Evaluating the simultaneous retention of organic contaminants and *Escherichia coli* (*E. coli*) in biochar-amended biofilters

Johanna Jernberg,[1,2] Tadele Haile,[1] and Bridget Ulrich[1,*]

[1]Natural Resources Research Institute, University of Minnesota Duluth, Duluth, MN, 55811

[2]Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN, 55812

[*]Corresponding author; email: ulrichb@d.umn.edu

ABSTRACT:

Though organic contaminants and *Escherichia coli* (*E. coli)* are very different contaminants, both are ubiquitous in urban runoff, mobile in conventional biofilters, and interact with biochar via hydrophobic interactions. However, limited information is available regarding their simultaneous retention in biochar-amended filtration systems, which was evaluated here via intermittently dosed column tests. Columns amended with commercial biochar (ABC-biochar or WF-biochar) were compared to sand-only controls over treatment of 100 empty bed volumes (EBVs) of creek water, which was augmented with dissolved organic carbon (DOC) and organic contaminants, as well as *E. coli* during three loading periods. While both biochars demonstrated similar DOC removal, effluents from ABC-biochar columns showed reduced specific ultraviolet absorption (SUVA) and improved organic contaminant retention relative to sand-only and WF-biochar columns. However, biochar-amended filters showed limited improvement in *E. coli* retention, as sand-only and biochar-amended columns demonstrated up to 1.0 ± 0.5 and 1.4 ± 0.4 log-removal of *E. coli*, respectively. *E. coli* log-removal for all columns was reduced to 0.5 ± 0.1 following a freeze-thaw cycle. Drainage rates for ABC-biochar columns were on average approximately 50% higher than the other columns, demonstrating the importance of considering hydraulic conditions when assessing overall filtration performance. Our findings warrant more rigorous validation of the effects of biochar amendment to filtration performance under environmentally relevant conditions and at the field scale.

INTRODUCTION:

Contamination of receiving waters by urban runoff, driven by increasing impervious cover and release of anthropogenic contaminants in urban areas, has caused growing environmental and public health concern (1). Conventional stormwater best management practices (BMPs) are often designed primarily to prevent flooding, while reductions in contaminant loads are secondary treatment goals (2). More recently, low impact development approaches such as biofiltration have emerged that seek to simultaneously manage water quantity and quality by filtering contaminants through bioretention media to retain contaminants. While such systems have proven effective for removal of hydrophobic and sediment-bound contaminants such as polyaromatic hydrocarbons and some metals, they are less effective for removal of various other more "mobile" contaminants present in urban runoff (3). For example, *Escherichia coli* (*E. coli)* can enter urban drainage systems via animal feces or leaking sewer or septic systems (4), and is poorly removed by conventional biofiltration systems (5). This has brought about substantial public health concern in addition to economic concerns associated with lost recreational opportunities, as exceedances of acute standards for *E. coli* have led to beach closures across the U.S. (6,7). Additionally, contamination of receiving waters with various dissolved organic contaminants present in urban runoff such as pesticides and chemicals from vehicle fluids has caused increasing concern, as these contaminants are widely present in urban runoff and poorly removed by conventional treatment systems (8,9).

Amendment of biofiltration systems with sorbent materials is an emerging approach to improve retention of mobile contaminants such as *E. coli* and organic contaminants. For example, biochar, a stable carbonaceous substance produced by thermally processing biomass (e.g., pyrolysis or gasification) has received substantial recent attention due to its multifaceted utility for soil health, carbon sequestration, and water quality management (10). Biochar can have widely varying material properties, including high intraparticle surface area and tunable surface chemistry, which are dependent on production conditions including feedstock and production temperature (11,12). While biochar has long been used as a soil conditioner, the application of biochar for water quality management is a growing field of research (13),

and biochars that are effective for soil conditioning may not necessarily be effective for water treatment. Biochar has demonstrated promising results for enhanced retention of mobile contaminants such as *E. coli* and dissolved organic contaminants from urban drainage in laboratory experiments (7,14). However, the observed retention performance for these contaminants varies widely and is strongly dependent on biochar production characteristics and resulting materials properties. For example, previous laboratory studies have found that wood-based biochars produced at high temperatures (e.g., >600 °C) are more effective for retention of both organic contaminants and *E. coli* (15,16).

Though *E. coli* and organic contaminants are very different in nature, hydrophobic interactions have been shown to contribute to retention processes for both types of contaminants. Accordingly, their enhanced retention has been attributed to increased intraparticle porosity and hydrophobic surface chemistry (e.g., more aromatic, fewer oxygen-containing functional groups) that arise for biochars produced at higher temperatures (17). Additionally, pi-pi interactions with aromatic biochar surfaces can also contribute to enhanced retention of aromatic organic contaminants by high-temperature biochars (18), while physical straining has been shown to affect retention of *E. coli* (19). However, while the individual removal mechanisms for these contaminants have been relatively well characterized under controlled laboratory conditions, few studies have evaluated their simultaneous removal, particularly under gravity-driven intermittent flow conditions typical of actual treatment systems. Further, results from a recent field-scale study have suggested that amendment of sand filters with biochar may not necessarily lead to improved retention of *E. coli* or dissolved organic carbon (DOC), potentially due to differences in operational conditions or biochar characteristics when transitioning from laboratory to field-scale (20).

In this study we evaluated the performance of biochar-amended sand filtration columns operating under intermittent flow conditions for the simultaneous retention of organic contaminants and *E. coli.* To simulate environmentally representative conditions, experiments were performed with urban creek water augmented with DOC, organic contaminants, and *E. coli*. We hypothesized that effectiveness for retention of the aromatic portions of DOC, as indicated by specific ultraviolet absorbance (SUVA), could be used to probe

for the presence of carbonaceous sorption sites that facilitate hydrophobic interactions, and ultimately as an indicator for retention performance for organic contaminants and *E. coli*. We compared the performance of two commercial, high-temperature, wood-based biochars marketed as soil conditioners against sand-only controls. Our findings demonstrate the challenges of translating results observed in the laboratory to systems operating under more environmentally representative conditions, and warrant more rigorous validation of the effects of biochar amendment to filtration performance at the field scale.

