Biochar role in improving pathogens removal capacity of stormwater biofilters

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Contents

Abstract

Stormwater treatment systems such as biofilters have been used to treat and reuse stormwater in water-stressed urban areas. However, the pathogen removal capacity of these systems is low and unreliable. Pathogens are difficult to remove because of many reasons: conventional biofilter amendments have low removal capacity, and previously removed pathogens can grow in biofilters or be remobilized during intermittent infiltration of stormwater. Variable climate affects removal and increases uncertainty to

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biofilter performance. Adding biochar to biofilter media can help overcome some of these challenges. Biochar removes pathogens because of hydrophobic interaction and straining, limits remobilization of previously attached bacteria during intermittent flow by increasing residual moisture content, and provides conditions for native microbial communities to strive and out-compete pathogens for nutrients. However, all biochars are not made equal. Thus, bacterial removal capacity varies with biochar properties: removal increases with surface area and fixed carbon content and decreases with volatile matter and ash content. Additionally, the removal efficiency also depends on biochar size and how they are applied such as the presence of compost and compaction conditions. Collectively, these results indicate that biochar with specific properties and application methods can effectively increase the pathogen removal capacity of biofilters in variable climate conditions.

Keywords: Biochar, Fecal indicator bacteria, Microbe, Water scarcity, Resilience, Runoff, Green infrastructure

1. Introduction

Groundwater and surface waters provide most of the water needs in public, industrial, and agricultural sectors.[1,2] However, rapid urbanization and climate change have depleted these water resources and exacerbated water scarcity issues. To alleviate the water deficit, the use of nontraditional water resources such as stormwater has been explored. In most places, gray infrastructures such as concrete canals and pipes have been used to convey stormwater rapidly to minimize flooding. In contrast, green infrastructures are designed to increase infiltration and minimize flooding.[3,4] Among the different types of stormwater treatment systems, infiltration-based systems such as biofilters are popular because of their low footprint and better pollutant removal performance than other GIs.[5] Biofilter consists of a planted top, filter layers, and a drainage layer; all layers serve different functions for pollutant removal (Fig. 1). Biofilters are good at removing suspended sediments but have limited capacity to remove dissolved pollutants including nutrients, some heavy metals, trace organics, and pathogens.[6,7] Among all the pollutants, pathogens or bacterial pathogens are the most difficult to remove because of their small size, persistence, and proliferation inside the stormwater treatment systems.[8,9] Bacterial pathogens can grow in biofilter media due to the presence of nutrients and detach from filter media during intermittent infiltration of stormwater, particularly during the first flush.[10] However, the addition of amendments to filter media can increase removal by adsorption, inactivation, and straining.[11,12]

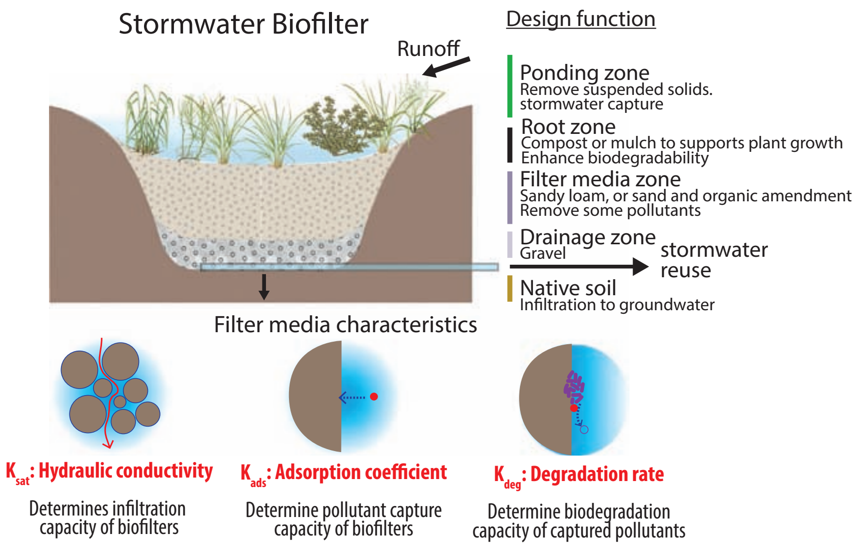

Fig. 1 Schematic of a traditional stormwater biofilter and functions of different components of biofilters and the filter media. *Reprinted and adapted from Mohanty SK, Valenca R, Berger AW, Yu IKM, Xiong X, Saunders TM, et al. Plenty of room for carbon on the ground: Potential applications of biochar for stormwater treatment. Sci Total Environ, 625:1644–1658, Copyright (2018), with permission from Elsevier.*

Amendments for biofilters can be chosen based on three properties, which indicate three unique functions: hydraulic conductivity (K_{sat}) to increase infiltration, adsorption capacity (K_{ads}) to increase pollutant removal from infiltrating water, and biodegradation capacity (K_{deg}) to biologically destroy adsorbed pollutants and recharge the adsorption capacity of the amendment. Normally, the particle size distribution of amendment affects hydraulic conductivity.[13] Compaction could also lower hydraulic conductivity.[14,15] Thus, bulking agents such as coarse sand is used as an amendment to increase infiltration and alleviate compaction with time. An increase in hydraulic conductivity of filter media increases the volume of stormwater infiltrated but it can also minimize the contact time of pollutants with the amendment, thereby reducing treatment of pollutants that exhibit slow removal kinetics.[16] The extent to which filter media could remove pollutants depends on the hydrophobic interaction,[17] cation exchange,[18] or electrostatic attraction.[19] Thus, surface area,[20] surface charge,[21] cation exchange capacity,[22] and organic carbon fractions[23] are used to predict the adsorption capacity of amendments. Natural microorganisms could degrade the pollutants and recharge the surface properties of the amendment.[24] Thus, amendments such as compost or mulch that provide an adequate environment for microbial growth are used to enhance biodegradation,[24] although they may export nutrients to effluent.[25]

