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Review

Indicator and Pathogen Removal by Low Impact Development Best Management Practices

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Abstract: Microbial contamination in urban stormwater is one of the most widespread and challenging water quality issues in developed countries. Low impact development (LID) best management practices (BMPs) restore pre-urban hydrology by treating and/or harvesting urban runoff and stormwater, and can be designed to remove many contaminants including pathogens. One particular type of LID BMP, stormwater biofilters (i.e., vegetated media filters, also known as bioinfiltration, bioretention, or rain gardens), is becoming increasingly popular in urban environments due to its multiple co-benefits (e.g., improved hydrology, water quality, local climate and aesthetics). However, increased understanding of the factors influencing microbial removal in biofilters is needed to effectively design and implement biofilters for microbial water quality improvement. This paper aims to provide a holistic view of microbial removal in biofilter systems, and reviews the effects of various design choices such as filter media, vegetation, infauna, submerged zones, and hydraulic retention time on microbial removal. Limitations in current knowledge and recommendations for future research are also discussed.

Keywords: biofilter; recreational water quality; indicator bacteria; pathogen; stormwater; urban runoff

1. Introduction

Urbanization, with concomitant increase in impervious surfaces, results in increased volume and rate of stormwater flow; reduces natural infiltration of stormwater; negatively impacts stream and coastal ecosystems; and often carries significant pollutant loads, including pathogens, into receiving waters [1], which provide significant values both as habitat and a recreational resource. Every year, millions of people recreate at beaches, creeks, lakes, and other water features, generating billions of dollars in economic revenue [2]. However, in the United States, microbial-contamination of recreational waters is one of the top causes of surface water quality impairment [3,4], despite decades of investigation, rule-making, and management efforts across the nation. Stormwater reuse is also becoming an increasingly attractive resource management strategy [5]. Yet, microbial contamination, among all stormwater contaminants, is the most problematic for water reuse [6].

Low impact development (LID), a planning and environmental management practice that focuses on restoring the hydrology of an urbanized watershed to its pre-development condition, has been increasingly used to improve human water security (e.g., by providing a 'fit-for-purpose' source of water), improve receiving water quality, and mitigate hydrological factors that contribute to the urban stream syndrome [1,5], including the symptoms associated with poor microbial water quality (e.g., [7–9]). LID best management practices (BMPs) include infiltration BMPs (bioretention, bioinfiltration swales, rain gardens, collectively called 'biofilters'), pervious surfaces (porous pavements, concrete, or asphalt), dry wells, detention ponds, and proprietary systems. Among these, biofilter systems are increasingly common features of the urban landscape and are often prioritized in LID implementation due to their multiple benefits, including filtration, infiltration/groundwater recharge, evapotranspiration, urban heat-island cooling, and aesthetics [5].

Typical biofilters are below-grade areas filled with a designed mix of soil media (sand, mulch, loam, etc.), vegetated, and underlain by sand or gravel with an underdrain and an overflow pipe (Figure 1). The bottom of the biofilter system may be pervious or impervious. Depending on the flow balance and the elevation of the underdrain and overflow pipe, there may be a submerged zone at the bottom that could provide additional pollutant removal [9,10]. If the stormwater flow is large enough, a layer of standing water may accumulate in a surficial ponding zone. Excess water can be released by an overflow drain to avoid flooding.

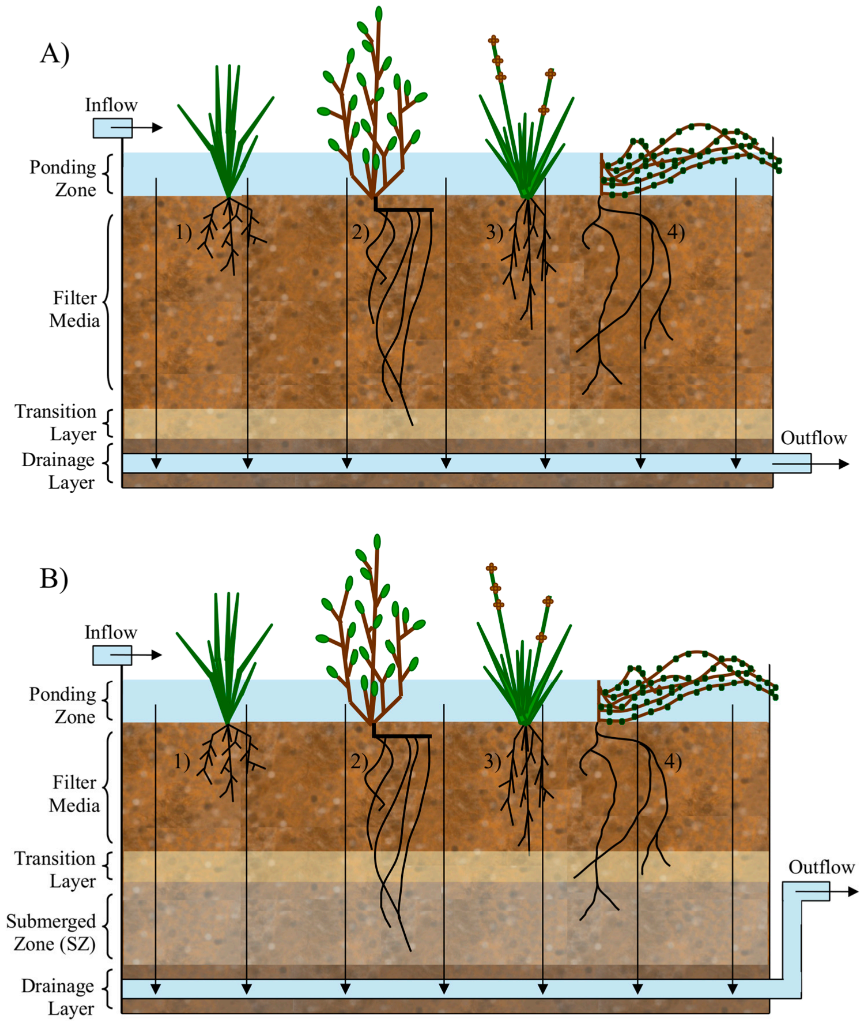

Figure 1. Schematics of a typical stormwater biofilter with (**B**) and without (**A**) a submerged zone (SZ). Both biofilters are planted with a variety of plant types reflecting different common above and below ground morphologies. These include herbaceous monocots with branching roots and shallow (1) vs. moderate (3) rooted depths, as well as upright (2) and creeping (4) dicots with deep rooted depths, low root branching, and high vs. low specific root density.

Since one major benefit of biofilters is water quality improvement, there has been great interest in studying the mechanism and efficiency of biofilter-mediated removal of stormwater contaminants. Grebel et al. [11] proposed three removal mechanism-based strategies to increase removal of common stormwater contaminants, including microbial contaminants, by engineered infiltration systems, including biofilters. These strategies include choice of infiltration media, manipulation of system hydraulic behavior, and manipulation of redox conditions. Rippy [12] summarized and conducted a meta-analysis to examine relationships between various design choices and removal of fecal indicator bacteria. Jiang et al. [13] focused on microbial risk assessment of stormwater harvesting by various LID, including biofilters. This review aims to build upon previous reports to provide a holistic view (Figure 2) of factors affecting the removal efficiency of microbial contaminants by biofilters. Particular attention is paid to biofilter design considerations and the removal of indicators (i.e., the current water quality compliance requirement) versus pathogens (i.e., the actual agents of public health risk).