EXPERIMENTAL SECTION:

Acquisition and characterization of filter materials

Filter materials. Two representative commercial biochars were selected to evaluate potential variations in material properties and performance among high-temperature, wood-based biochars available in bulk quantities. Biochars were obtained from the American Biochar Company (ABC-biochar, Niles, Michigan, marketed as Naked Char) and Wakefield Biochar (WF-biochar, Valdosta, Georgia). Both biochars are marketed primarily as soil conditioners and are available in bulk at comparable prices. While the ABC-biochar had a moderately higher cost (approximately $250/CY versus $190/CY for WF-biochar, as of November 2024), the company provides more publicly available documentation for technical specifications (Figure S1) on their website (21) relative to the Wakefield Biochar website (22). The ABC-biochar is a steam-activated biochar produced from Southern Yellow Pine with pyrolysis temperatures ranging from 550–900 °C (particle sizes ranging from 0.5–2.0 mm). This biochar was selected because it was previously evaluated in laboratory up-flow saturated column experiments (16) and demonstrated effective *E. coli* retention (i.e., up to 3 log-removal). The WF-biochar is also a high-temperature, wood-based biochar, and is produced from loblolly pine waste wood from sawmill operations at a pyrolysis temperature of 600 °C or higher (particle size ranging from 0.3–2.4 mm). As a base for the biochar-amended filtration media, concrete sand was purchased from Plaisted Companies (Elk River, Minnesota, technical data for particle size and mineral composition provided by Plaisted are shown in Tables S1 and S2). Gravel used for drainage

and media support purposes (particle size ranging from 2.38–4.76 mm) was thoroughly washed with deionized water prior to use.

Biochar characterization. Ultimate and Proximate analyses were carried out by Timber Products Inspection (Conyers, GA) following ISO standard methods (Table S3). Biochar pH was measured following equilibration with deionized water using a Fisher Brand Accumet AB150 pH electrode (23). To quantify intraparticle porosities, dual N_2/CO_2 gas adsorption experiments were performed with a Micromeritics 3 Flex according to a previously published method (24), and cumulative pore volumes were determined for the microporous (0.36-0.99 nm) and mesoporous (2.00–50 nm) ranges. Intraparticle pore volumes are provided as a metric for intraparticle surface area, as previous studies have shown that Brunauer-Emmett-Teller (BET) surface area values for biochars determined via N_2 physisorption are biased by degassing temperature (25). The utility of intraparticle pore volume as an indicator for the availability of intraparticle sorption sites is supported by previous work showing that pore volume is a better predictor than BET surface area for the uptake of organic contaminants by biochars in batch tests (15).

Experimental approach

Experimental apparatus. Columns consisted of 2-inch ID x 24-inch length (5.1 cm x 61 cm) PVC pipe with an open top and bottom cap and a 1 cm outlet, such that the drainage rate was controlled by the filter media and back pressure within the column was minimized. Columns were packed (from bottom to top) with a gravel drainage layer, a filtration layer including sand or sand amended with one of the two biochars, and an upper gravel layer to fix the media in place and facilitate flow distribution. Following compaction during initial dosing events, each column contained approximately 8–10 cm of ponding space above the filter beds. Three different column configurations were evaluated in triplicate: sand-only control columns, sand amended with the ABC-biochar, and sand amended with the WF-biochar. Sand and biochar were mixed at a 70:30 volume ratio prior to being dry-packed into columns, such that the filtration layers had an empty bed volume (EBV) of approximately 700 mL. The bulk density of the sand and sand/biochar mixtures were

1.6 g/cm^3 and 1.21 g/cm^3, respectively. All columns had similar porosities following initial equilibration via flushing with deionized water (40% ± 2% v/v).

Preparation of dosing solution. Natural creek water (20 L) was collected from Tischer (Duluth, MN) 24–72 hours prior to each test. To augment DOC levels, creek water was equilibrated overnight with 5 g of dried leaf litter prior to each dosing event. Following equilibration, equal volumes of creek water (2.1 L, equivalent to three empty bed volumes, EBVs) were added to nine separate influent containers, each of which were charged with organic contaminants (four analytes: atrazine, diuron, imidacloprid, and methyl benzotriazole, selected due to their high detection frequency in urban runoff (8)) via a methanol carrier solution to achieve influent concentrations between 10–20 µg/L. Organic contaminant concentrations were approximately an order of magnitude above typical environmental concentrations to facilitate observation of breakthrough within the experimental duration, reflecting procedures from various previous studies (14,15,26,27).

To augment influent *E. coli* levels for a subset of dosing tests (i.e., *E. coli* loading periods), a concentrated *E. coli* culture was initially prepared from stock *E. coli* (reference strain ATCC#25922) in sterilized LB media and stored in an incubator at 35 °C for at least 24 hours prior to use. Fresh *E. coli* subcultures were prepared one to two days prior to dosing tests by diluting *E. coli* cultures 100x in 1x phosphate-buffered saline (PBS). Immediately prior to dosing tests, 5 mL of dilute subculture were added to each influent reservoir to achieve target *E. coli* concentrations of roughly 200,000 colony forming units (CFU) per 100 mL, though actual measured concentrations varied from 100,000–500,000 CFU/100 mL. While these concentrations are approximately an order of magnitude higher than maximum reported environmental levels (3), these high concentrations were presumed necessary to enable differentiation between treatment conditions with large differences in removal performance. Note that Valenca *et al.* reported *E. coli* retention up to 3 log-removal for ABC-biochar in saturated up-flow column tests (16). Therefore, an influent concentration of 200,000 CFU/100mL would enable quantification differentiation between conditions

achieving between 1 and 3 log-removal (effluent concentrations between 200–20,000 CFU/100mL) using the *E. coli* plating described a subsequent section.

Dosing procedure. A total of 34 dosing tests (3 EBV each, 102 EBV total) were performed twice per week over a six-month period, representative of 2.5 years of equivalent runoff volume based on an approximate average annual rainfall of 30 inches (76 cm) for the Duluth, MN area. The first dosing period consisted of 18 dosing tests performed during the fall (0–54 EBVs). The columns were then subject to a freeze-thaw cycle, during which the column manifold was stored outside under a tarp for 6 weeks (average temperature of -6.1 °C, ranging from -22 to +6.9 °C), then allowed to thaw at room temperature and subject to an additional 18 dosing tests (dosing period 2, 57–102 EBVs). Columns were allowed to drain but remained moist prior to the freeze-thaw cycle. Note that an unseasonably warm winter in Duluth, MN enabled collection of creek water for the entire duration of the experiment, though the water was at near-freezing temperatures upon collection during winter months and did not fully equilibrate to room temperature overnight during the second dosing period.

E. coli loading periods were performed at the beginning of the experiment (loading period 1, five dosing tests, 0–15 EBVs), at the end of the first dosing period prior to the freeze-thaw cycle (loading period 2, five dosing tests, 42–54 EBVs), and near the end of the experiment (81 EBVs), though *E. coli* concentrations were only augmented in the influent during a single dosing test for the final loading period. During each dosing test 3 EBVs (2.1L) of water (equivalent to a two-inch storm for a treatment system sized to 5% of the catchment area) were delivered from each influent reservoir to the ponding reservoir for each column, and the water was allowed to drain by gravity through each column. Samples were collected from effluent reservoirs once the reservoir volume reached approximately 1 L. Samples for CECs were collected in 20 mL glass scintillation vials and stored frozen until analysis. UV 254 analyses were performed for each doing test, and samples were collected in 50 mL falcon tubes and analyzed on the same day. Aliquots for *E. coli* analyses were also taken from the 50 mL Falcon tubes and *E. coli* analyses were initiated on the day

of the experiment. Samples for dissolved organic carbon (DOC) analysis were collected in 250 mL poly bottles and stored frozen.