One amendment may not fit all the criteria, so it is typical to mix amendments in biofilters to serve different functions.[11] Among different types of engineered-geomedia, biochar has been used in stormwater treatment systems because they can be produced from raw or waste biomass at any place[26] and remove a wide range of pollutants.[27,28] By comparing 6 peer-reviewed studies that investigated pathogens removal using biochar-augmented biofilters[29–34] against typical biofilters constructed without biochar that were reported on the BMP Database,[35] we show that the addition of biochar to biofilters significantly ($P < 0.05$) increase the removal of pathogens (Fig. 2). However, not all biochar is made equal. The removal can vary widely based on biochar properties,[36] design,[37] and conditions at the site.[38] Thus, it is critical to understand why and how biochar improves pathogen removal from stormwater. This chapter describes recent advances in understanding how biochar improves pathogen removal in stormwater treatment systems.

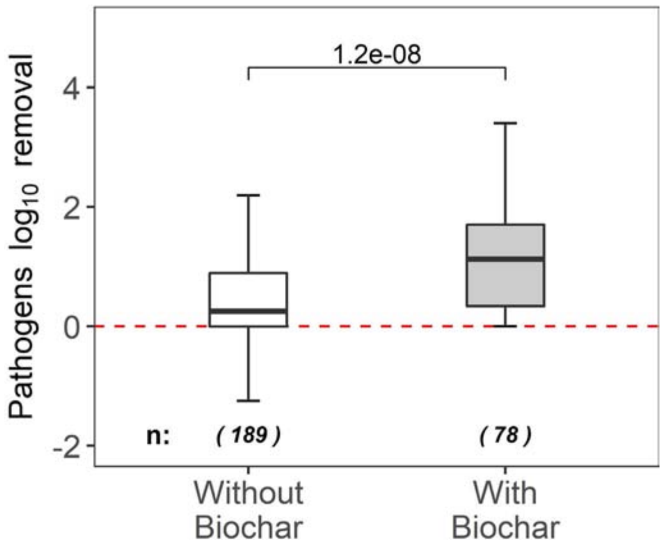

Fig. 2 Removal of varying pathogens using biofiltration systems built with and without the addition of biochar. The horizontal dashed red line indicates no removal of pathogens. Negative values of $\log_{10}$ removal values indicate that biofilters are a source of pathogens, while positive values indicate net removal of pathogens. The number of observations of each boxplot (n-value) is represented between parenthesis below the boxplot. Statistical analysis was conducted through the Wilcoxon rank-sum test and is indicated above the boxplot.

2. Testing methods to evaluate biochar capacity to remove pathogens in stormwater

The capacity of biochar to remove pathogens from stormwater is typically assessed by conducting bench-top column experiments under controlled conditions (Table 1). These flow-through column studies are designed to simulate stormwater infiltration in nature,[33] whereas batch experiments are useful to understand how changes in surface or water chemistry can affect maximum sorption capacity or inactivation of pathogens.[49] The capacity of biochar to remove pollutants has been tested without mixing it with other amendments[41,42,45] or with other media in layered and mixture configurations.[37,42,46] The typical application rate for biochar varies between 2% to 33% by volume with soil or sand. The mixture is packed in columns with a diameter ranging from 1.0 cm[32] to 15 cm.[46] The height of the geomedia may vary from 4.5 cm[29] to 180 cm.[42] The depth of the amended layer in the field is typically 45 cm. Thus, the laboratory setup can simulate the designed depth of biofilters. In field studies, rectangular plots are used.[30,37] The mixture is gently packed in biofilters to prevent breaking. However, in some cases such as roadside biofilters, compaction may be necessary or required for soil stability.[14,15] The columns packed with biochar in laboratory are typically subjected to intermittent infiltration of synthetic or natural stormwater pre-contaminated with pathogens or fecal indicator bacteria with concentrations varying from 10^2 to 10^8 colony forming units (CFU) per milliliter. High influent concentration in the laboratory is necessary to determine maximum adsorption capacity. Synthetic stormwater provides greater control on conditions, whereas natural stormwater is useful to form biofilm in the biofilters.[44] Stormwater can be applied on the top of filter media to ensure downward flow by gravity or injected from the bottom with upward flow through the column to simulate saturated flow. While downward flow mimics the flow of stormwater runoff in real-world conditions, upward flow is often adequate to determine maximum removal capacity.[10] Unsaturated flow, which occurs when stormwater is applied from the top, causes underutilization of geomedia[50] as the presence of air and air-water interface may affect bacterial adsorption on biochar.[39] While laboratory column experiments provide an estimate of the biochar's performance in removing pathogens, they do not simulate many real-world field conditions such as variation in stormwater chemistry, influent pathogen concentration, and changing weather patterns such as dry-wet cycles, intense rainfall, and hot climate. These field conditions either reduce[40] or improve the removal of bacteria by biochar.[37]

Table 1 Summary of flow-through column studies that examined the potential of biochar in removing pathogens from stormwater.

