of biochar-augmented filters during 10 infiltration events. Yellow and grey shaded areas represent clean-bed ($n = 12$) and long-term removal ($n = 12$), respectively. Red dashed line represents detection limit of 1 colony per plate (20 CFU mL^{-1}); and (b) GDI between two consecutive infiltration events as a function of drying duration between infiltration events. GDI was calculated as $-\log_{10}(C_b = C_a)$, where C_b and C_a represent the concentration of *E. coli* in the effluent before and after flow interruption, respectively. Positive GDI values (grey shaded area) represents net-growth of bacteria during flow interruption, while negative GDI values represents net die-off (or decay) or bacteria. Positive GDI values (grey shaded area) represents net-growth of bacteria during flow interruption, while negative GDI values represents net die-off (or decay) or bacteria.

($p > 0.05$) between the clean-bed and the long-term removal was observed for the biofilters packed with NAKED Char.

The *E. coli* concentration in the pore water of sand-biochar filters mostly decreased (with few exceptions) during intervals between infiltration events, resulting in a GDI below zero [Fig. 1(b)]. The log GDI values appear to be independent of the interval between infiltration events, indicating a lack of growth of *E. coli*. The result indicates that, despite the presence of nutrients in pore water, *E. coli* did not grow in pore water or on biochar between infiltration events (Valenca et al. 2020). Thus, biochar could continue to remove or inactivate *E. coli* from pore water between infiltration events, thereby replenishing filter media for the removal of pore *E. coli* in subsequent infiltration events.

It was shown that the removal in clean-bed biofilters can be different from the removal after long-term exposure. Compared to other studies that examined long-term *E. coli* removal by biochar, this study used stormwater without native bacteria and dissolved organic carbon (DOC) that might have exhausted the biochar faster. Thus, the results presented here may overestimate the capacity of biochar in removing *E. coli*. Another possible overestimation of these results relates to flow direction. Although an upward injection was used in the current study, actual field application is usually subject to several complexities, such as downward flow, the presence of DOC and other bacteria, and biochar aging, all of which may affect the removal capacity of the filter. Here, the objective was to select the best biochar and not to measure actual removal capacities. Nonetheless, the best biochar in this controlled study will likely be the best biochar in field conditions.

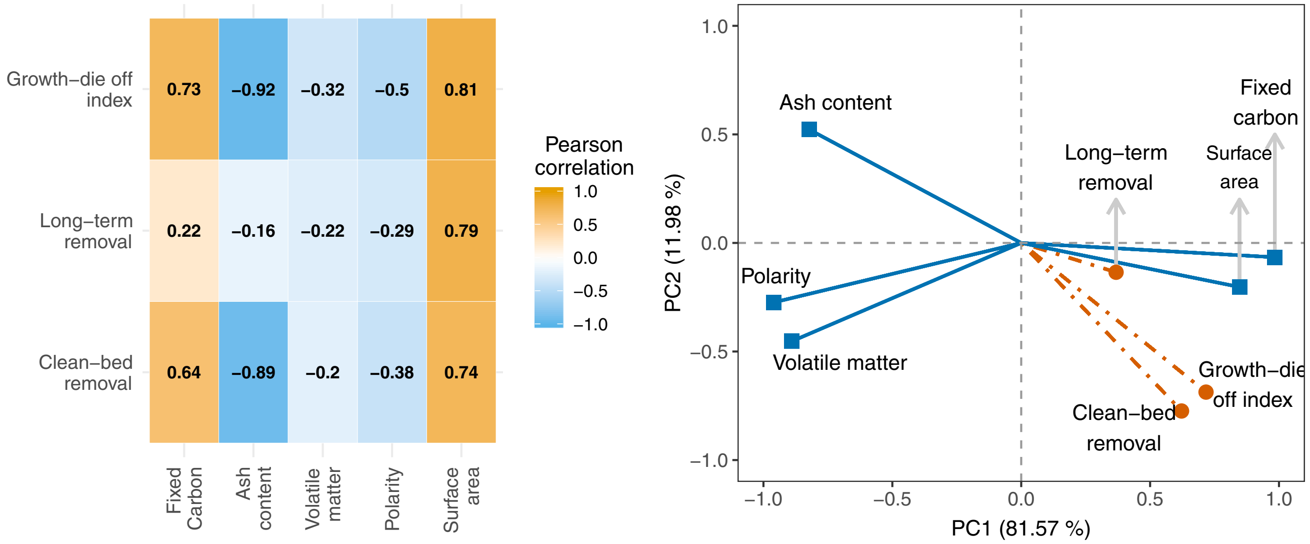
Correlation of Removal with Biochar Properties

Removal was positively correlated with surface area (SA) and fixed carbon (FC) and negatively correlated with polarity, volatile matter (VM), and ash content (AC) (Fig. 2). The AC was found to be the most important indicator of bacterial removal in biochar. The vendors can lower AC in biochar by optimizing the production condition such as feedstock type and pyrolysis temperature (Ahmed et al. 2016), or they can also be washed with strong acids to dissolve and remove the ash (Sun et al. 2013).