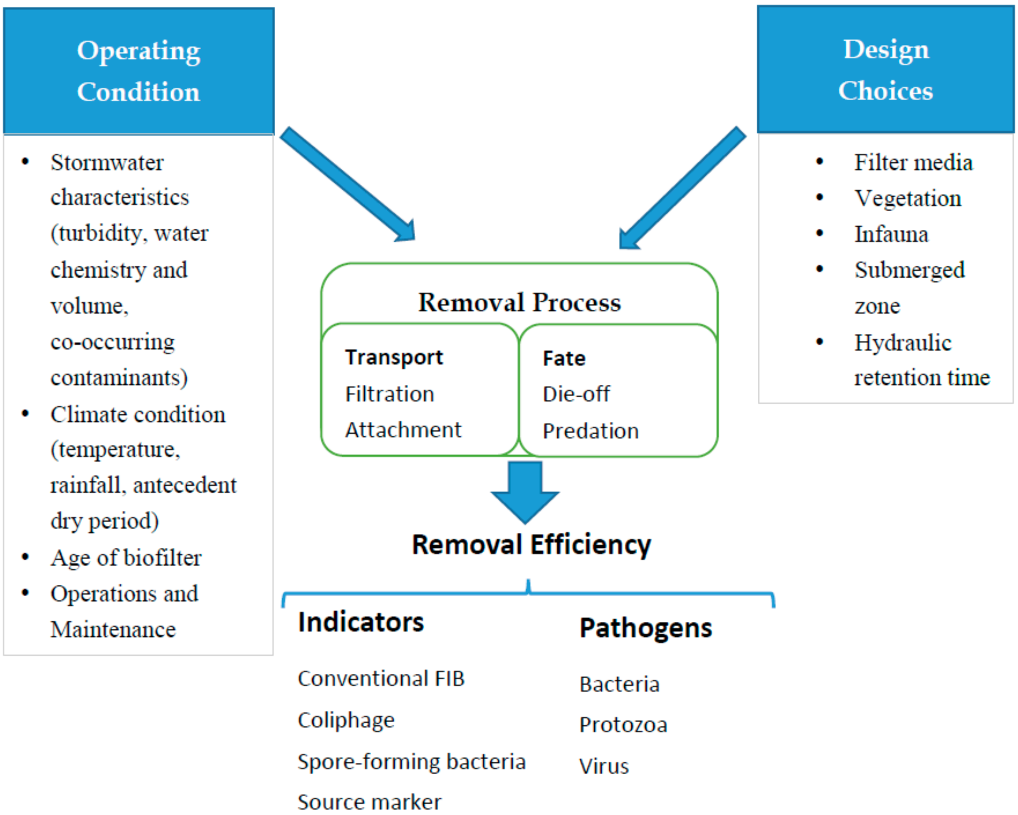

Figure 2. Conceptual model for removal of indicators and pathogens by biofilters. Spore-forming bacteria are usually used as process indicators for protozoan pathogens. Source markers refer to markers (e.g., genetic markers for microbial source tracking that identify fecal sources of microbial contamination).

2. Conceptual Model for Removal of Microbial Contaminants

Figure 2 provides a conceptual model for removal efficiency of microbial contaminants by biofilters. At the center of the model are the *processes* (or mechanisms) responsible for removing microbial contaminants; *design choices* (filter media, plants, infauna, hydraulics) and *operation conditions* (stormwater characteristics, climate condition, age of biofilter, operations and maintenance) effect removal *processes*; and all three (*processes, design choices*, and *operation conditions*) collectively affect the removal efficiency of fecal indicators and pathogens.

Transport and Fate describe processes that remove indicators and pathogens by physical retention and through biological die-off and/or predation, respectively, in the biofilter. Briefly, transport includes capture of microbial contaminants by filtration and by attachment. Note that, filtration solely refers to capture by size exclusion, while attachment (also known as physicochemical filtration in some literature) refers to capture through microbial attachment to biofilter media. Filtration processes include mechanical filtration (entrapment at the top of the biofilter media) and straining (at narrow pore throats or grain junctions inside media layers) of particle-attached and/or free-living stormwater microbes. For a biofilter with median grain diameters ranging from approximately 150 to 1000 µm, mechanical filtration is expected to remove particles >75–100 µm (i.e., mostly fine sand particles-attached microbes), whilst straining removes particles 27–100 µm in diameter (at narrow pore throats) or 0.75–5 µm (at grain junctions) [12].

Attachment occurs when microbes stick to biofilter particle grains. Compared to size exclusion-based filtration processes, attachment plays an important role for microbial removal as it provides the means to capture pollutants of much smaller size than filter media size exclusion allows. Whether the microbes successfully stick to filter grains or not depends upon the physicochemical properties of the suspending fluid (e.g., ionic strength, pH and presence of dissolved organics in stormwater), the collector (e.g., diameter, chemical composition, electrostatic properties, presence/absence of biofilm, and adsorbed organics), and the microbes (e.g., surface properties, size, and shape) [11,12,14–16]. Attachment also occurs at particle–air and air–water interfaces under unsaturated conditions when air, water, and solid phases are all present inside the biofilter. Microbes can attach to the air–water interface due to strong capillary forces, be pinned to media grains by the thinning water film, or be captured in water pockets between grains that are disconnected from the bulk flow [12,17].

Fate refers to biological processes such as die-off and predation where microbes decay or are consumed instead of being physically captured by the biofilter. Many abiotic and biotic conditions such as sunlight/Ultraviolet (UV) radiation, temperature, osmotic stress, moisture content, nutrient availability, and biotic competition can affect persistence of microbes in the environment [11,18,19]. Sunlight/UV exposure and moisture content were found to be important for *E. coli* survival in biofilter surface layers, while temperature and the presence of indigenous microbial communities greatly affected *E. coli* at all biofilter depths [20]. Rippy [12] summarized two competition mechanisms by which indigenous microbial community in the biofilters could impact fecal indicator bacteria die-off. Predation by protozoa or bacteriophage may also play an important role in removing bacteria from the environment [21,22].

Indicators vs. Pathogens. Historically, little effort has been made by stormwater practitioners, even by regulators, to distinguish amongst fecal indicator bacteria (FIB) and human pathogens. However, it is important to understand such distinctions because of the disconnection between current compliance requirements, which are based on FIB, and public health protection, which is rooted in human pathogens.

FIB include total coliform, fecal coliform, *E. coli*, and enterococcus. FIB themselves are generally not pathogens, but are used as proxies to infer presence of human waste and associated human pathogens through the "chain of inference" from FIB to public health risk [23]. A wide array of bacterial, protozoan and viral pathogens can cause human illness. These pathogens are usually present in the environment at low concentrations, but also tend to have low infectious doses. Although eight common waterborne pathogens including protozoa (e.g., *Cryptosporidium* spp., *Giardia lamblia*), bacteria (e.g., *Campylobacter jejuni*, *Salmonella enterica*, *E. coli* O157:H7), and viruses (e.g., norovirus, adenovirus, and rotavirus) were reported to account for over 97% of non-foodborne illnesses [24], detection and quantification of even this limited set of pathogens is operationally difficult and costly. Because epidemiological studies show significant correlations between FIB and recreational waterborne illness [25], FIB which are usually more abundant and easier to measure than pathogens, are used in lieu of pathogens in many regulatory microbial water quality requirements.

Despite their widespread use, major limitations exist for FIB as water quality criteria. FIB can originate from non-human or even non-fecal sources and multiply in environmental habitats (perhaps even in biofilters) under a wide range of climatic conditions [26]. In some biofilters, negative removal efficiency has been reported for *E. coli*, possibly due to re-growth of this indicator [7]. Fate and transport of FIB can also differ greatly from that of human pathogens, particularly human viruses [27]. As the regulatory framework shifts from fixed numeric criteria for FIB to a risk-based framework that allows for the establishment of alternative criteria based on equivalent public health protection [28], microbial water quality regulations may embrace alternative indicators and even direct measurement of pathogens [26]. Indeed, coliphages (viruses that infect *E. coli*) are being considered as a viral fecal indicator because bacteria viruses are expected to mimic fate and transport of human viruses better than FIB [29]. Human source markers (e.g., genetic markers from bacteria associated predominantly with human fecal material) have also attracted attention as potential alternative indicators because they represent human fecal material that is of much greater public health concern than other fecal waste [30–32]. It is therefore important to consider microbial removal by biofilters in its broadest sense. This means that engineering design choices and future predictions of treatment efficiency should not be based on FIB removal alone.

