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Biofiltration technique for removal of waterborne pathogens

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1. Introduction

Water is an important natural resource utilized for domestic, industrial, recreational, and agricultural purpose by human society. Utility of water is negatively affected by contamination of various pollutants. Pollutants are physical, chemical, and biological in nature and they deteriorate respective qualities of water after contamination. Physical properties of water includes electrical conductivity (EC), total dissolved solids, and suspended solids. Chemical properties are given by composition of various minerals, carbon content, dissolved oxygen, nitrogen and phosphorus. Biological property refers to presence of various types of microbes and pathogens specially viruses, bacteria, algae, protozoan, nematodes, insects, and their propagules.

Based on the source of pollution, wastewater is broadly classified as stormwater runoff, agricultural runoff, industrial wastewater, and domestic wastewater. Stormwater is a kind of raw water formed by natural contamination of pollutants in rain catchment areas like agricultural field, pond, and forest, etc. Other examples of raw water are groundwater abstracted through borehole, rivers, natural and man-made lakes, and reservoirs (Scholz, 2006). The raw water can be supplied for potable use after simple filtration steps and disinfection. After domestic or industrial usage, water is discharged in sewage system. This water, burdened with pollutants and pathogenic microbes, is called as wastewater. Domestic wastewater is categorized as greywater and blackwater. Former is generated from kitchen, laundry, and washrooms, while latter includes human excreta, i.e., feces and urine discharged from toilets. Well-engineered wastewater treatment plant and zero-energy constructed

wetlands are employed to treat domestic wastewater so water can be discharged back to waterbodies (Lewandowski and Boltz, 2010).

2. Waterborne disease

Waterborne disease refers to outbreaks or cases of disease of which pathogenic agents spread through ingestion of water. Human excreta contains wide variety of pathogenic agents including protozoa, bacteria, and viruses which can contaminate water source. Ingestion of water polluted with pathogen causes infection. Infection can be categorized as symptomatic (with clinically observable syndrome) and asymptomatic (without clinical manifestation). Disease is a symptomatic infection in which cause agent is known. Asymptomatic infection is also a serious concern for public health. Many bacteria and viruses that cause asymptomatic infections may spread directly in person via food or water and may result in an epidemic condition (Table 7.1) (Riley et al., 2011; Bridle, 2013; Leclerc et al., 2002).

Diverse kinds of pathogens are impractical to monitor for assessment of a wastewater treatment system. Therefore, suitable indicator organism which may be pathogenic or nonpathogenic is selected for assessment of pathogen contamination level. One widely used indicator organism is coliform bacteria, quantified either as total coliforms or fecal coliforms. Fecal coliform indicates the contamination of fresh fecal matter. Possible indicators for protozoa used in the various studies include aerobic and anaerobic spores and particle size distribution. Similarly, helminths' ovum is implicated as indicator for monitoring helminths abundance in wastewater. T4 coliphage virus is used as indicator for viruses as it is similar to adenoviruses, reoviruses, rotaviruses, and coronaviruses.

Most common indicator organism for pathogen removal is coliform. The coliforms designate the genera *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter* belonging to family Enterobacteriaceae. EC (*Escherichia coli*) is considered as a more specific indicator organism because several of coliforms are abundant in unpolluted soils and water and their presence cannot specify fecal pollution. *E. coli* is considered the most sensitive, reliable, and specific indicator of fecal pollution because it is nearly exclusive to fecal microflora and constitutes more than 90% of the coliform flora of the human gut.

2.1 Log removal

Because of huge number of pathogenic microorganisms, their removal is expressed in terms of log removal value (LRV). LRV can be defined as follows:

$$\text{Log Removal} = -\log \left(\frac{\text{inflow concentration}}{\text{outflow concentration}} \right)$$

Thus, 1-log removal designates 90% reduction in microorganisms and 2-log removal is a 99% reduction and so on.

TABLE 7.1 Pathogenic agents for waterborne diseases.