Hydraulic conductivity measurements. Hydraulic conductivity tests were performed on six occasions throughout the dosing experiment as described previously (28). Briefly, while maintaining a constant height of water over the filter media, the discharge volume from each column was measured over 60-second increments. The average hydraulic conductivity for each column was calculated from the average of six individual measurements according to Darcy's law.

Quantitative analysis

E. coli analysis. *E. coli* concentrations were determined by a plating method using Coliscan® Easygel® kits (Micrology Laboratories, Granger, Indiana) according to manufacturer-recommended procedures. Briefly, Easygel media containing 1–5 mL aliquots of sample were incubated at 35 °C in sterile petri dishes for 24 hours. Highly concentrated samples (e.g., influent samples during *E. coli* loading periods) were pre-diluted 10x with PBS buffer. Following incubation, plates were documented photographically, and colonies were counted based on the images using OpenCFU, an open-source colony counting software (see Figure S2 for example images and details regarding counting procedures). While higher variability is expected for these measurements relative to more robust quantitative approaches (i.e., Colilert tests), this method was selected to enable identification of large differences in *E. coli* retention performance (i.e., orders of magnitude differences in concentration) among experimental conditions over numerous treatment events while accommodating budget constraints. Note that effluent concentrations below 200 CFU/100 mL (i.e., <10 CFU for an undiluted 5 mL sample) or above 300,000 CFU/100 mL (i.e., >300 CFU for a 10x diluted 1 mL sample) can be considered semi-quantitative estimates due to inconsistencies in colony counting and plating at very high and low concentrations, respectively. However, effluent *E. coli* concentrations during *E. coli* loading periods largely fell within the quantitative range (e.g., between 1000 and 100,000 CFU/100 mL).

194 Specific ultraviolet absorbance (SUVA). Filtered (0.45 μm, nylon) influent and effluent samples were
195 analyzed by ultraviolet visible absorbance spectroscopy at 254 nm (UVA 254) using a Perking Elmer
196 Lambda 25 Spectrophotometer. DOC (mg/L) was also measured for select samples to determine the
197 specific ultraviolet absorbance (SUVA, UVA 254/DOC, L/mg*m). DOC analysis was performed using a
198 Shimadzu TOC-L Total Carbon Analyzer and a high temperature combustion method (SM 5310-B 2014).

199 Organic contaminant analysis. A detailed description of the sample preparation, instrumental analysis, and
200 quality assurance/ quality control procedures for analysis of organic contaminants is provided in the
201 Supporting Information (Table S4). Briefly, samples were diluted with isotope-labeled extraction
202 surrogates and analyzed by a multiple-reaction-monitoring high resolution (MRM^{HR}) acquisition method
203 using an AB Sciex X500R quadrupole time-of-flight mass spectrometer (QTOF-MS) coupled to an AB
204 Sciex ExionLC AD liquid chromatography system. Two transitions (one quantitative, one qualitative) were
205 monitored for five analytes (atrazine, imidacloprid, methyl-1H-benzotriazole, diuron, and 1-(3,4-
206 dichlorophenyl)-3-methylurea; DCPMU). Select samples were analyzed as necessary to identify any
207 contaminant breakthrough that occurred within the experimental duration. Influent samples were analyzed
208 in triplicate from three randomly selected influent containers. Effluent samples from sand-only controls
209 were analyzed in triplicate (i.e., individual samples from each of the three replicate columns). Effluent
210 samples for biochar-amended columns were initially measured in duplicate to identify instances of
211 breakthrough, and were analyzed in triplicate if any of the five analytes were detected for a given dosing
212 test. Reporting limits ranged from 0.025–0.1 ng/mL depending on the analyte (Table S4). No analytes were
213 detected above the reporting limits in the laboratory blanks, which were performed during each preparatory
214 batch.

215 Statistical analysis. Statistical comparisons were performed via two-tailed, paired student *t* tests, where a
216 threshold of $p < 0.05$ was used to identify statistically significant differences between data sets (assuming
217 equal variance unless noted otherwise). Values for *n* indicate the number of data points for each
218 experimental condition included in statistical calculations ($n > 3$ indicates inclusion of experimental

replicates over multiple dosing tests). Mean values are reported with the standard deviations for three experimental replicates as error, unless indicated otherwise. For example, combined means and standard deviations from more than one column type or from multiple dosing tests are reported for instances where student *t* tests indicated that the data sets were statistically equivalent.

RESULTS AND DISCUSSION:

Biochar characterization

The biochar characterization results are shown in Table S3. The ABC-biochar had a relatively high moisture content (14%, as expected for the steam-activated biochar), while its fixed carbon, ash, and volatile matter contents (82%, 11%, 7% on a dry weight basis, respectively) were in line with previously reported values for high-temperature, wood-based biochars (16). In contrast, the WF-biochar had an unusually high ash content (77%, dry) and very low fixed carbon content (15%). Analysis of WF-biochar from a package obtained from a separate order showed similar results. While high ash contents are generally considered undesirable for commercial biochars, the presence of alkaline chemical species could potentially be beneficial for remediating acidic soils (29). Results for pH indicate that the WF-biochar was circumneutral (pH = 7.17), while the ABC biochar was alkaline (pH = 9.67).

Technical data provided by Wakefield Biochar suggested that these characterization results were atypical, and thus may have been due to batch-to-batch variations that can arise during biochar production. For example, introducing oxygen or exceeding temperature or residence time specifications can cause partial combustion, leading to high ash contents and low carbon contents. The intraparticle pore volume data further support this reasoning. For example, the intraparticle pore volume for the ABC-biochar (0.44 cm^3/g cumulative pore volume) was relatively high (e.g., values ranging from 0.070–0.62 cm^3/g were reported for high-temperature wood-based biochars in a previous study (15)). In contrast, the intraparticle pore volume for the WF-biochar (0.072 cm^3/g) was very low, potentially because the intraparticle pore structure for the WB-biochar collapsed, as could be expected for a partially combusted, high-ash material. These results

suggest that the WF-biochar likely contained fewer intraparticle carbonaceous sorption sites available to facilitate hydrophobic interactions relative to the ABC-biochar.

Regarding elemental composition, ratios of H/C and O/C are indicative of aromatic and polar surface character, respectively (i.e., lower H/C indicates higher aromaticity, higher O/C indicates higher polarity). The presence of similar H/C (ABC: 0.042, WFB: 0.12) and O/C (ABC: 0.031, WFB: 0.047) ratios suggest that the carbonaceous portions of the materials may have had somewhat similar surface chemistries, though the ABC-biochar appeared to be slightly more hydrophobic (i.e., more aromatic, less polar). Therefore, though the WF-biochar likely contained few intraparticle carbonaceous sorption sites, the chemistry of available surface sites and hence their ability to facilitate hydrophobic interactions may have been similar between the two biochars. Considering that the two biochars have similar particle size ranges and hence similar external surface area, these results suggest that the ABC-biochar possessed properties that could be advantageous over the WF-biochar for sorption of small hydrophobic contaminants that can access intraparticle pore spaces (e.g., organic contaminants), but that the WF-biochar may still have capacity to facilitate hydrophobic interactions with larger species that interact primarily with external particle surfaces (e.g., *E. coli*), at least on a carbon-normalized basis.