Design Choices include design elements that engineers can manipulate to enhance pollutant removal. Indicators and pathogens (protozoa, bacteria and viruses) range in size from micrometers to tens of nanometers, comparable in size to fine silt (4–10 μm), clay (1–4 μm), and colloidal particles (<1 μm) (Table 1). Microorganisms have a wide variety of shapes, surface characteristics, and cellular appendages (cilia, flagella, etc.) and therefore different mobility and motility [33,34]. Upon entering a biofilter, microbial contaminants will be subject to the various removal processes (described above), which in turn are affected by design considerations (e.g., filter media, vegetation, infauna, submerged zone, and hydraulic retention time), and potential effect modifiers, including stormwater characteristics (e.g., turbidity, stormwater chemistry and volume, and co-occurring contaminants), climate conditions (e.g., temperature, rainfall, sunlight, and storm antecedent dry period), biofilter age, and operations and maintenance, collectively termed operating conditions (Figure 2).

Table 1. Comparative size scale of particles, infauna, and microbial contaminants.

Dimension (mm)	Particle Size	Microbe Type
100	cobble	macrofauna
10	pebble	meso- to macrofauna
1	coarse sand	mesofauna
0.1	fine sand	protozoa
0.01	fine silt	protozoa/bacteria
0.001	clay/colloid	bacteria
0.0001	colloid/macromolecule	virus

Another less obvious operating condition is the state of microbial contaminants in stormwater: either freely "floating" or particle-associated. Viruses are often attached to or enmeshed within suspended particles and dissolved solids (such as submicron natural organic matter) in ambient waters [35]. A significant portion (8%–55% depending on indicator and weather type) of FIB can be attached to particles in stormwater [36,37]. Studies also indicate preferential attachment of microbes to particles of smaller size, presumably due to increased surface area and organic material on the surface. Such microbe-particle association not only changes the effective size, shape, and surface properties of microbial contaminants (impacting filtration and attachment processes), but also affords microorganisms protections against biotic and abiotic factors affecting die-off and predation [38,39]. Section 3 (below) discusses in detail how various design choices may affect microbial removal efficiency under different operating conditions.

3. Biofilter Design Consideration for Removal of Microbial Contaminants

3.1. Filter Media

The removal of microbes in a biofilter depends strongly on the physicochemical nature of the filter media, and operational conditions. The filter media in stormwater biofilters traditionally consists of a mixture of coarse and fine sand, compost, and an overlying layer of mulch. While this recipe provides ample hydraulic conductivity, good removal efficiencies for a number of pollutants, and adequate support for vegetative growth, traditional stormwater biofilters demonstrate poor microbial removal efficiency under field conditions, as reported by multiple case studies [40]. Log_{10} removal of indicator bacteria in laboratory-scale sand biofilters varies [9,41–44] from 0.45 to 2.5 depending on the filter media properties (e.g., particle size distribution, organic content and filter depth), stormwater composition, microbe strain, and biofilter operating conditions (e.g., extent of saturation, presence of vegetation). Lower microbial removal in these filter media under field conditions can be attributed to the complex operating conditions in the field, possible maintenance issues, such as clogging and short circuiting, stormwater overloading, and microbial regrowth. It should also be noted that most LID BMPs are not designed to remove bacteria only. However, data regarding the log_{10} removal of protozoan and viral indicators in traditional biofilters are limited. To the best of our knowledge, only one such study has been published [9] so far, which reported Log_{10} removal values of 3.2 and 3.9 for protozoan (i.e., *Clostridium perfringens*, a bacterium used as indicator for protozoan due to its size and spore-production) and viral indicators (i.e., F-RNA Coliphages), respectively.

One approach for improving the removal of microorganisms by biofilters might be altering the surface properties and grain size range of the filter media. This could involve (for example) the use of filter media with smaller average grain sizes, the inclusion of secondary geomedia (activated carbon, zeolite, or biochar) to improve filtration rates, or chemical modifications of media grain (also called collector) surfaces (e.g., with biocides to promote microbial die-off) [11,45]. The following section discusses studies on the effects of filter media amendments and filter media surface modification on microbial removal in stormwater biofilters.

3.1.1. Amendments to Sand Biofilters

Activated Carbon is a porous carbonaceous material produced via pyrolysis of biomass and subsequently activated using thermal, chemical, or physical processes. Because of their high surface area and excellent sorption capacities, activated carbon has been used for nearly a century to remove organic contaminants during drinking water treatment. Despite the potential utility of granular activated carbon (GAC) to remove contaminants from stormwater, its use could be cost-prohibitive. Moreover, as an organic material and electron donor, activated carbon makes GAC-amended filter media an excellent surface for microbes to grow and potentially leach during intermittent flow conditions [46,47]. Multiple studies [48–50] have investigated the microbial removal capacity of GAC-amended biofilters, with inconsistent results (0.02 log_{10} net leaching to more than 3 log_{10} removal of *E. coli*; see Table 2).

Zeolites are porous alumino-silicates with exceptional sorption and ion-exchange properties [51]. Zeolites can be found as natural geological deposits or can be chemically synthesized via crystallization followed by binding. Zeolite acts as an effective adsorbent for chemical compounds because of its high specific surface area and porosity, with an abundance of micropores [52,53]. Modification of the sand biofilters with zeolite has been reported in multiple studies [54]. Primary mechanisms of microbial removal in zeolite-based filter media are attachment and straining [54,55]. Only three studies have investigated the microbial removal performance of zeolite-modified stormwater biofilters [49,56,57]. These studies explored the effect of zeolite particle size, surface modification, and the presence of vegetation on *E. coli* removal (Table 2).

Biochar has been used for centuries to boost soil fertility [58,59], but only recently has been considered for stormwater treatment applications [60]. Like activated carbon, biochar is a pyrogenic carbonaceous material, however, no activation process is applied during the char making process where the pyrolysis occurs in oxygen-limited conditions [61]. Depending on the feedstock, pyrolysis method, duration and temperature, the physicochemical properties of biochar greatly vary [62]. Biochar removes microbial contaminants via multiple mechanisms, including pore-diffusion and attachment due to hydrophobic and electrostatic forces [60,63,64]. Physical weathering (wet–dry or freeze–thaw cycles) of biochar biofilters in the field is likely to enhance their microbial removal capacity for *E. coli*, as shown in in a recent study [65]. Furthermore, the amendment of sand biofilters with biochar has been reported to reduce the remobilization of *E. coli* under intermittent flow conditions [64]. However, the microbial removal efficiency of biochar depends on its particle size. Biochar fines (<125 µm) are necessary to attain enhanced removal [63]. This makes mobilization and leaching of these fines a concern for field scale implementation. While biochar amendment has demonstrated significant promise for improving FIB removal efficiency in sand-biofilters (Table 3), the efficacy of biochar is largely dependent on its physicochemical properties.

Table 2. Fecal indicator bacteria removal in sand biofilters with or without amendment (GAC: granular activated carbon, Zeolite), with or without various surface modification of the sand or of the amendment.

Sand Type	% Sand	Amendment, Modification [a]	Particle Size (mm)	Log_{10} Removal		Column Size (cm)	Reference
				E. coli	Enterococci		
Fine Sand	100%	-	$d_{10} = 0.33$ $d_{50} = 0.46$	0.69	-	2.5 × 23	[22]
Ottawa Sand	100%	-	0.6-0.8	0.52	0.36	2.5 × 15	[65]
Coarse Sand	100%	Iron oxide	$d_{10} = 0.61$ $d_{50} = 0.85$	0.86	-	2.5 × 23	[21,22]
Fine Sand	100%	Iron oxide	$d_{10} = 0.33$ $d_{50} = 0.46$	1.92	-		
Ottawa Sand	100%	Iron oxide	0.6–0.8	2	1.52	2.5 × 15	[66]
Ottawa Sand	50%	Iron oxide	0.6–0.8	2	1.52		
Fine Sand	53%	GAC, -	0.3–0.6	0.58	-	2.5 × 15	[48]
Fine Sand	20%	GAC, Cu	0.3–0.6	1.13	-	2.5 × 15	
Fine Sand	53%	GAC, TiO_2	0.3–0.6	0.42	-	2.5 × 15	
Fine Sand	53%	GAC, $Zn(OH)_2$	0.3–0.6	1.93	-	2.5 × 15	
Fine Sand	53%	GAC, $Cu(OH)_2$	0.3–0.6	0.4	-	2.5 × 15	
Fine Sand	53%	GAC, Si-QAC	0.3–0.6	0.47	-	2.5 × 15	
Sand	50%	GAC, $ZnSO_4.7H_2O$	0.3–0.6	1.70	-	2.8 × 10	[49]
Fine Sand	53%	Zeolite, -	0.3–0.6	0.40	-	2.5 × 15	[57]
Fine Sand	20%	Zeolite, -	0.1–0.3	0.20	-	1.8 × 20	
Fine Sand	20%	Zeolite, -	0.1–0.3	0.64	-	1.8 × 20	
Fine Sand	53%	Zeolite, Cu	0.3–0.6	3.44	-	2.5 × 15	
Fine Sand	20%	Zeolite, Cu	0.3–0.6	2.13	-	1.8 × 20	
Fine Sand	53%	Zeolite, Zn	0.3–0.6	0.92	-	2.5 × 15	
Fine Sand	53%	Zeolite, TiO_2	0.3–0.6	0.42	-	2.5 × 15	
Fine Sand	53%	Zeolite, Zn $(OH)_2$	0.3–0.6	0.41	-	2.5 × 15	
Fine Sand	53%	Zeolite, TPA	0.3–0.6	0.81	-	2.5 × 15	
Fine Sand	20%	Zeolite, CuO	0.3–0.6	0.20–2.04	-	1.8 × 20	