Biochar characteristics			Influent water chemistry			Column/Biofilter Setup				
Feedstock	Pyrolysis (°C)	Addition (%)	Type	Pathogen	CFU/mL	Layered or mixed	Internal diameter (cm)	Media Height (cm)	Removal range (%)	References
Poultry litter	350	2 (w/w)	Synthetic stormwater	*E. coli*	1.0×10^7	mix	2.5	10	0–99.9	31
	700									
Pine chips	350									
	700									
Hardwood	500	5–25	Natural wastewater	*Total coliform*	2.6×10^6	mix	50 × 50 rectangular	65	88.7–99.8	30
Wood dust	300	2 (w/w)	Natural stormwater	*E. coli*	10^5	mix	1	10	25.6–87.1	32
	500									
	700									
Softwood	900	30 (v/v)	Synthetic stormwater	*E. coli*	10^3–10^7	mix	2.5	15	61.8–95.2	34
Macadamia Shell	450	10 (w/w)	Synthetic stormwater	*E. coli*	10^7	mix	1.9	5	5.3–67.8	34
Oil Mallee	450									
Phragmites Reed	460									
Rice Husk	650									
Wheat Chaff	550									

Pine wood	350	1–20 (w/w)	Synthetic stormwater	*E. coli*	1.2×10^{8}	mix	NA	4.5	10–80	29
	600									
Pine bark	350									
	600									
Softwood	900	5 (w/w)	Synthetic stormwater	*E. coli*	10^{5}	mix	2.5	17	80–99.9	39
Sonoma Biochar	350	5 (w/w)	Synthetic stormwater	*E. coli*	$1.2–1.7 \times 10^{6}$	mix	2.5	15	83–99.9	38
	700									
Wood chips	350									
	700									
Mix of Monterey pine, Eucalyptus, Bay Laurel, Hardwood and Softwood	395	30 (v/v)	Synthetic stormwater	*E. coli, Staph, Salmonella, Bacteriophage MS2*	10^{5}	mix	2.5	15	94.9–99.9	17
Mix of Monterey pine, Eucalyptus, Bay Laurel, Hardwood and Softwood	395	30 (v/v)	Synthetic stormwater	*E. coli*	$1.5–5.3 \times 10^{5}$	mix	2.5	10	71.8–97.6	40

Continued

Table 1 Summary of flow-through column studies that examined the potential of biochar in removing pathogens from stormwater.—cont'd

Biochar characteristics			Influent water chemistry			Column/Biofilter Setup				
Feedstock	**Pyrolysis (°C)**	**Addition (%)**	**Type**	**Pathogen**	**CFU/mL**	**Layered or mixed**	**Internal diameter (cm)**	**Media Height (cm)**	**Removal range (%)**	**References**
Hardwood	NA	100	Natural wastewater	*E. coli, Enterococci spp, Bacteriophage MS2, Bacteriophage* ϕ*X174, Saccharomyces cerevisiae*	10^2–10^3	NA	7.5	60	20–99.9	41
Softwood	700	100	Natural wastewater	*E. coli, Enterococci*	1.9×10^4	layer	5	180	79–95.2	42
Acacia confuse and *Celtis sinensis* and chemically modified biochars	700	5 (w/w)	Synthetic stormwater	*E. coli*	0.3–3.2 × 106	mix	2.5	15	87.9–99.8	43
Softwood	900	5 (w/w)	Natural stormwater	*E. coli*	10^6	mix	5.1	30.5	93–99.9	14
Softwood	900	15 (v/v)	Natural stormwater	*E. coli*	10^5	mix	5.1	30.4	60–99.9	15
Oak hardwood	540	30 (v/v)	Synthetic stormwater	*E. coli*	10^5	mix	2.54	30	87–99.9	36
Wood-based	550									
Yellow pine	990									
Softwood	900									

Mix of Monterey pine, Eucalyptus, Bay Laurel, Hardwood and Softwood	395	30 (v/v)	Natural stormwater	*E. coli, Enterococci*	$1.5–5.5 \times 10^{4}$	mix	2.5	15	74–94.9	44
Carbon Terra GmbH	NA	100	Natural wastewater	*E. coli, Total coliform*	8.5×10^{7}	NA	14	60	99–99.5	45
Mix of Monterey pine, Eucalyptus, Bay Laurel, Hardwood and Softwood	395	33.3 (v/v)	Natural stormwater	*E. coli, Enterococci, F+ coliphage*	7.8×10^{4}	layer	50 × 40 rectangular	30	52–99	37
Pinewood	NA	33 (v/v)	Natural stormwater	*E. coli, Total coliform*	$0.1–5.1 \times 10^{4}$	layer	15.2	50	43–93.7	46
Poultry litter	350 700	2–10 (w/w)	Synthetic stormwater	*E. coli*	1.3×10^{7}	mix	2.5	10	9.7–49	47
Wood derived	900 550	30 (v/v)	Synthetic stormwater	*E. coli*	2.2×10^{7}	mix	7.2	30	99–100	22
Waste wood pellets	520	100	Synthetic stormwater	*E. coli*	$0.1–4.7 \times 10^{5}$	layer	7.0	23	20–25	48

3. Pathogen removal processes in biochar-amended filter media

Biochars are porous materials with high surface area, but their surface properties can vary widely. The removal of pathogens by biochars depends on the biochar's characteristics as depicted in Fig. 3. The detail of how biochar enables each process is described in the sections below.