The GDI was negatively correlated with biochar polarity ($r = -0.5$) and AC ($r = -0.92$), but positively correlated with biochar SA ($r = 0.81$). These results are attributed to the biochar's ability to continue to remove bacteria by inactivation (Gurtler et al. 2014) and adsorption (Mohanty et al. 2014) or to reduce the availability of growth metabolites (Hill et al. 2019).

PCA showed that while SA and FC positively affected the GDI and clean-bed removal, polarity and AC negatively affected the GDI and the clean-bed removal capacity. Long-term removal appears to be uncorrelated or weakly correlated with most variables.

Based on PLS regression, an empirical model was developed to predict clean-bed removal [R_S, Eq. (1)] and GDI [Eq. (2)] based on SA, FC, AC, and VM. The proposed model was able to predict the removal capacity of another commercially available biochar entitled Sonoma Biochar (Sonoma Compost, Nicasio, California) which was used in a previously published study (Mohanty et al. 2014). The model proposed here predicts that Sonoma Biochar would remove 99.90% of *E. coli*, while the reported data showed removal of 99.52%. The interaction between variables was



(a)

(b)

Fig. 2. (a) Correlation of clean-bed removal capacity, long-term removal capacity, and GDI with specific biochar properties, including fixed carbon, ash, volatile matter, polarity, and surface area; and (b) PCA between biochar properties, removal capacities, and GDI. Orange dot-dashed lines represent dependent variables, and blue solid lines represent explanatory variables. The contribution of each component is as follows: PC1 (23.76% fixed carbon, 16.57% ash content, 19.42% volatile matter, 22.66% polarity, and 17.48% surface area) and PC2 (0.73% fixed carbon, 45.53% ash content, 34.33% volatile matter, 12.50% polarity, and 6.91% surface area). PCA was created using XLSTAT (version 2020.2.3).

analyzed and it was found that the interactive effect between the variables was either statistically insignificant for the prediction of GDI or yielded an unrealistic outcome for the prediction of the clean-bed removal capacity (Table S5). This result indicates that the interaction effect between variables is minimal. Overall, the results presented here show that the proposed model could be used to indicate the *E. coli* removal capacity of biochars from different suppliers, although validating the model with more biochars could improve the model:

$$R_S = 0.0045 \times \mathrm{SA} + 0.0097 \times \mathrm{FC} - 0.113 \times \mathrm{AC} + 0.104 \times \mathrm{VM} + 0.531 \quad (1)$$

$$GDI = 0.0008 \times \mathrm{SA} + 0.0023 \times \mathrm{FC} - 0.019 \times \mathrm{AC} + 0.015 \times \mathrm{VM} - 0.157 \quad (2)$$

Conclusions

The *E. coli* removal capacity of fresh biochar and used biochar varied with biochar types. Despite the presence of nutrients in infiltrating water and pore water, *E. coli* was removed over the course of drying between infiltration events, suggesting that biochar limits the growth of attached *E. coli*. The *E. coli* removal capacities of sand-biochar filters were positively correlated with the surface area and organic carbon content of biochar and negatively correlated with ash content and volatile matter content. A model relating biochar removal capacity with these commonly measured biochar properties was developed based on PLS regression, which has the potential to predict the *E. coli* removal capacity of commercially-available biochar. Thus, the model can not only help stormwater managers to select biochar for biofilters but also inform biochar production companies to tailor their production methods to produce biochar possessing specific properties, that aid in *E. coli* removal from contaminated stormwater.

Data Availability Statement

Some or all data, models, or code generated or used during the study are available in an online repository (https://doi.org/10.6084/m9.figshare.12955217) in accordance with funder data retention policies.

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Supplemental Materials

Tables S1–S5 and Fig. S1 are available online in the ASCE Library (www.ascelibrary.org).

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