Notes: [a] The iron oxide coating is a modification on the sand media itself. All the rest of the surface modifications are applied on the GAC or Zeolite amendment.

Table 3. Removal of *E. coli* in sand stormwater biofilters amended with biochar.

Biochar Source and Properties						Log_{10} Removal	Reference
Feedstock	Production Process, Temperature	% Sand	Particle Size (mm)	Surface Area (m^2/gm)	Column Size (cm × cm)		
Waste Wood Pellets	Gasification, 520 °C	0%	0.45–4.75	-	7 × 23	0.14	[67]
Wood Chips	350 °C 700 °C Sonoma	78%	0.6–0.8	65.9 ± 1.2 64.9 ± 6.5 326.2 ± 5.9	2.5 × 15	1.18 ± 0.22 0.83 ± 0.05 1.16 ± 0.20	[64]
Softwood + Bark	Pyrolysis, 815–1315 °C	70% 70%	<1 0.125–1	- -		1.3 ± 0.01 0.42 ± 0.02	[63]

Other media amendments, such as expanded shale (ES) and red cedar wood chips (RC), have also been investigated as potential biofilter amendments. Like other amendments described above, the introduction of ES and RC for microbial removal is inspired by their promise in removing organic contaminants and heavy metals from urban stormwater [68–70]. The efficacies of these amendments are found to be dependent on the influent microbial concentration. Log_{10} removal efficiencies for *E. coli* in ES and RC media have been reported to be 0.2–0.9 and 0.1–1.0 respectively, depending on the influent *E. coli* concentrations (from 10^2 to 10^6 CFU/100 mL).

3.1.2. Surface Modification of the Filter Media and Amendments

In addition to introducing a secondary media to sand-based biofilters, efforts have also been made to modify both sand and the amending filter media surfaces to improve microbial removal. Such modifications were motivated by lessons learned from drinking water filtration studies. To date, multiple metal oxides, metal hydroxides, and chemicals with antimicrobial properties have been reported [71–74] to increase microbial removal during sand filtration in drinking water systems.

Chemically coated filter media may increase microbial attachment in multiple ways [75]: (a) by creating positively charged surface sites for favorable microbial immobilization (i.e., metal oxide coating); (b) by lowering the negative surface charge on filter media grains and thus reducing the electrostatic barrier to microbial attachment (i.e., metal hydroxide or polymeric modification); or (c) by imparting strong antimicrobial properties to the collector surface where microbes get inactivated by cell rupture or other biocidal mechanisms (i.e., nano-metallic or poly-cationic coating).

The most commonly used surface modifier for biofilter sand is iron-oxide coating. A number of studies [21,22,66] have investigated the efficiency of iron oxide-coated sand in removing FIB from urban stormwater runoff. In all of these studies, coated sand media removed *E. coli* and Enterococci from urban stormwater runoff more effectively than uncoated sand media (Table 2).

Multiple studies have also investigated if modifying secondary media amendments with surface coating improved microbial removal efficiency. Performance of metal (i.e., Cu, Zn)-, metal oxide (i.e., CuO, TiO_2)-, or antimicrobial (i.e., 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride, $ZnSO_4 \cdot 7H_2O$)-modified GAC and zeolite amendments were compared to unmodified GAC and zeolite amendments, respectively, and always demonstrated better FIB removal (Table 2).

Similarly, 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride (TPA) modification of RC and ES has been shown to increase *E. coli* log_{10} removal in RC- and ES-amended biofilters by 2.0 and 2.90 units, respectively. The same laboratory-scale study investigated the effects of silver nanoparticle (AgNP) modification on the performance of RC- and ES-amended biofilters. Both amendments increased average *E. coli* log_{10} removal (2.0 and 2.10 unit, respectively) [68,70]. Importantly, the effectiveness of antimicrobial modification depends on the antimicrobial loading (mg/g of filter media), temperature, and the residence time of stormwater. Recently, a full-scale field-study with TPA-modified RC and ES was attempted [69] using a tree box filter—a biofilter installed beneath a tree. Unlike the laboratory-scale study, TPA-modified biofilters in the field did not demonstrate any significant improvement of *E. coli* removal compared to the biofilters containing unmodified RC and ES, possibly due to reduced hydraulic residence time (too short for antimicrobial action to occur). This study presents an example of placing surface-modified biofilter media in the field. However, we would like to note that incorporating surface-modified biofilter media at larger scale could be somewhat challenging due to high material cost and increased complexities related to material preparation and transport.

3.1.3. Biofilm

Biofilm is a densely-packed community of surface-associated microbial cells bonded by extracellular polymeric substances (EPS). The growth of biofilm in stormwater biofilters is likely (given their near ubiquity in natural unsaturated soils) and encouraged by repeated exposure to natural stormwater throughout a biofilters' design life. Biofilms may grow on filter media grain surfaces or within pore spaces, and can be considered a natural modification of filter media. Like engineered modification of filter media, natural biofilms are likely to influence microbial removal in stormwater biofilters.

Biofilm growth inside the biofilter may change removal of microbial contaminants in several ways: (a) by altering the porosity [76] thereby influencing the hydraulics of flow through the porous media; (b) by modifying surface properties [77–79], impacting surface heterogeneity, roughness, hydrophobicity, and electrokinetic properties of the media grain surface and (c) by introducing additional microbial removal mechanisms [43,80], for example, microbial predation or physical straining. While these are the primary mechanisms through which biofilm might influence microbial removal, the magnitude and direction of effect (i.e., increase or decrease in removal) is as of yet unknown.

Although studies describing the effects of biofilm on microbial removal in stormwater biofilters are rare, literature discussing microbial transport in drinking water systems has identified several factors that govern the role of biofilm in microbial fate and transport [38,81–84]. The effects of biofilm on microbial removal depend on the porous media type, the microbial diversity of the biofilm, nutrient availability, and the thickness or maturity of the biofilm. The interplay among these factors is complex, making it difficult to reliably predict the effects of biofilm in stormwater biofilters without field-scale investigation over an extended period of time. On one hand, biofilm may accelerate stormwater microbial die-off through competition for nutrients or microbial predation [84]. On the other hand, naturally-occurring indicator bacteria may become incorporated into biofilms increasing survival during dry periods [39]. Further studies—both field- and laboratory-scale—are needed to better understand how biofilm growth in stormwater biofilters impact their efficacy for microbial removal.