3.1 Attachment and straining

Bacteria can be removed by filter media initially by a reversible step governed by weak forces such as van der Waals, electrostatic, and hydrophobic interactions, followed by an irreversible second step that involves direct attachment of bacteria wall or flagella to the surfaces.[51] The surfaces of the

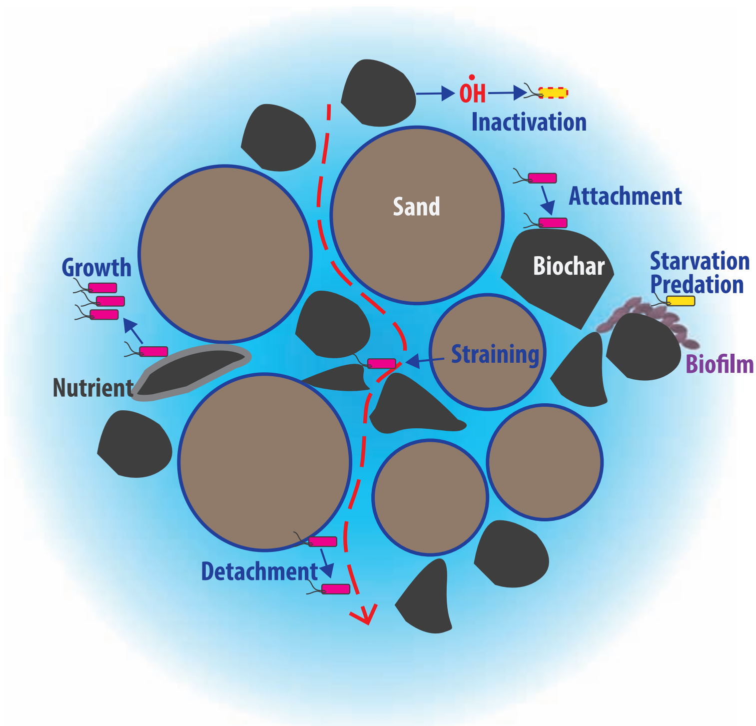

Fig. 3 Mechanisms related to the fate of pathogens in biochar-amended filter media: straining, attachment, detachment, growth, inactivation, predation, and biofilm development.

bacterial cell and the filter media must have opposite charges for electrostatic attraction to be relevant. The biochar surface has hydroxyl and carboxylic acid groups, along with phenolic, quinones, and condensed aromatics groups that make the biochar's surface a net-negative surface.[27] This results in net electrostatic repulsion. Dissolved ions from salts can mask the surface charge and lower the repulsion. Biochar can also increase removal by straining. Grain-to-grain interaction and surface roughness can affect bacterial removal by straining.[34] The extent to which straining is relevant depends on biochar's grain size, roughness, and media porosity along with the size and concentration of bacteria.[52] Biochar's grain size can vary widely based on feedstock size.[53,54] Smaller grain size is helpful to improve bacterial removal by straining. For instance, Mohanty and Boehm[33] examined the removal of *E. coli* in three different types of biofilters: (a) sand-only, (b) sand-biochar, and (c) sand-biochar without fine ($<125\,\mu m$) particles (Fig. 4). They concluded that the addition of biochar to sand increased the removal of *E. coli* significantly when compared to sand-only columns. However, the removal of fine biochar particles ($<125\,\mu m$) increased the transport of *E. coli* in biofilters potentially due to a decrease in straining and surface area available for sorption.[33]

3.2 Die-off and inactivation

Biochar not only removes pathogens from infiltrating stormwater but also affects the fate of removed pathogens. For instance, biochar could prevent the growth of bacteria inside stormwater biofilters.[38] Biochar's feedstock type and pyrolysis temperature can affect the extent to which biochar can prevent pathogen growth. Depending on biochar's pyrolysis temperature, biochar may disrupt the communication between growing bacterial cells by inhibiting the signal of acyl-homoserine lactone which regulates gene expression and alter the extent to which biofilm can form.[55] Biochar could support diverse microbial communities,[56] which can inactivate or kill pathogens via starvation or predation.[57] On the other hand, biochar may induce the growth of bacteria by providing nutrients such as phosphate,[58] although the extent of the growth depends on biochar's feedstock type and biochar properties.[59] A previous study showed that ash in biochar could suppress the growth of previously removed *E. coli* between rainfall events.[36] In between rainfall events, biochar could adsorb more *E. coli* due to an increase in residence time[38] or help inactivate *E. coli*.[60] Biochar could also adsorb metabolites produced by *E. coli*,[56] thereby limiting bacterial growth.