DOC concentration and quality

Results for UVA 254, DOC, and SUVA analyses are shown in Figure 1. The influent UVA 254 values (Figure 1A) were higher during the first dosing period (conducted during the fall) than the second dosing period (conducted during the winter following a freeze-thaw cycle). The influent DOC concentration appeared to be relatively consistent throughout the experiment (65 ± 3 mg/L, n = 3), with DOC from leaf litter accounting for the majority of the overall DOC (creek water DOC was 13 ± 1 mg/L over three separate dosing tests). The cause for this apparent seasonal difference in the resulting influent DOC quality is unclear, though cooler water temperatures during the second dosing period may have affected how the DOC leached from the leaf litter. Figure 1B shows that effluent DOC concentrations were relatively consistent

throughout the experiment. Effluent DOC concentrations for the sand-only columns (55 ± 4 mg/L, n = 21) were 14% higher on average than those for the biochar-amended columns (ABC-biochar 47 ± 2 mg/L, WF-biochar 48 ± 4 mg/L, n = 21), which showed equivalent effluent DOC concentrations throughout the

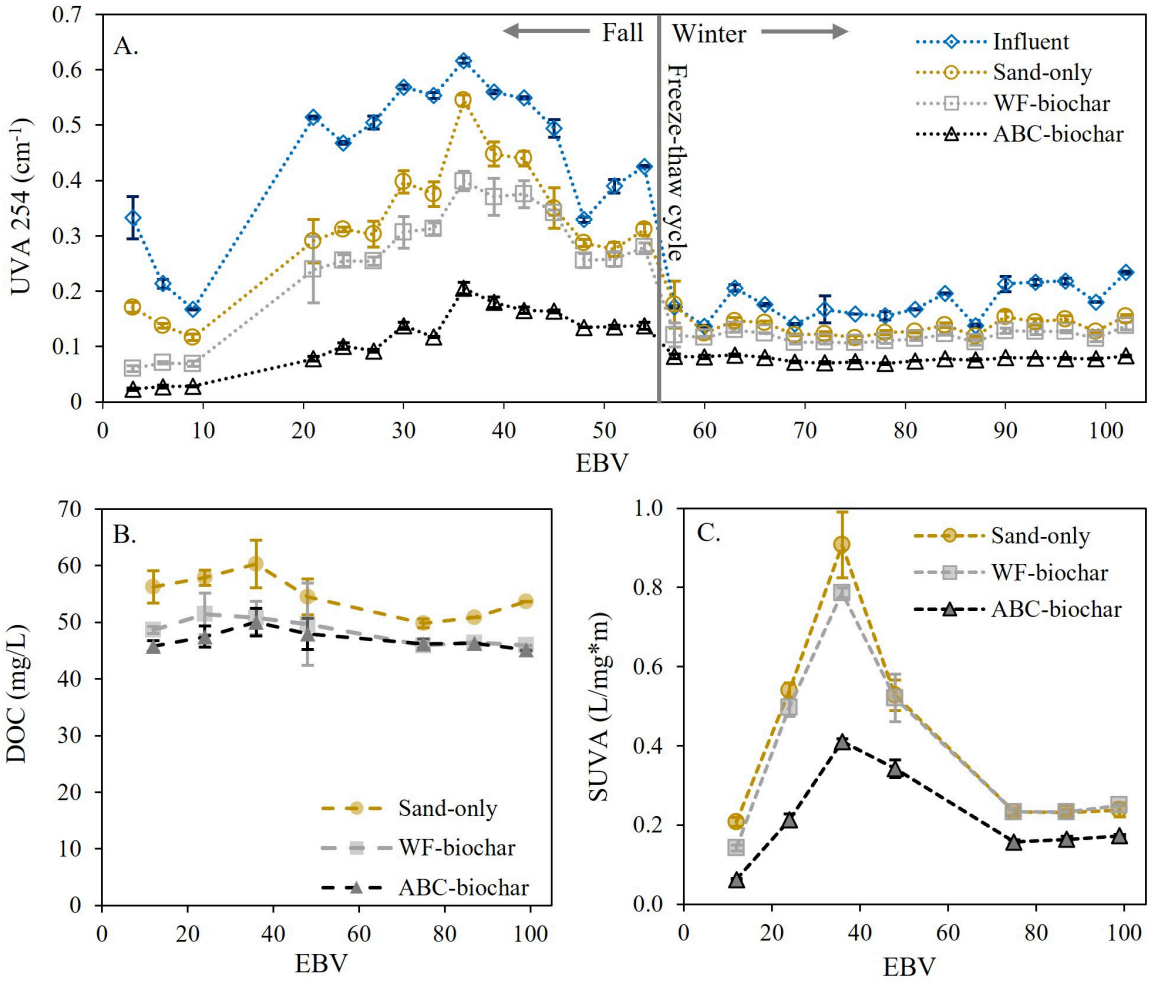

Figure 1. Results for influent and effluent UVA 254 from column dosing tests (A.); and DOC (B.) and SUVA (C.) results for column effluents from select dosing tests. Note that influent DOC concentrations were only measured during three dosing tests but appeared to be relatively consistent throughout the experiment (65 ± 3 mg/L). Dosing tests up to 54 EBVs were conducted in the fall, and tests for EBVs 57 and above were conducted in the winter following a freeze-thaw cycle.

experiment. This suggests that both biochars consistently retained the same proportion of DOC, or that any differences in DOC retention were balanced by leaching of DOC present on the biochars. However, there were clear differences in the effluent SUVA values between the biochar-amended columns (Figure 1C), as the ABC-biochar columns had much lower UVA 254 values than the WF-biochar columns.

These results suggest that though both biochars appeared to retain less than 20% of the total DOC, the ABC-biochar more effectively retained the aromatic portion of the DOC. This interpretation is consistent with the characterization results, as the ABC-biochar appeared to possess more intraparticle carbonaceous sorption sites in the micro- (< 2 nm) and mesoporous (2–50 nm) ranges that may be accessible to aromatic fulvic (0.2–1 nm) and humic (1–50 nm) acid components of the DOC (30). Overall, these findings demonstrate that differences in biochar properties may result in substantial differences in retention of aromatic DOC components, reflecting the potential utility of SUVA to probe for biochar properties that may enhance retention of hydrophobic contaminants.