3.2. Vegetation

Although biofilters are often planted for aesthetic reasons, vegetation plays a key role in regulating important soil processes such as carbon and nitrogen cycling as well as soil structure, moisture content, and stability. Vegetation also confers many pollutant treatment benefits such as nutrient uptake; physical straining of macro-pollutants including sediment and trash; attenuating/distributing stormwater flow; and controlling erosion, among others [81–84]. As vegetation changes soil physicochemical properties and impacts the soil microbiome [81,85], it has the potential to affect removal of microbial contaminants via all four removal processes: filtration, attachment, die-off/growth and predation (Figure 2). For example, changes in soil porosity and creation of preferential flow paths may alter filtration of stormwater microbial contaminants (Figure 3). Similarly, vegetation-mediated changes in soil moisture content, biofilm growth, and nutrient availability may affect removal by attachment, die-off/growth, and predation. Vegetation may also impact microbial removal through interactions with the soil macro-, meso-, and micro-fauna (see sections below).

Thus far, few experimental studies have been conducted that examine the effects of vegetation on microbial removal in biofilters, reflecting our relative inattention to plant–microbe interactions in these systems. Moreover, these studies are FIB centric and have inconsistent findings. Indeed, while some studies report higher removal efficiency in unplanted biofilters [86], others have observed improved removal performance linked to particular plant species (e.g., the shrubs *Melaleuca incana* and *Leptospermum continentale* [43] and the grasses *Paspalum conjugatum* [43] and *Buchloe dactyloides* [87]). Work by Chandrasena et al. [43] showed that biofilter vegetation that improved *E. coli* removal also reduced biofilter infiltration rates, suggesting that the effects of plants on microbial removal may be indirect through biofilter residence time. This finding is consistent with work by Parker et al. (personal communication), who found that FIB removal by biofilters when fully saturated (storm conditions) was lower in vegetated systems planted with *Carax appressa* than non-vegetated controls due to residence time effects (e.g., FIB spent less time in planted than unplanted biofilters; see Hydraulic Residence Time section for a further discussion of this issue). Importantly, because plant effects on microbial removal are a function of prevailing climate conditions such as inter-storm duration and storm frequency as well as biofilter design (e.g., submerged zone presence/absence; Figure 1), climate and design-related considerations must also be considered when selecting plants for biofilters in the field [9,43].

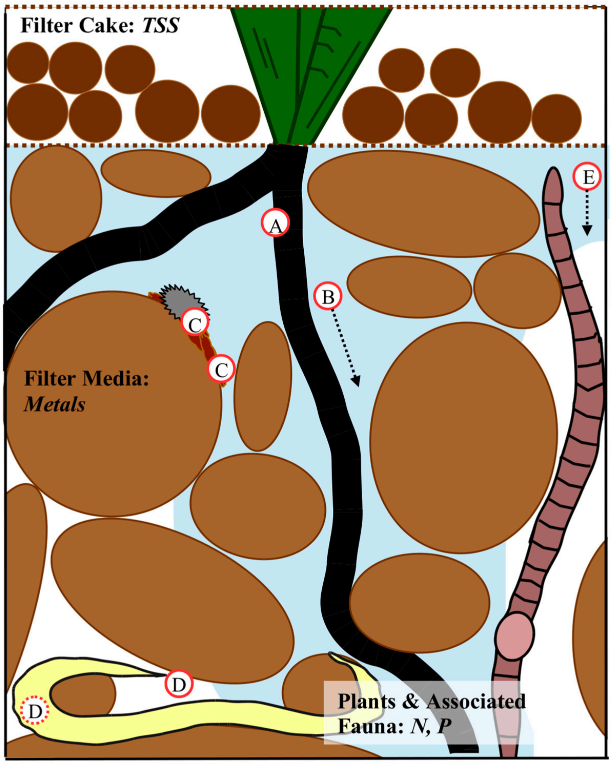

Figure 3. Microscopic cartoon of a typical biofilter filter media showing the filter cake, media grains, pore water, plant roots, and infauna (figure not drawn to scale). Fecal indicator bacteria FIB (white circle, red border), and processes relevant to FIB removal are denoted by letters A–E: (**A**) shows FIB capture via adsorption to plant roots; (**B**) shows transport in a preferential flow path alongside plant roots; (**C**) shows grazing by protozoa; (**D**) shows physicochemical attachment to filter media grains (solid red border) and subsequent ingestion by nematodes (dashed red border); and (**E**) shows transport in a preferential flow path made by a burrowing earthworm. Dominant biofilter features pertinent to the removal of other pollutants are denoted in plain text (e.g., filter cake: total suspended solids (TSS), filter media: metals, or plants and associated fauna: nutrients).

Nevertheless, general plant ecological theories may guide plant selection in stormwater biofilters. A growing body of evidence suggests that plant-regulation of soil processes can be linked back to different plant resource economic strategies (e.g., favoring resource acquisition or resource conservation), each of which is associated with specific plant traits (a subset of common plant traits are detailed in Figure 1A,B) [88,89]. For example, plants with a resource acquisition strategy (e.g., *Carax appressa* and *Juncus* sp.) tend to promote fast nutrient/carbon cycling and stabilize soils. These plants often (but not always) have the following architectural, morphological, physiological and/or biotic traits: high specific root length, low leaf and root tissue density, high root nutrient uptake and leaf/root nutrient content, low root carbon content, high photosynthetic capacity, high leaf and root respiration, low leaf and root lifespan, associations with arbuscular micorrhizal (not ectomycorrhizal) fungi, and bacterial (not fungal) dominated soil microbial communities [88,89]. Indeed, Read et al. [90] found that plants with root traits consistent with a resource acquisition strategy (e.g., high total root length, longest root, and rooted depth) removed significantly more nitrate and phosphate from stormwater biofilter influent than their resource conservative counterparts. The same pattern was not observed for metals, however, where high removal across all biofilter designs was attributed to filter media effects. Importantly, while resource-acquisitive plants are likely to facilitate nutrient removal, resource-conservative plants may provide superior protection against filter media clogging if their thicker, woodier roots promote macropore formation [90,91]. This suggests that incorporating a combination of resource-acquisitive and resource-conservative plants in biofilter design is warranted to achieve both pollutant removal and longevity endpoints. Additionally, when considering biofilter plant selection, it is also important to recognize that plant morphology and physiology are quite plastic, meaning that resource economic strategies can change when exposed to adverse conditions. For instance, during drought, many plants alter both root diameter and length (producing thinner, longer roots) to increase their water acquisition capacity [89]. Essentially, water-stressed plants assume resource acquisition traits even if they are typically more resource conservative. Another important consideration regarding biofilter plant selection concerns biotic traits related to the root microbiome (e.g., plant–bacteria and plant–fungal interactions). At present, our metrics for plant selection in biofilters are limited to consideration of architectural, morphological, and (in some cases) physiological plant traits [90]. Given that many important soil processes (including nutrient cycling and porosity) are strongly regulated by the soil microbiome [89,92], further work concentrating on identifying biotic root traits associated with enhanced biofilter performance is a research priority.

3.3. Infauna

Meso-Macrofauna. To date, few studies (five since 1990) have evaluated the composition and/or role of meso- or macro-invertebrate infauna (hereafter referred to as invertebrates) in stormwater biofilters [93]. Those that have suggest that the most common taxa include earthworms, potworms, springtails, mites, fly larvae, beetles, millipedes, centipedes, isopods, ants, spiders, and snails [94,95]. Earthworms, potworms, springtails and mites alone can constitute 80% of total invertebrate abundance, with earthworms contributing most substantially to total biomass [94]. Although biofilter community composition can be stable in time (at least over short timeframes; <1 year), significant variability amongst systems has been observed [94]. If associated with changing invertebrate functional roles, this variability could underlie differences in biofilter performance. Indeed, work by Mehring et al. [94] suggests invertebrate infauna most likely contribute to the following biofilter functions (in order of decreasing importance): decomposition, fragmentation of coarse organic matter, facilitation of plant nutrient uptake, plant growth, and infiltration. While FIB and pathogen removal is notably absent from this list, this more likely reflects the current state of knowledge regarding the effects of invertebrate infauna on microbial removal than any true assessment of invertebrate–microbe interactions in biofilters. Indeed, infiltration and plant growth are on the list, suggesting that invertebrates could affect microbial removal indirectly by impacting biofilter residence time and/or plant root architecture (see mechanisms discussed above). Earthworms in particular are organisms of interest from a microbial removal perspective, as they are abundant in biofilters, burrow extensively (in some cases increasing soil infiltration rates 2–15-fold), increase plant growth and vertical and lateral spread of plant roots, and have significant but sometimes contradictory effects on pathogen and viral persistence when used for vermicomposting of biosolids or sludge (being variously associated with reduced levels of fecal coliforms, *Salmonella*, enteric viruses, and parasitic worm eggs; increased microbial diversity/activity; and enhanced pathogen transport through soils) [12,93,96].