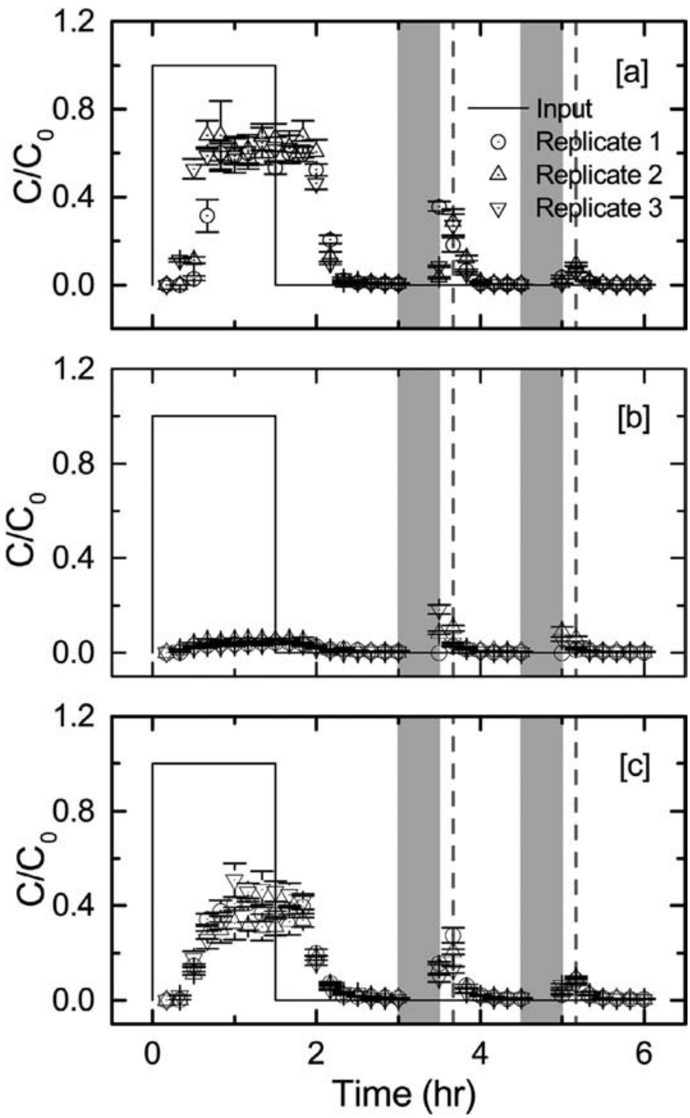

Fig. 4 Transport and mobilization of *E. coli* through columns packed with (a) sand, (b) mixture of sand and biochar, and (c) mixture of sand and biochar where biochar particles smaller than 125 μm were removed. The gray area indicates the 0.5 h pause during which the column was drained, and the dashed lines indicate the timing of the first samples after the pause. The error bar indicates one standard deviation of measurements. *Reprinted (adapted) with permission from 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;48(19): 11535–11542. Copyright (2021) American Chemical Society.*

However, it is expected that a longer duration between rainfall events would allow the bacteria to grow on carbon adsorbent utilizing nutrients in the infiltrating water.[61–63] Excess of bacterial growth[38] and mobilization of bacteria during intermittent infiltration events[10,39] can result in negative removal or net export of indicator bacteria from biofilters.[28] Because biochar can reduce the availability of growth metabolites[56] and remove bacteria by inactivation[64] and adsorption,[38] an addition of biochar to stormwater biofilters would decrease the growth or kill pathogens between rainfall events. But these processes can vary with biochar types. To examine how different biochars may inactivate pathogens during intermittent rainfall, Valenca, Borthakur[36] tested 4 types of biochar and found that *E. coli* did not grow inside the biofilters despite the presence of nutrients. The result indicates that biochar may continue removing pathogens through inactivation, starvation, or predation in between rainfall events (Fig. 5).

Biochar can also remove pathogens by inactivation. Biochar surface can produce hydroxyl radical through the reduction of oxygen and the oxidization of phenolic hydroxyl groups on biochar.[65] These radicals can kill pathogens by compromising the cell wall.[60] Bacterial cell wall properties can affect the inactivation rate. While gram-positive bacteria cell wall is composed of a thick but simple peptidoglycan layer, the cell wall of gram-negative bacteria is composed of a multi-layer of lipid, membrane, and peptidoglycan.

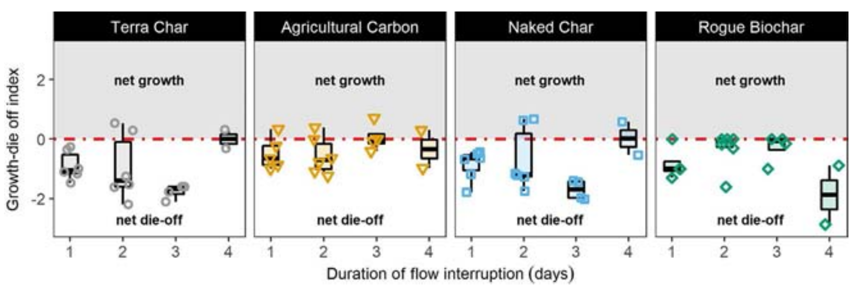

Fig. 5 Growth-die off index (GDI) of filter media as a function of drying duration between infiltration events. GDI was calculated as $-\log_{10}(C_b/C_a)$, where C_b and C_a represent the concentration of *E. coli* in the effluent before and after flow interruption, respectively. Positive GDI values (gray shaded area) represent net-growth of bacteria during flow interruption, while negative GDI values represent net die-off (or decay) or bacteria. *Republished with permission from Valenca, R. et al., Biochar selection for* Escherichia coli *removal in stormwater biofilters, Am Soc Civil Eng, 2021:147(2); permission conveyed through Copyright Clearance Center, Inc.*

3.3 Low remobilization during intermittent flow

Biochar removal capacity can decrease if some of the attached bacteria are remobilized during the infiltration of stormwater. In fact, intermittent infiltration of stormwater is shown to increase mobilization of attached bacteria from conventional filter media because of an enhanced detachment of bacteria by moving air-water interfaces.[10] Previous studies show that biochar lowers the remobilization of previously attached bacteria by keeping the biofilter moist and increasing the strength of bacterial binding to filter media.[38] The authors analyzed the effect of flow interruption (0.5 h and 21 h) on the remobilization of *E. coli* using sand-only and sand-biochar columns (Fig. 6) and showed that, while sand-only columns remobilized between 10% and 20% of attached *E. coli*, sand-biochar columns remobilized less than 0.1% of attached *E. coli*. However, the presence of natural organic matter (NOM) increased the remobilization of *E. coli* in both columns. The mobilization of bacteria may be enhanced if biochar particles are broken or mobilized and if particulate organic matters are released carrying bacteria.[66] In addition, NOM may compete for the attachment sites and provides a physical barrier for bacteria to access the sites on biochar.[40] The mobilization is sensitive to antecedent weather conditions.[67–69] Weathering processes