Retention of organic contaminants

Data for all detectable influent and effluent concentrations for the five monitored organic contaminants are compiled in a box-and-whisker plot in Figure 2, and Figure S4 shows the organic contaminant concentrations versus EBV. In general, little-to-no retention of organic contaminants was observed for the sand-only columns, with a few exceptions. For example, for two dosing tests near the end of the experiment (treated EBVs = 90, 102) concentrations of methyl-benzotriazole in the sand-only effluents (4 ± 1 ng/mL, n = 6) were significantly lower than the influents (14 ± 2 ng/mL, n = 6, p = 1.8×10^{-5}, Figure S4A). This may have been due to either biodegradation (31) or sorption to biofilms that developed on the sand over time. Further, though DCPMU was not added to nor detected in the influent, it was detected in 54% of the sand-only effluent samples, with concentrations ranging up to 2.1 ± 0.6 ng/mL (Figure S4B). The highest concentrations were observed during the final dosing test from the first dosing period (i.e., at 54 treated EBVs), where diuron concentrations in the sand-only effluents (18 ± 1 ng/mL) were on average 26% lower than influent concentrations (25 ± 1 ng/mL). These results suggest that diuron biodegraded to form DCPMU within the sand-only columns, reflecting previous literature documenting aerobic biodegradation of diuron and accumulation of DCPMU in aerobic soils (32,33). Generation and release of DCPMU from biofiltration

systems is a potential concern, as diuron is widely present in urban runoff (8), and DCPMU is suspected to be more mobile in the environment and more toxic to aquatic organisms relative to diuron (34).

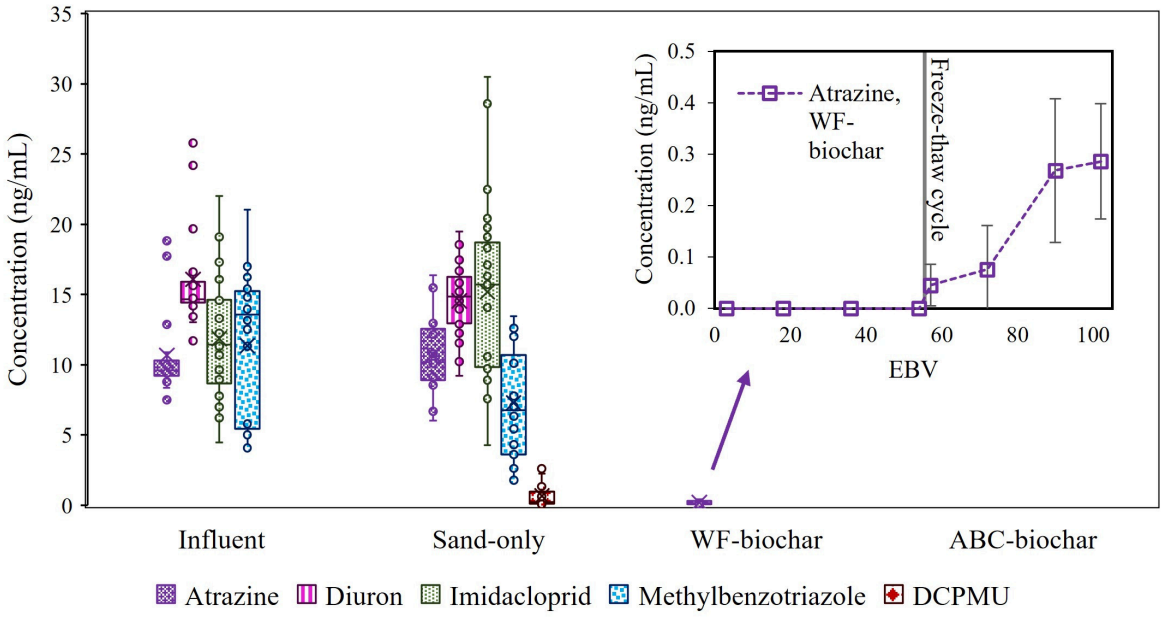

Figure 2. Box and whisker plot depicting concentrations of organic contaminants in column influent and effluent samples throughout the experiment (non-detects not included). Medians (horizontal lines) are included in the quartile calculations (whiskers), and "X" markers indicate mean values. No analytes were detected in the effluent samples from the ABC-biochar columns. The inset figure depicts the initial breakthrough of atrazine following the freeze-thaw cycle for the WF-biochar column.

Columns amended with both the WF-biochar and ABC-biochar demonstrated effective retention of organic contaminants throughout the first dosing period, during which no organic contaminants were detected in effluents from the biochar-amended columns. However, initial breakthrough of atrazine was observed for the WF-biochar columns during the dosing test immediately following the freeze-thaw cycle (57 EBVs, Figure 2, inset graphic), and effluent atrazine concentrations reached 0.3 ± 0.1 ng/mL (approximately 3% of the average influent concentration for atrazine) by the final dosing test (102 EBVs). No atrazine breakthrough was observed for the ABC-biochar columns, while no other organic contaminants were detected in the effluents of the biochar-amended columns.

These results suggest that disturbances to the WF-biochar columns caused by the freeze-thaw cycle may have facilitated the early breakthrough of atrazine. For example, soil expansion can cause deformation of

soil microstructures that may lead to channeling, which is associated with accelerated breakthrough (35). Further, a recent study showed that freeze-thaw cycles can accelerate the disintegration of biochar particles, leading to the release of dissolved and colloidal carbonaceous substances which may carry sorbed contaminants (36). These results further suggest that the retention capacity of the ABC-biochar for organic contaminants was more resilient to the disturbances caused by the freeze-thaw cycle relative to the WF-biochar. This may be attributed to a higher overall retention capacity for the ABC-biochar due to its higher carbon content and intraparticle pore volume, while the ABC-biochar also may have been less fragile than the WF-biochar and more resistant to disintegration. As SUVA results also indicated that the ABC-biochar showed improved retention of aromatic DOC components, these results further support the potential utility of SUVA as a probe for desirable biochar properties.

Retention of *E. coli*

Figure 3A shows the *E. coli* influent and effluent concentrations throughout the dosing experiment, with the grey-shaded areas indicating the three periods where *E. coli* levels were augmented in the influent (i.e., *E. coli* loading periods). Note that during dosing tests between *E. coli* loading periods, *E. coli* were present in the creek water during the fall months at concentrations up to approximately 3000 CFU/100 mL, while no *E. coli* were detected during winter months following the freeze-thaw cycle. Following the loading periods, *E. coli* concentrations in effluents from the biochar-amended columns appeared to recede somewhat more slowly relative to the sand-only columns, potentially due to release of initially retained *E. coli*. During the *E. coli* loading periods, effluent concentrations were lower than influent concentrations for all columns, indicating that all columns demonstrated some degree of *E. coli* retention.