Micro-Mesofauna. Perhaps the most important micro-mesofaunal control over microbial populations in soils is grazing by protozoa (2–50 µm) and nematodes (30 µm–1 mm). Because their size and primary modes of feeding differ, these two groups can have disparate effects on microbial community structure (e.g., indigenous and microbial contaminants from stormwater), metabolic activity, and abundance in soils. The most important modes of protozoan grazing in soils are raptorial (e.g., targeted capture of suspended microbes) and grasping (e.g., grazing of attached microbes, including biofilms), both of which are selective [97,98]. Nematodes, in contrast, are primarily filter feeders that ingest all suspended bacteria (as well as protozoa) within a given size range, limiting their capacity to target specific prey [98,99]. This means that while protozoa can restructure microbial communities based on individual cell characteristics as well as size, nematodes primarily restructure microbial size distributions. Importantly, both protozoa and nematodes can increase microbial activity in soils. This is due (at least in part) to excreted ammonium, the metabolic waste product of their feeding, which stimulates new microbial growth [98]. The relative importance of nematode and protozoan grazing in soils in part reflects soil characteristics. For instance, soils with high silt and clay content often exclude nematodes but not protozoa, which tend to be smaller [100]. Similarly, dry soils also favor protozoan grazing, as large pores tend to drain faster than small pores meaning that nematodes are excluded at a higher soil-water content than their protozoan counterparts [98]. This effect is somewhat mitigated by the greater mobility of nematodes, which allows them to seek (and migrate into) moist regions when water availability is low [98,101]. To date, there is very little information regarding the effects of micro-mesofaunal grazers on FIB (or microbes in general) in stormwater biofilters, and what we do know is limited to protozoa–*E. coli* interactions in unplanted microcosms (e.g., 2.5×23 cm^2 glass chromatography columns) [12]. Indeed, microcosm experiments with protozoa and native soil microbiota performed by Zhang et al. [22,102], suggest that *E. coli* removal is elevated in sediments with high concentrations of protozoa relative to (1) sterile sediments; (2) sediments amended with protozoan poor microbial communities; or (3) un-aged sediments representative of immature biofilters (where protozoan recruitment is presumed limited). Given the significant effects of protozoa on FIB removal in laboratory microcosms, extending our evaluation of FIB–protozoa interactions to field-scale, vegetated columns, and other indicators and pathogens, is a promising research direction. Furthermore, because nematodes are important grazers of soil microbes alongside protozoa, the scope of current research efforts should be expanded to include nematode–FIB or nematode–pathogen interactions.

3.4. Submerged Zone

While a submerged zone (SZ) may result from operating conditions, they are also a deliberate design choice that one may implement. We limit our discussion to a deliberately designed SZ system in this paper. The submerged zone is a saturated layer near the base of lined and piped biofilters that forms when the collection pipe outlet is elevated to the level of the transition layer, allowing water to saturate the biofilter from base to outlet (Figure 1B). The resulting system has a vertical gradient of moisture and redox conditions (unsaturated and aerobic at the surface and saturated and anaerobic near the base). SZ designs provide a water reservoir for plants when the interval between storms is long (compare plants 2 and 4 in panels A and B of Figure 1) [9,43]. SZs are also often amended with organic carbon (e.g., sugar cane mulch, wood chips, straw [103,104]). These amendments are intended to stimulate anaerobic metabolic processes, particularly denitrification, but their overall contribution to pollutant removal by field biofilters remains controversial [105]. Relative to nitrate, information regarding the effects of SZs on microbial removal by stormwater biofilters is sparse, and FIB-centric. This said, a 2015 meta-analysis of log10 FIB removal rates across biofilter designs found that SZs significantly increase removal of all FIB groups (e.g., enterococci, *E. coli*, and total coliform) [12]. Indeed, on average, FIB removal was 10-fold higher in SZ designs, with log removal being 1.9 (0.9) for biofilters with (without)

SZs. Currently, there is no consensus mechanism for the effects of SZs on FIB removal, although several have been proposed [12]. Three of these mechanisms concern altered microbial attachment processes and fluid flow, as SZs affect (1) pore water velocity, which impacts the single collector contact efficiency (also called the ideal filtration efficiency) [14,15]; (2) the degree of re-suspension by propagating wetting fronts under transient flow conditions [16,66]; and (3) the formation of fissures and preferential flow paths during dry inter-storm periods [9]. SZs may also impact FIB removal through perturbing the "bio" component of biofilters (e.g., via promoting growth of plant roots (facilitating attachment), the mobility/abundance of protozoan grazers (increasing predation), and expansion of sediment biofilm communities (impacting filtration, attachment and regrowth), amongst others [12,22,43,102]). Importantly, these mechanisms are unlikely to operate in isolation and should not be viewed as independent alternatives (e.g., the effects of SZs on pore water velocity and attachment could be the result of changes in biofilm formation and root growth and architecture, or not). Given the plethora and trans-disciplinary complexity of mechanisms involved, a coupled physical, chemical, and ecological perspective is likely required to advance our understanding of SZ effects on removal of FIB, other indicators and pathogens.

3.5. Hydraulic Retention Time

In this context, the hydraulic residence time (HRT) is defined as the time stormwater spends passing through the biofilter. At the outset, it is important to stress that a biofilter's HRT is not a constant. First, mixing processes (caused by, for example, mechanical dispersion, molecular diffusion, and preferential flow paths within the biofilter) will cause water passing through the biofilter to have a range (or distribution) of residence times. Put simply, water parcels taking different routes through the biofilter will, in general, spend different amounts of time in the biofilter [106]. Second, transients in the volumetric flow rate of stormwater applied to a biofilter, together with transients in media saturation, will add to the HRT variability already noted. Transients can occur over the course of individual storms as ponding zones fill and drain (intra-storm variability), and between storms as biofilters age and experience, for example, reduced hydraulic conductivity (inter-storm variability) [107]. For all of these reasons, biofilter HRTs are likely to be highly variable. Importantly, this HRT variability has significant implications for residence time dependent treatment processes, such as physicochemical filtration of bacteria and heavy metals, micropollutant destruction, and nutrient retention and removal.

Given the complications described above, how can HRT be factored into biofilter design? Here, we argue for the following two design philosophies: (1) design for scenarios that result in the smallest range of HRTs likely to be encountered in practice; and (2) assume simplified operating conditions amenable to analytical treatment. The first design philosophy is premised on the idea that biofilters should achieve pollutant treatment goals even when HRT is minimal as might occur, for example, when the flow of stormwater through a biofilter is at the upper limit of its design range. The second design philosophy is advocated because, for simplified scenarios, it is often possible to estimate pollutant removal based on simple design equations developed from chemical engineering reactor theory, as illustrated next.