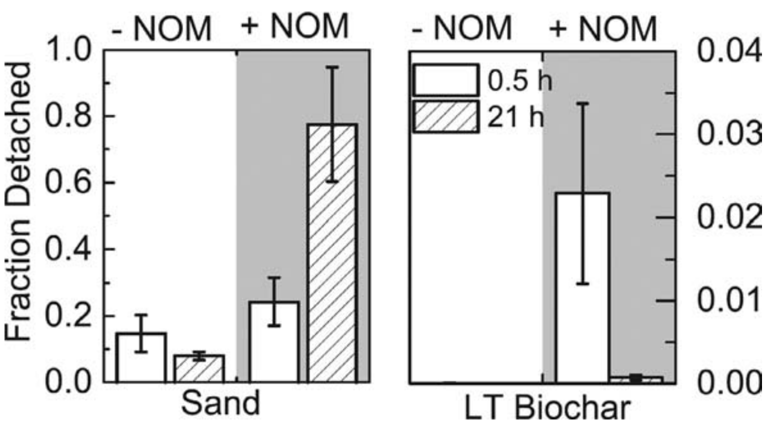

Fig. 6 Fraction of attached *E. coli* mobilized from sand (left) and low-temperature (LT) biochar columns (right) in stormwater with and without NOM during two intermittent flows. The gray background represents results from experiment with NOM. The error bar indicates one standard deviation of results obtained from four replicate column experiments. Note that the scale of y-axis is magnified for LT biochar. *Reprinted from Mohanty SK, Cantrell KB, Nelson KL, Boehm AB, Efficacy of biochar to remove* Escherichia coli *from stormwater under steady and intermittent flow, Water Research, 61:288–296, Copyright (2014), with permission from Elsevier.*

could affect moisture conditions in biofilter and affect biochar state in filter media, both of which could affect bacterial removal.[39] For example, dry–wet and freeze–thaw cycles have been shown to increase bacterial removal by biochar,[70] potentially due to the breaking of biochar by expanding ice or other change in surface properties.[39] Moreover, breakage of large biochar particles may expose newly available active sites for bacterial attachment.[14]

4. Challenges

4.1 Not all biochars are made equal

Biochar's capacity to remove pathogens or pathogen indicators varies by orders of magnitude,[28] which makes it difficult for biofilter designers to select a biochar available in the market. The variability has been attributed to a variation in biochar properties and stormwater chemistry.[17,29,38,71] Unlike activated carbon, biochar properties can vary widely based on preparation conditions and feedstock types.[72] Generally, it is recommended to use wood-based biochar prepared at high pyrolysis temperature[47,73] without removing fine particle size.[33,34,71] Despite constraining these conditions, a previous study[36] showed that bacterial removal could vary (Fig. 7). They showed that the *E. coli* removal capacity of biochar is positively correlated with surface area and carbon content and negatively correlated with ash and organic matter. High removal capacity of biochar has been attributed to an increase in surface hydrophobicity[38,40,43] and surface area[40,43] of biochar, whereas a low removal capacity has been attributed to an increase

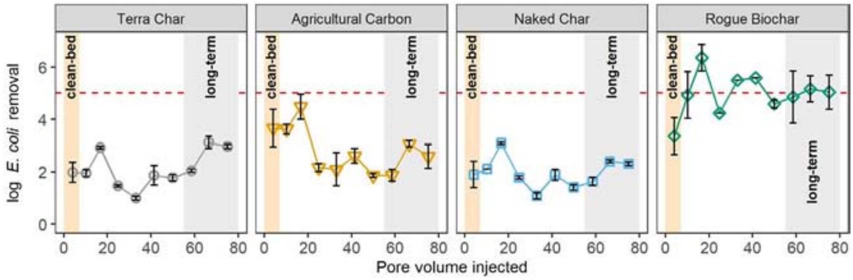

Fig. 7 *E. coli* removal capacity varies with biochar from different vendors. Removal capacity of biochar-augmented filters was investigated during 10 infiltration events. Yellow and gray shaded areas represent clean-bed removal ($n = 12$) and long-term removal ($n = 12$), respectively. Red dashed line represents detection limit of 1 colony per plate (20 CFU/mL^{-1}). *Republished with permission of the Valenca, R. et al., Biochar selection for Escherichia coli removal in stormwater biofilters, Am Soc Civil Eng, 2021:147(2); permission conveyed through Copyright Clearance Center, Inc.*

in oxidation of biochar[29] that increase net negative surface charge, and volatile carbon content.[38] These surface properties are influenced by bulk chemical properties of biochar including carbon content, ash content, volatile carbon content, and physical property such as surface area.[74] Thus, these attributes can be used by field managers to select a reliable biochar to remove pathogens.[28]

4.2 Chemical weathering could affect removal capacity

Biochar in biofilters is naturally exposed to dry-wet or freeze-thaw cycles, which can affect bacterial removal by altering surface properties of biochar.[39,75] Under these conditions, the biochar's surface is gradually oxidized, increasing the aliphatic carbon, especially carboxylic acids, and decreasing the aromatic carbon content.[75,76] Additionally, aged biochar has less total carbon and electrical conductivity. Aged biochar particles also have less potassium but more O, Si, N, Na, Al, Ca, Mn, and Fe on their surface due to their interactions with the soil.[77] Finally, biochar may lose fine particles due to weathering cycles.[39,78] The release of particles from biochar can also release the pollutants sorbed onto the particles, making the biochar a secondary source of pollutants in the long term. For instance, Mohanty and Boehm[39] exposed biochar-amended biofilters to varying weather conditions including freeze-thaw cycles, dry-wet cycles in cold (4 °C), and warm (37 °C) conditions (Fig. 8), and found that weathering conditions improved *E. coli* removal. Other studies showed that the weathering of biochar could increase the ability of biochar to adsorb trace metals[79] and nitrogenous compounds.[76]