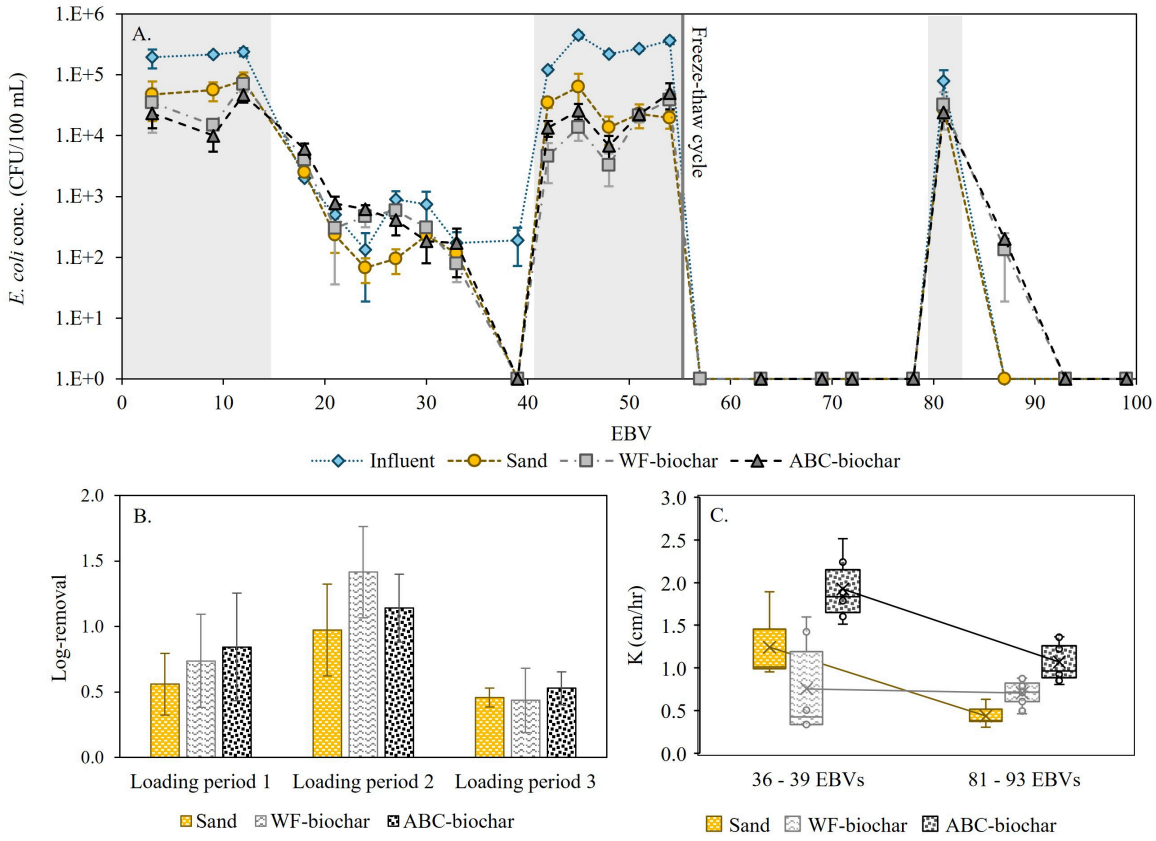

Figure 3. (A.) Concentrations of *E. coli* in the influent and effluents throughout the experiment. Grey-shaded areas indicate *E. coli* loading periods when E. coli concentrations were augmented in the influent. Instances where no colony forming units (CFU) were detected are reported as "1" to allow presentation on a log scale. (B.) Mean log-removal values during each loading period. (C.) Results from hydraulic conductivity tests conducted during before and after the freeze-thaw cycle.

During the first *E. coli* loading period (considering three of four dosing tests from 3 to 12 EBVs, as plates did not gel properly during the second dosing test) the ABC-biochar and WFB-biochar columns showed similar *E. coli* retention performance (Figure 3B, $p = 0.12$ for comparison for log-removal values). Though log-removal values were higher on average for the biochar-amened columns relative to the sand-only columns, the difference was not statistically significant when the biochar-amended columns were considered equivalent ($p = 0.06$, unequal variance). During the second loading period following conditioning with 40 EBVs, all columns achieved higher log-removal values relative to the first loading period, potentially due to biofilm development (for the sand-only columns) or increased straining due to compaction over time. The ABC-biochar and sand-only columns showed similar retention performance (p

= 0.19), while log-removal values were higher for the WFB-biochar columns (p = 0.003 and 0.02 for comparison to ABC-biochar and sand-only columns, respectively). While the third *E. coli* loading period following the freeze-thaw cycle only consisted of a single dosing test, *E. coli* retention was reduced by up to an order of magnitude relative to the second loading period. This may have been due to disturbances in the filter beds associated with the freeze-thaw cycle, reflecting observations of atrazine breakthrough for the WF-biochar columns.

Overall, while *E. coli* retention performance for all columns appeared to change throughout the dosing experiment, biochar-amended columns showed limited improvement in retention performance relative to sand-only columns. These results are somewhat in contrast to previous studies based on results from laboratory up-flow columns (7,16,19). Interestingly, a previous study also observed diminishing retention of *E. coli* during intermittent dosing with natural creek water, reporting no discernable improvements for biochar-amended columns by the end of a conditioning period (37). However, when *E. coli* concentrations were augmented during challenge tests via addition of wastewater, the *E. coli* retention capacity appeared to be restored. The authors suggested that this may have been due to differences in the behavior of *E. coli* indigenous to wastewater versus stormwater. While limited information is available regarding *E. coli* treatment performance for full-scale systems, particularly relative to control conditions without biochar, a recent study also reported little-to-no improvement in retention of *E. coli* from parking lot runoff in the presence of biochar (20).

Overall, these results, in combination with previous studies (20,37), suggest that biochar-enhanced *E. coli* retention observed in laboratory up-flow saturated columns may not necessarily translate to systems operating under gravity-driven intermittent flow conditions. For example, differences in hydraulic conditions may affect *E. coli* retention performance, as saturated column tests are often operated under controlled flow rates. While drainage rates for the columns evaluated here generally decreased throughout the experiment, the ABC-biochar columns consistently drained more quickly than the other columns (Figure 3C shows results for hydraulic conductivity measurements conducted before and after the free-thaw

cycle). Therefore, as the ABC-biochar columns achieved similar *E. coli* retention despite lower contacts times, the ABC-biochar appeared to improve the overall filtration function if both hydraulic and *E. coli* retention performance are taken into account.