One design scenario that fulfills both conditions above is the case where a biofilter is operated for an extended (multi-day) period of time at the maximum ponding depth. Under these operating conditions, steady-state conditions will likely prevail for both the volumetric flow rate of stormwater through the biofilter and saturation of the biofilter media (i.e., the biofilter media is likely to be fully saturated). If one further assumes that pollutant removal in the biofilter follows first-order kinetics, then the following expression can be used to estimate the pollutant concentration exiting the biofilter (C_{exit}, units of kg or mol·m^{-3}) based on the pollutant concentration in the ponding zone (C_{pond}, units of kg or mol·m^{-3}) and the two non-dimensional quantities Peclet Number ($\boldsymbol{P_e}$) and Damkohler Number ($\boldsymbol{D_a}$) [106,108]:

$$\frac{C_{exit}}{C_{pond}} = \frac{(4a)\exp\left(\frac{P_e}{2}\right)}{(1+a)^2\exp\left(\frac{aP_e}{2}\right) - (1-a)^2\exp\left(-\frac{aP_e}{2}\right)} \tag{1a}$$

$$a = \sqrt{1 + 4\boldsymbol{D_a}/\boldsymbol{P_e}} \quad (1b)$$

$$\boldsymbol{P_e} = \frac{\overline{u_s} d}{D} \quad (1c)$$

$$\boldsymbol{D_a} = k\overline{\tau}_{HRT} \quad (1d)$$

Physically speaking, these two non-dimensional parameters represent the relative strength of the three fate and transport processes considered in the model: (1) pollutant removal by a first-order reaction rate (e.g., due to physicochemical filtration and/or protozoan grazing); (2) pollutant transport by advection; and (3) pollutant mixing by mechanical dispersion. The Peclet Number represents the relative importance of advection and dispersive mixing on the transport of mass through the biofilter. The Damkohler Number, on the other hand, represents the relative importance of first-order removal and advective mass transport through the biofilter. Variables appearing here include the mean interstitial velocity ($\overline{\mu_s}$, units of m·s^{-1}), biofilter depth (d, units of m), dispersion coefficient (D, units of m^2·s^{-1}), first-order removal rate constant (k, units of s^{-1}), and mean HRT ($\overline{\tau}_{HRT}$, units of s). The dispersion coefficient is a measure of how quickly constituents in the stormwater are mixed longitudinally as they travel through the biofilter.

Equation (1c) can be simplified by noting that the dispersion coefficient can be expressed as $D = \alpha\overline{\mu_s}$, where α is the biofilter media's dispersivity (units of m):

$$\boldsymbol{P_e} = \frac{d}{\alpha} \quad (2)$$

Furthermore, dispersivity increases like a power-law with the distance over which transport occurs (in our case, the distance traveled is equal to the depth of the biofilter): $\alpha = c \cdot d^{\mathrm{m}}$. Substituting this scaling law into Equation (2), we observe that the biofilter's Peclet Number depends on only the depth of the biofilter and the two scaling constants c and m:

$$\boldsymbol{P_e} = \frac{d^{1-m}}{c} \quad (3)$$

For unconsolidated sediments, Schulze-Makuch [109] recommend the following numerical values for the pre-factor and exponent, respectively: $c \approx 0.01$ and $m \approx 0.5$. Substituting these values into Equation (3), we arrive at a simple relationship between the Peclet Number and the depth of the biofilter:

$$\boldsymbol{P_e} \approx 100\sqrt{d(m)} \quad (4)$$

The parenthetical on the right hand side of Equation (4) indicates that the depth of the biofilter should be reported in units of meters. For biofilters with a depth of around 1 m, Equation (4) implies that the Peclet Number will be around 100. Thus, pollutant transport through biofilters will, in general, be advectively dominated (i.e., $\boldsymbol{P_e} > 1$).

The Damkohler Number can be calculated from the product of the first-order rate constant for pollutant removal k and the mean HRT $\overline{\tau}_{HRT}$ (see Equation (1d)). The first-order removal rate constant will depend on the mechanism by which a particular pollutant is removed by the biofilter (e.g., see discussion of physicochemical filtration earlier). The mean HRT can be estimated experimentally, for example, by measuring the mean transport time of a conservative dye through the biofilter. Alternatively, the mean HRT can be calculated from the depth of the biofilter and the mean interstitial velocity, $\overline{\tau}_{HRT} = d/\overline{\mu_s}$. The mean interstitial velocity can be calculated from the mean porosity of the biofilter ($\overline{\theta}$, unitless) and Darcy flux of stormwater moving through the biofilter (q, units of m·s^{-1}): $\overline{\mu_s} = q/\overline{\theta}$. For the scenario under consideration here (steady flow through a fully saturated biofilter), the Darcy flux can be calculated from Darcy's Law given the biofilter's hydraulic conductivity K_h (units of m·s^{-1}) and the pressure head drop across the biofilter (h, units of m):

$$q = K_h \left(1 - \frac{\Delta h}{d}\right) \tag{5a}$$

$$\Delta h = h_{bottom} - h_{top} \tag{5b}$$

In the event that the biofilter is lined and the outlet is raised (e.g., for the purpose of creating a saturation zone, see earlier sections), Equation (5a) can be written explicitly in terms of the depth of the ponding zone (ℓ_p) and the elevation of the outlet above the biofilter base (ℓ_o):

$$q = K_h \left(\frac{\ell_p - \ell_o}{d} + 1\right) \tag{6}$$

In writing out the last equation, we have assumed that frictional losses associated with piping, valves, and fittings downstream of the biofilter outlet can be neglected, and there are no pumps connected to the biofilter outlet (although such details can be easily addressed as needed). Combining these results, we have the following prediction for the Damkohler Number as a function of the removal rate constant k, depth of the biofilter d, average hydraulic conductivity K_h, mean porosity $\overline{\theta}$, ponding depth ℓ_p, and elevation of the outlet above the base of the biofilter ℓ_o:

$$\boldsymbol{D_a} = \frac{k\overline{\theta}d^2}{K_h(\ell_p - \ell_o + d)} \tag{7}$$

For a biofilter depth of d = 1 m, ponding depth of ℓ_p = 50 cm, outlet elevation of ℓ_o = 50 cm, saturated hydraulic conductivity ideal for temperate climates [107] of $K_h = 6 \times 10^{-5}$ m·s^{-1}, mean porosity of $\overline{\theta}$ = 0.37, and first-order rate constant of $k = 10^{-3}$·s^{-1} (an upper limit for the physicochemical filtration of fecal indicator bacteria) we predict D_a = 6.2 and C_{exit}/C_{pond}, which corresponds to 2.5 $\log_{10}$ units of pollutant removal.

The influence of Damkohler Number on pollutant removal (predicted from Equation (1a)) is evaluated for two different choices of the Peclet Number (corresponding to biofilter depths of d = 0.5 and 1 m, see Equation (4)) in Figure 4. As can be seen from the figure, the Peclet Number has little effect on predicted pollutant removal. Instead, variation in pollutant removal is determined primarily by the magnitude of the Damkohler Number. Thus, for all practical purposes, the magnitude of the Damkohler Number, estimated using Equation (7), can diagnose if biofilter treatment performance goals are likely to be achieved under the conservative scenario outlined here—steady-state operation and fully saturated conditions.

It should be noted that Equation (1a) assumes that the filter is homogeneous, when in fact biofilters are heterogeneous in several respects: (1) they are layered systems consisting of fine sand, coarse sand, and gravel units (see Figure 1 and related discussions); and (2) the "bio" component of biofilters (i.e., the flora and fauna) can structure both the biofilter's permeability and reaction fields [110]. For the range of Peclet Numbers expected for biofilters (on the order of 100 or larger, see above), layered systems can be analyzed using the approach outlined here, with the understanding that all media parameters (e.g., porosity, interstitial velocity, hydraulic conductivity) are weighted by the thickness of the individual layers [106]. Ongoing research should elucidate how pollutant removal in biofilters is affected by heterogeneity in the permeability field and reaction field. Judging based on analogies to pollutant treatment in river sediments, such heterogeneity (sometimes referred to as "microzone heterogeneity") could play a key role in pollutant destruction and sequestration [111].

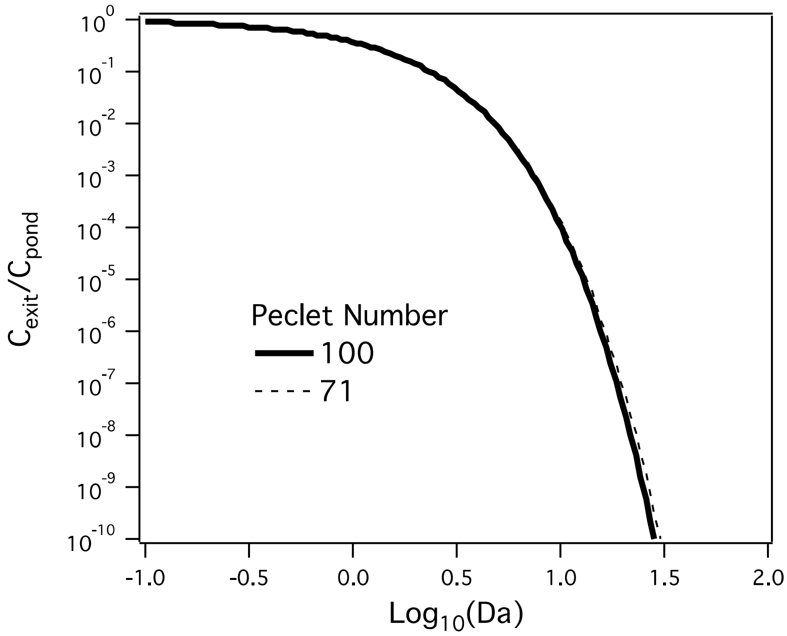

Figure 4. Relationship between Peclet number, Damkohler number and percentage of removal of microbial contaminants.