4.3 Biological weathering or biofilm development could affect biochar performance

Biological weathering occurs via the development of biofilm on biochar's surface,[80] which can alter the performance of biochar in removing contaminants.[39] Biofilm is defined as a broad community of microorganisms–single or multiple species of gram-positive and/or gram-negative bacteria–that grows irreversibly attached to a surface depending on environmental conditions such as nutrient availability.[81] Biofilm development depends on the bacteria's hydrophobicity, surface charge, and outer membrane protein, along with the filter material's charges, chemistry, hydrophobicity, roughness, topography, and stiffness.[82] Compared to smooth surfaces, rough surfaces are more likely to adhere to bacteria and form biofilm because the

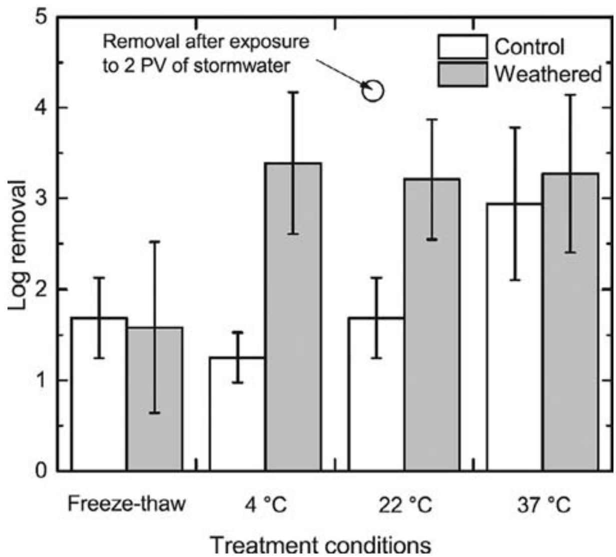

Fig. 8 Removal of *E. coli* by sand-biochar biofilters when exposed to freeze-thaw cycles and dry-wet cycles at 4 °C, 22 °C and 37 °C. *Reprinted from Mohanty SK, Boehm AB., Effect of weathering on mobilization of biochar particles and bacterial removal in a stormwater biofilter, Water Research, 85:208–215, Copyright (2015), with permission from Elsevier.*

enhanced atomic and molecular-scale of a rough surface increases the surface reactivity.[83] Biochar provides excellent support for biofilm development because, besides having high surface roughness and porosity, biochar is less likely to adsorbs metabolites including L-asparagine, L-glutamine, and L-arginine.[56] Afrooz and Boehm[40] investigated the removal of *E. coli* in sand-biochar columns with and without biofilm (Fig. 9) and found that the formation of biofilm may reduce the *E. coli* removal properties of biochar possibly due to the alteration of the hydraulic properties of the biofilter, reduction of the number of attachment sites, and increment of biofilm detachment. However, the laboratory study is typically carried out by a model microorganism that lacks the diverse community to simulate processes such as predation and starvation of pathogen in field conditions. Biofilm in field conditions could increase bacterial removal by offering a polymer-mediated adhesion to bacteria and producing a nutrient-limited environment which could indirectly kill or inactivate the surrounding bacteria. Thus, future studies should evaluate community abundance and diversity on biochar and link them with predation or other removal mechanism.

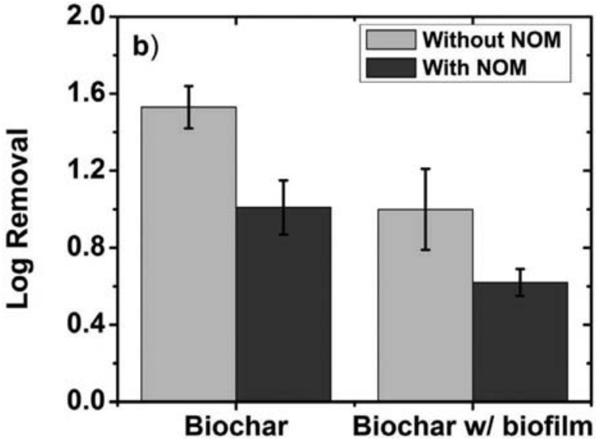

Fig. 9 Log removal of *E. coli* in laboratorial sand-biochar (70% sand, 30% biochar: by volume) columns in presence or absence of biofilm and natural organic matter (NOM). *Reprinted from Afrooz ARMN, Boehm AB,* Escherichia coli *removal in biochar-modified biofilters: effects of biofilm, PLoS One 11(12), open-access, © 2016 Afrooz, Boehm.*

4.4 Pathogen removal depends on how biochar is applied in biofilters

Biochar's capacity to remove pathogens can also depend on how they are packed in biofilters. The packing conditions including compaction energy, presence of other amendments, moisture content during packing, biochar particle size selected, and its application rates. Previous studies showed that varying biochar application rates may alter *E. coli* removal from 60% to 98% depending on the application rate.[38,43,47] The removal varied widely based on the amount of biochar added.[22,33,34,36,40,47] Even biofilters with 100% biochar show a wide removal range: 27%[48] to 99.5%.[45] Low removal occurs when biochar size is larger than 1 mm. Thus, the removal of fine biochar particles can reduce removal capacity significantly.[33,34,41,48] The presence of other amendments in the media filter, such as compost, could also decrease the bacteria removal capacity of the biofilter.[33] However, such effect may be reversed if the biofilter is compacted during packing.[15] Compaction can improve bacteria removal by straining. It appears that compacting biochar with moisture in it improves bacterial removal by minimizing the occurrence of preferential flow.[15] Irrespective of compaction, smaller biochar removes more bacteria.[14] Thus, the particle size distribution of biochar should be standardized to enhance pathogen removal in biochar-amended biofilters.