E. coli retention may have also been affected by differences in water composition, as previous laboratory column studies have largely used laboratory-generated synthetic stormwater (prepared with deionized water salts to mimic typical ion concentrations, with or without added DOC), in contrast to the natural creek water used for this study. The presence of DOC in natural or synthetic waters can enhance the transport of bacteria through saturated porous media and hence reduce bacteria retention (38,39). Moreover, the use of natural water may have facilitated more rapid biofilm development, which also may have affected *E. coli* retention. For example, biofilms can fill pore spaces and decrease surface area available for interactions with *E. coli*. One study found that the presence of biochar caused more rapid biofilm growth relative to sand-only columns, but that biofilm development was associated with reduced *E. coli* retention for biochar-amended columns (40). In contrast, biofilm growth on sand-only columns has been associated with improved *E. coli* retention (41). These variable effects to *E. coli* retention have been attributed to differences in surface chemistry and associated impacts to hydrophobic interactions, as the biofilms were less hydrophobic than the biochar surfaces but more hydrophobic than the sand surfaces (40,42). Therefore, the biofilms that developed on the filter media for this study may have reduced the *E. coli* retention performance for the biochar-amended columns but enhanced performance for the sand columns. This interpretation is further supported by the *E. coli* concentration data in Figure 3B, as the differences in the *E. coli* retention from the sand-only columns between the first and second *E. coli* loading period may have been due in part to biochar development as the experiment progressed.

CONCLUSIONS:

Regarding retention of organic contaminants, the findings of this study demonstrate the importance of carefully considering biochar properties when selecting biochars to be incorporated into stormwater

treatment systems. Our results suggest that readily measurable properties of effluent DOC such as SUVA and UVA 254 may be useful to identify biochars that may be effective for retention of organic contaminants. For example, while direct measurement of organic contaminants is often cost-prohibitive, simple filtration tests to compare effluent UVA results among candidate materials could be useful as a screening measure during biochar selection. This has practical implications, considering the wide variability of biochar quality among suppliers and on a batch-to-batch basis, and that available technical data may not reflect the full variability of the biochar product.

However, we observed limited improvement in *E. coli* retention that could be attributed to the presence of biochar, suggesting that the benefit of biochar amendment for retention of *E. coli* may be diminished under more environmentally relevant conditions representative of passive filtration systems (i.e., intermittent, gravity-flow conditions, presence of DOC and biofilms). This prospect has notable practical implications, as municipalities have begun to adapt biochar-amended filtration systems as solutions to *E. coli* pollution in watersheds, and biochar amendment represents an additional cost to implementation. However, as amendment with the ABC-biochar appeared to improve hydraulic performance and retention of organic contaminants, it is likely that amendment of field-scale filtration systems with biochar still provides substantial treatment benefits, though these benefits may not necessarily be related to the intended function of the filter. Overall, the findings of this study warrant more rigorous verification of the effects of biochar amendment to filtration performance under environmentally relevant conditions, particularly regarding field-scale systems in comparison to control conditions without biochar.

ACKNOWLEDGEMENTS: This project was supported by the Minnesota Stormwater Research and Technology Transfer Program administered by the University of Minnesota Water Resources Center. Financial support was provided through an appropriation from the Clean Water Fund established by Minnesota Clean Water Land and Legacy Amendment and from the Minnesota Stormwater Research Council (MSRC/WRC award # 2022-DP203). The authors graciously acknowledge the contributions of NRRI staff and students, without whom this study would have not been possible. We acknowledge

undergraduate student Riley Brunner for assistance with performing the dosing experiments, and staff at the NRRI Central Analytical Laboratory for performing DOC analyses. We also thank Brian Barry and Alexey Kacharov of the Chemistry and Materials team for performing the gas adsorption analyses, as well as Chan Lan Chun and Katie Edblad for providing guidance regarding *E. coli* incubations and dilutions.

REFERENCES:

1. Müller A, Österlund H, Marsalek J, Viklander M. The pollution conveyed by urban runoff: A review of sources. Science of The Total Environment. 2020 Mar;709:136125.

2. Dhakal KP, Chevalier LR. Urban Stormwater Governance: The Need for a Paradigm Shift. Environmental Management. 2016 May;57(5):1112–24.

3. Grebel JE, Mohanty SK, Torkelson AA, Boehm AB, Higgins CP, Maxwell RM, et al. Engineered Infiltration Systems for Urban Stormwater Reclamation. Environmental Engineering Science. 2013 Aug;30(8):437–54.

4. Tiefenthaler L, Stein ED, Schiff KC. Levels and patterns of fecal indicator bacteria in stormwater runoff from homogenous land use sites and urban watersheds. Journal of Water and Health. 2011 Jun 1;9(2):279–90.

5. Hathaway JM, Hunt WF, Wright JD, Jadlocki SJ. Field Evaluation of Indicator Bacteria Removal by Stormwater BMPs in North Carolina. In: World Environmental and Water Resources Congress 2009 [Internet]. Kansas City, Missouri, United States: American Society of Civil Engineers; 2009 [cited 2024 Nov 12]. p. 1–10. Available from: http://ascelibrary.org/doi/10.1061/41036%28342%29112

6. Ahmed W, Hamilton K, Toze S, Cook S, Page D. A review on microbial contaminants in stormwater runoff and outfalls: Potential health risks and mitigation strategies. Science of The Total Environment. 2019 Nov;692:1304–21.

7. Mohanty SK, Boehm AB. *Escherichia coli* Removal in Biochar-Augmented Biofilter: Effect of Infiltration Rate, Initial Bacterial Concentration, Biochar Particle Size, and Presence of Compost. Environ Sci Technol. 2014 Oct 7;48(19):11535–42.

8. Masoner JR, Kolpin DW, Cozzarelli IM, Barber LB, Burden DS, Foreman WT, et al. Urban Stormwater: An Overlooked Pathway of Extensive Mixed Contaminants to Surface and Groundwaters in the United States. Environ Sci Technol. 2019 Sep 3;53(17):10070–81.

9. Zhang K, Randelovic A, Page D, McCarthy DT, Deletic A. The validation of stormwater biofilters for micropollutant removal using in situ challenge tests. Ecological Engineering. 2014 Jun;67:1–10.

10. Spokas KA, Cantrell KB, Novak JM, Archer DW, Ippolito JA, Collins HP, et al. Biochar: A Synthesis of Its Agronomic Impact beyond Carbon Sequestration. J Environ Qual. 2012 Jul;41(4):973–89.

11. Tripathi M, Sahu JN, Ganesan P. Effect of process parameters on production of biochar from biomass waste through pyrolysis: A review. Renewable and Sustainable Energy Reviews. 2016 Mar;55:467–81.

12. He X, Liu Z, Niu W, Yang L, Zhou T, Qin D, et al. Effects of pyrolysis temperature on the physicochemical properties of gas and biochar obtained from pyrolysis of crop residues. Energy. 2018 Jan;143:746–56.

13. Glaser B, Parr M, Braun C, Kopolo G. Biochar is carbon negative. Nature Geosci. 2009 Jan;2(1):2–2.

14. Ulrich BA, Loehnert M, Higgins CP. Improved contaminant removal in vegetated stormwater biofilters amended with biochar. Environ Sci: Water Res Technol. 2017;3(4):726–34.

15. Ulrich BA, Im EA, Werner D, Higgins CP. Biochar and Activated Carbon for Enhanced Trace Organic Contaminant Retention in Stormwater Infiltration Systems. Environ Sci Technol. 2015 May 19;49(10):6222–30.