4. Summary and Future Research Needs

Our understanding of key design factors that maximize stormwater microbial removal is still limited, especially in the long term and under various operating conditions (e.g., various stormwater characteristics, climatic conditions and age-related characteristics of the biofilter system). Ideally, design choices should be made based on mechanistic understanding of contaminant removal [11], and then evaluated under variable operating conditions. If large variations in certain operating parameters are expected at a given biofilter site, design optimization measures that are robust to fluctuations in those parameters should be selected. Alternatively, conservative boundary conditions could be adopted broadly across biofilter sites for design parameter selection (e.g., see the HRT section). While this review constitutes a broad roadmap for how individual design factors could impact microbial removal, it is not a definitive work, and further site- and design-specific biofilter studies are required to validate our conceptual thinking and provide empirical removal efficiency values for use in future biofilter modeling, design and implementation efforts. Here, we summarize limitations in current literature, and discuss future research needs.

Indicators vs. Pathogens. Most microbe-centric studies of stormwater biofilters are on FIB (measured by culture-based, and occasionally genetic, methods). There are very few studies on pathogens, particularly viruses. Only two publications and one conference proceeding [9,112] investigated the removal of viral and protozoan indicators (e.g., F-RNA coliphage and *Clostridium perfringens*) from urban stormwater. With the exception of Sidhu et al. [112] and Chandrasena et al. [113] examining adenovirus, enterovirus, Campylobacter, Cryptosporidium, and Giardia removal, studies describing the removal of viral and protozoan pathogens in stormwater biofilters are rare. Given the difference in size and surface properties among indicators and pathogens, it is unlikely we can extrapolate results from FIB to viral and protozoan pathogens, or even to bacterial pathogens [114]. Fecal decay studies also reveal significant differential die-off among microbes [27]. It is therefore important that future studies on microorganism removal by biofilters include evaluation of pathogen removal, with an immediate focus on virus removal (as viruses are a leading cause of waterborne gastrointestinal illness).

In situations where it is difficult to study pathogens directly, perhaps due to low pathogen concentrations coupled with relatively insensitive measurement techniques and/or safety concerns, proper process indicators or deactivated viruses might be selected as proxies for different reference pathogens and used to evaluate treatment efficiency [115]. Where pathogen concentrations might be too low for available monitoring technologies, proper process indicators may be selected to mimic removal efficiency for each of the three groups of pathogens (e.g., FIB for bacterial pathogens; spore-forming microbes such as *Cryptosporidium* and *Bacillus* spp. for protozoan pathogens; and bacterial viruses such as somatic and male-specific coliphage for viral pathogens) [115].

Moreover, as PCR-based technology offers many advantages over culture-based methods for ambient water quality monitoring [116], PCR-based methods are now allowed to be used in routine water quality monitoring [28] and are indeed the recommended [117], if not only option (e.g., norovirus) for evaluation of certain microbial contaminants in stormwater. The ability of these methods to analyze, in high throughput, archived samples, simplifies logistics during short-term but often intensive storm studies, allowing flexible and extensive monitoring practices to become a standard part of performance assessment. Future biofilter studies should consider adopting these new methods of monitoring.

Microbial Sources for Spiking Experiments. Most biofilter spiking studies are performed with laboratory grown cultures. However, most laboratory cultures are single strain and grown in nutrient-rich media. These cultures have substantial physiological differences relative to environmental organisms (such as those in wastewater and stormwater) and often have very different fate and transport characteristics. The culturing methods and media also cause significant differences in microbial attachment efficiency and in die-off rates [118]. In short, a single laboratory growth strain is rarely representative of even the same species of microbial contaminant in real stormwater. This issue needs to be considered in future studies of stormwater biofilters.

Generally, laboratory-grown culture is the least (and sewage or raw wastewater the most) preferred spiking cocktail for evaluating treatment efficiency [115]. Where laboratory-grown cultures have to be used, care should be taken to grow the culture in such a way to mimic environmental sources. For example, a mixture of environmental isolates (e.g., from sewage sources) grown in less nutrient-rich media would be better than American Type Culture Collection (ATCC) strains grown to exponential phase for use in spiking experiments. In some cases, inoculum types might even be included as an experimental design factor to control for this confounding issue in biofilter studies.

Microbial Communities and Biofilm. It is also clear that we lack information regarding the natural biofilter microbiome and microbial succession in biofilters in response to storm events and biofilter age. Given the potential importance of microbe–microbe interactions and biofilm for removal of stormwater microbial contaminants, as well as fauna–microbe interactions, further research in this area is warranted. Such research is also likely to prove critical for understanding removal of other stormwater contaminants such as nutrients and metals.

Laboratory vs. Field Studies. Most biofilter studies are benchtop column experiments with relatively small column size and limited permutations of design factors and operating conditions. For example, nearly all studies on the effects of filter media and amendments on microbial contaminant removal have been conducted in a laboratory setting over a short time period (i.e., less than 30 days) using very small-scale (i.e., less than 3 cm diameter and 25 cm depth) column experiments under continuous flow conditions (Table 2). In contrast, field biofilters are much larger scale with variable operating conditions and a complex relationship with the social (e.g., human perception, governance, and maintenance) and ecological (e.g., patchy networks of plant and animal communities) features of the urban environment. These differences make it unclear whether findings from benchtop experiments can translate to field performance [119]. More studies with realistic column designs (size and operating conditions) as well as manipulative experiments using well instrumented field biofilters are clearly needed.

A related issue is that many stormwater biofilter studies are limited to using synthetic stormwater as the influent. While this approach offers greater control on influent water quality, it precludes reliable prediction of the performance of biofilters in the field because of the inherent variability and complexity of urban stormwater runoff. Stormwater characteristics (Figure 2) can vary greatly depending on the local sources of contaminants, frequency and duration of the storm events, and the antecedent dry period [11,120]. For arid or semi-arid regions, irrigation of biofilters with potable or recycled water is needed during prolonged dry periods, adding another layer of complexity to the issue.

Nevertheless, full scale field challenge tests with microbial pollutants are challenging due to safety concerns and resource limitations, which can be extensive given the long-term nature of the experiments required as well as the plethora of complex permutations of different design factors and operating conditions. However, new tools are available and should be utilized in future studies. For example, in situ (or modular) columns are promising for studying biofilter field performance [121], as are experiment-informed modeling approaches that allow for comparative evaluation (and rejection) of various microbial removal mechanisms [119]. Models are also particularly useful in that they can be used to generate biofilter performance estimates that are of great value to stormwater management communities (1) for watershed BMP selection and optimization; and (2) for calculating credit for alternative compliance approaches.

Biofilter implementation, along with other LID BMPs, needs to be planned on a watershed level in order to effectively improve microbial water quality. Before they are implemented, biofilters and other LID BMPs require a priori investigation of soil type, groundwater contamination, groundwater level, and high priority pollutants in the drainshed in order to optimize the biofilter design and to avoid pitfalls that lead to failure. A watershed model capable of simulating runoff and microbial pollutant loading/mass balance and incorporating LID BMP performance metrics is a powerful tool to support such watershed management strategies. As each research project accumulates data and knowledge and brings us closer toward developing effective watershed-wide or even regional stormwater management tools, research study design and data collection should be conducted in such a way that favors its integration with and utility for the development and refinement of future stormwater management tools.

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