4.5 Limited removal capacity for virus

Studies investigating virus removal by biochar are scarce.[17] Adsorption mechanism is the main pathway of virus removal in natural or engineered systems. The low size of viruses makes it difficult to remove them by straining.[41] A previous study showed that biochar-augmented biofilters removed more bacteriophage MS2 virus than sand biofilters probably due to the electrostatic and hydrophobic interactions.[17] But another study showed opposite results: sand removed more virus than biochar.[34] It should be noted that the removal of virus from pore water does not eliminate the risk of virus infection. Thus, inactivation of the virus is necessary.[84] A recent study showed that the treatment of greywater with biochar filters reduces the risks of virus infection by 90%.[85] However, the dominant mechanism by which biochar can remove viruses is less clear.

5. Opportunities

5.1 Selection of best biochar based on biochar properties

Choosing biochar with high capacity is essential in meeting the design goal of the removal of bacterial pollutants from contaminated waters. Understanding which properties are related to removal is critical in the selection of appropriate biochar. Generally, an increase in carbon content and surface area, and a decrease in ash content and volatile carbon can increase biochar performance.[36] Thus, there is an opportunity for the vendors to optimize the production condition to produce biochar with these qualities. For instance, washing of biochar[86] and modifying temperature during pyrolysis[87] could lower ash content.

5.2 Modifying biochar surface properties

Modification of biochar's surface could increase their pollutant removal capacity.[88] Biochar is oxidized using strong acids such as phosphoric acids to increase the acidity of the surfaces and to modify their porous structure.[89] Similarly, biochar is also treated with NaOH to increase the oxygen content and basicity[90] and remove ash and condensed organic matter.[89,91,92] Biochar can also be chemically treated to change the functional groups to suit environmental applications. Treating biochar with HNO_3 can form amine groups on the biochar for this purpose.[93] Coating biochar with metal oxides can enhance the sorption capacity of the biochar.[94] Treating biochar with steam increases the surface area of the biochar[95] and also increases the sorption capacity.[96] Moreover, treating biochar with a mixture of CO_2 and ammonia gas increases biochar surface area and pore volume.[97] However, any modification of the biochar's surface could increase the cost of biochar.

5.3 Lack of field studies

Limited studies examined biochar capacity to remove pathogens in the field. A recent study showed that biochar-amended biofilters in the field underperformed compared to the biofilters in the laboratory setting.[37] Another study by Minnesota Pollution Control Agency investigated the feasibility of using biochar in stormwater treatment systems such as stormwater pond filter bench, filter box, and catch basin and they found *E. coli* removal efficiencies of 72% to 93%.[98] The result is similar to the removal reported in lab-scale studies.[22,33,36,40,43] Thus, more field studies should be conducted.

5.4 Change in biofilter microbiome after biochar addition

Biofilter microbiome can affect pathogen removal by predation and competition for resources. Biochar addition can shift in the microbiome in soils by altering the pore water chemistry such as pH,[99] organic carbon fraction and quality,[100] and nutrient availability.[101] Biochar can increase the retention of nitrogen-based contaminants in biofilters. Biochar addition can increase pH and carbon-nitrogen ratio, which could affect the abundance, richness, and diversity of the fungal community and shape fungal association with plants in biofilters. Biochar can also protect the soil microbiome by retaining moisture during dry seasons. Biochar can also adsorb pollutants such as polyaromatic hydrocarbons, trace organics, and heavy metals from stormwater,[102,103] where it can pose toxicity to pathogens. However, the bioavailability of adsorbed heavy metals[104] and sulfamethoxazole[103] could be lower. A slow release of these pollutants could affect pathogen concentration on biochar's surface. A study has used this concept to improve the bacterial removal capacity of biochar by impregnating copper into biochar.[105] Accumulation of toxic material on biochar surface can affect the microbiome responsible for the biodegradation of organic pollutants.[99,101,106–109] Future studies should investigate the role of accumulated toxic pollutants on the biochar microbiome and its impact on pathogen removal.

6. Summary

Biochar is a promising amendment for biofilters to improve pathogen removal from stormwater runoff. Biochar can remove pathogens by multiple processes: straining, attachment, inactivation, growth suppression, and reduced remobilization during intermittent flow. However, the relevance of each process on pathogen removal by biochar can vary based on biochar properties and their weathering under natural conditions. The particle size of biochar plays a critical role in pathogen removal as fine particles significantly increase the overall surface area and increase pathogen removal by straining. Other properties that increase removal include high surface area, high carbon content, whereas volatile carbon content and ash diminish biochar capacity to remove pathogens from stormwater. The removal capacity can further vary with how biochar is applied in biofilters: biochar size distribution, the fraction of biochar in the media mixture, layered or mixing configuration, compaction level, and moisture content during compaction. Although biochar is a recommended amendment to increase bacterial pathogen removal, biochar capacity in removing viruses is limited. In this case, modifying the biochar's surface to inactivate the virus can improve removal. Most results are based on laboratory column studies where some of the field-relevant conditions are not tested. Future studies should validate biochar capacity in field studies.

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