16. Valenca R, Borthakur A, Zu Y, Matthiesen EA, Stenstrom MK, Mohanty SK. Biochar Selection for *Escherichia coli* Removal in Stormwater Biofilters. J Environ Eng. 2021 Feb;147(2):06020005.

17. Tomczyk A, Sokołowska Z, Boguta P. Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. Rev Environ Sci Biotechnol. 2020 Mar;19(1):191–215.

18. Chen B, Zhou D, Zhu L. Transitional Adsorption and Partition of Nonpolar and Polar Aromatic Contaminants by Biochars of Pine Needles with Different Pyrolytic Temperatures. Environ Sci Technol. 2008 Jul 1;42(14):5137–43.

19. Mohanty SK, Cantrell KB, Nelson KL, Boehm AB. Efficacy of biochar to remove Escherichia coli from stormwater under steady and intermittent flow. Water Research. 2014 Sep;61:288–96.

20. Ulrich BA, Weelborg K, Haile TM, Singh UB, Magner J. Field evaluation of a biochar-amended stormwater filtration system for retention of nutrients, metals, and *Escherichia coli*. Environ Sci: Water Res Technol. 2024;10(10):2546–58.

21. Naked Char Activated Biochar Technical Data Sheet [Internet]. [cited 2024 Nov 11]. Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://blazing-star.com/wp-content/uploads/NAKED-Char-TDS.pdf

22. Wakefield Biochar — organic soil conditioner for outdoor and indoor plants, gardens and lawns. [Internet]. [cited 2024 Nov 11]. Available from: https://wakefieldbiochar.com/products/premium-biochar-soil-conditioner/

23. Li X, Shen Q, Zhang D, Mei X, Ran W, Xu Y, et al. Functional Groups Determine Biochar Properties (pH and EC) as Studied by Two-Dimensional 13C NMR Correlation Spectroscopy. Motta A, editor. PLoS ONE. 2013 Jun 19;8(6):e65949.

24. Jagiello J, Ania C, Parra JB, Cook C. Dual gas analysis of microporous carbons using 2D-NLDFT heterogeneous surface model and combined adsorption data of N2 and CO2. Carbon. 2015 Sep;91:330–7.

25. Sigmund G, Hüffer T, Hofmann T, Kah M. Biochar total surface area and total pore volume determined by N2 and CO2 physisorption are strongly influenced by degassing temperature. Science of The Total Environment. 2017 Feb;580:770–5.

26. Ulrich BA, Vignola M, Edgehouse K, Werner D, Higgins CP. Organic Carbon Amendments for Enhanced Biological Attenuation of Trace Organic Contaminants in Biochar-Amended Stormwater Biofilters. Environ Sci Technol. 2017 Aug 15;51(16):9184–93.

27. Spahr S, Teixidó M, Gall SS, Pritchard JC, Hagemann N, Helmreich B, et al. Performance of biochars for the elimination of trace organic contaminants and metals from urban stormwater. Environ Sci: Water Res Technol. 2022;8(6):1287–99.

28. Erickson AJ, Gulliver JS, Weiss PT. Capturing phosphates with iron enhanced sand filtration. Water Research. 2012 Jun;46(9):3032–42.

29. Liao W, Thomas SC. Biochar Particle Size and Post-Pyrolysis Mechanical Processing Affect Soil pH, Water Retention Capacity, and Plant Performance. Soil Systems. 2019 Feb 14;3(1):14.

30. Gaffney JS, Marley NA, Clark SB, editors. Humic and Fulvic Acids: Isolation, Structure, and Environmental Role [Internet]. Washington, DC: American Chemical Society; 1996 [cited 2024 Jul 9]. (ACS Symposium Series; vol. 651). Available from: https://pubs.acs.org/doi/book/10.1021/bk-1996-0651

31. Abu-Dalo MA, O'Brien I, Hernandez MT. Effects of Substitutions on the Biodegradation Potential of Benzotriazole Derivatives. IOP Conf Ser: Mater Sci Eng. 2018 Feb;305:012020.

32. Egea TC, Da Silva R, Boscolo M, Rigonato J, Monteiro DA, Grünig D, et al. Diuron degradation by bacteria from soil of sugarcane crops. Heliyon. 2017 Dec;3(12):e00471.

33. Li J, Zhang W, Lin Z, Huang Y, Bhatt P, Chen S. Emerging Strategies for the Bioremediation of the Phenylurea Herbicide Diuron. Front Microbiol. 2021 Aug 12;12:686509.

34. Tixier C, Bogaerts P, Sancelme M, Bonnemoy F, Twagilimana L, Cuer A, et al. Fungal biodegradation of a phenylurea herbicide, diuron?:?structure and toxicity of metabolites. Pest Manag Sci. 2000 May;56(5):455–62.

35. Li T, Kong L, Guo A. The deformation and microstructure characteristics of expansive soil under freeze–thaw cycles with loads. Cold Regions Science and Technology. 2021 Dec;192:103393.

36. Zhu L, Chen N, Zhang X, Ren L, Zou R, Xie J, et al. Freeze–Thaw Cycle Events Enable the Deep Disintegration of Biochar: Release of Dissolved Black Carbon and Its Structural-Dependent Carbon Sequestration Capacity. Environ Sci Technol. 2024 Nov 14;acs.est.4c07262.

37. Kranner BP, Afrooz ARMN, Fitzgerald NJM, Boehm AB. Fecal indicator bacteria and virus removal in stormwater biofilters: Effects of biochar, media saturation, and field conditioning. Paz-Ferreiro J, editor. PLoS ONE. 2019 Sep 25;14(9):e0222719.

38. Pieper AP, Ryan JN, Harvey RW, Amy GL, Illangasekare TH, Metge DW. Transport and Recovery of Bacteriophage PRD1 in a Sand and Gravel Aquifer: Effect of Sewage-Derived Organic Matter. Environ Sci Technol. 1997 Apr 1;31(4):1163–70.

518 39. Johnson WT, Logan BE. Enhanced Transport of Bacteria in Porous Media by Sediment-Phase and
519 Aqueous-Phase Natural Organic Matter. Water Res. 30(4):923–31.

520 40. Afrooz ARMN, Boehm AB. Escherichia coli Removal in Biochar-Modified Biofilters: Effects of
521 Biofilm. Nerenberg R, editor. PLoS ONE. 2016 Dec 1;11(12):e0167489.

522 41. Liu Y, Li J. Role of Pseudomonas aeruginosa Biofilm in the Initial Adhesion, Growth and
523 Detachment of Escherichia coli in Porous Media. Environ Sci Technol. 2008 Jan 1;42(2):443–9.

524 42. Janjaroen D, Ling F, Monroy G, Derlon N, Mogenroth E, Boppart SA, et al. Roles of ionic strength
525 and biofilm roughness on adhesion kinetics of Escherichia coli onto groundwater biofilm grown on
526 PVC surfaces. Water Research. 2013 May;47(7):2531–42.

527

528