Group	Pathogen	Diseases caused	Relative infectivity	Resistance to disinfection
Virus	Enteroviruses (polio, echo, coxsackie)	Meningitis, paralysis, rash, fever, myocarditis, respiratory disease, and diarrhea	High	Moderate
	Hepatitis A and E human caliciviruses	Infectious hepatitis	High	Moderate
	Norwalk viruses, Sapporo, rotavirus	Diarrhea/gastroenteritis	High	Moderate
	Astroviruses	Diarrhea	High	Moderate
	Adenovirus	Diarrhea (types 40 and 41), eye infections, and respiratory disease	High	Moderate
	Reovirus	Respiratory and enteric	High	Moderate
	Putative enteropathogens (coronavirus, enterovirus, torovirus, parvovirus, and reovirus)	Causal relationship is still not proven	High	Moderate
Bacteria	<i>Salmonella</i>	Typhoid and diarrhea	Low	Low
	<i>Shigella</i> , <i>Yersinia enterocolitica</i>	Diarrhea	High	Low
	<i>Campylobacter</i>	Diarrhea-leading cause in foodborne outbreaks	Moderate	Low
	<i>Escherichia coli</i> O157:H7 and other certain strains	Bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans	High	Low
	<i>Legionella pneumophila</i>	Legionnaires (acute purulent pneumonia) and Pontiac fever (a self-limiting nonpneumonic disease)	Moderate	Moderate
	<i>Pseudomonas aeruginosa</i>	Pulmonary disease, skin infection	Low	Low
	<i>Vibrio cholerae</i>	Cholera	Low	Low

Continued

TABLE 7.1 Pathogenic agents for waterborne diseases.—cont'd

Group	Pathogen	Diseases caused	Relative infectivity	Resistance to disinfection
Protozoa	<i>Naegleria</i>	Amebic meningoencephalitis	Moderate	Low
	<i>Entamoeba histolytica</i>	Amebic dysentery	High	High
	<i>Giardia lamblia</i>	Giardiasis (chronic diarrhea)	High	High
	<i>Cryptosporidium parvum</i>	Cryptosporidiosis (acute diarrhea, fatal for immunocompromised individuals)	High	High
	<i>Cyclospora</i>	Diarrhea	High	High
	Microsporidia (<i>Enterocytozoon</i> spp., <i>Encephalitozoon</i> spp., <i>Septata</i> spp., <i>Pleistophora</i> spp., <i>Nosema</i> spp.)	Chronic diarrhea and wasting, pulmonary, ocular, muscular, and renal disease		
	<i>Toxoplasma gondii</i>	Toxoplasmosis	High	High
	<i>Acanthamoeba</i> spp.	Keratitis, encephalitis	High	Low
Cyanobacteria	<i>Microcystis</i> , <i>Anabaena</i> , <i>Aphanizomenon</i>	Diarrhea from ingestion of the toxins; these organisms produce microcystin; toxin is implicated in liver damage		
Helminths	<i>Ascaris lumbricoides</i>	Ascariasis		
	<i>Trichuris trichiura</i>	Trichuriasis (whipworm)		
	<i>Taenia saginata</i>	Beef tapeworm		
	<i>Schistosoma mansoni</i>	Schistosomiasis (affecting the liver, bladder, and large intestine)		

2.2 Turbidity

In some reports, turbidity is also taken as a crude parameter for pathogen removal because suspended microorganisms behave as colloidal particles. However turbidity includes concentration of suspended solids along with microbes, cysts, and ova.

3. Biofiltration

Biofiltration has been proved an essential constituent of treatment process for air, wastewater, and raw water. Major sources of potable water in megacities are treated surface water. Raw water is not suitable for human consumption because of unwanted micropollutants, pathogenic microorganisms, organic matter, and other growth-supporting nutrients present in it. Storage raw water may lead to biofouling, adverse taste and odor development, and pathogen growth. In multistep treatment process of centralized wastewater treatment plant, biofiltration constitutes one of the steps primarily for removal pollutant and in part for removal of pathogen. Biofilter is applied as sole process to make raw water potable in remote areas. Trickling filter or granular activated carbon (GAC) or sand filter is a common form of biofilter in wastewater treatment plant; GAC and sand filter are applied for treatment of raw water; rain garden and soil filter are examples of low-impact development filters for management of stormwater; horizontal rock filters are applied to mitigate polluted stream (Hammes et al., 2011; Lewandowski and Boltz, 2010).

A biofilter can be defined as any type of filter media with attached microbial community on surface in the form of biofilm performing at least one essential functions of treatment process. However, some studies referred filter media as biofilter without confirming role of indigenous microorganisms or biofilm in removal of pollutants and pathogens. Biofilter was introduced in wastewater treatment plant as trickling filter where filter media was slag or rock. Nowadays, various kinds of filter media such as rock stones, gravel, sand, GAC, or synthetic plastic media, etc., have been successfully applied for treatment of domestic and industrial wastewater. Necessity of applying biofilter for treatment of drinking water arose after the discovery of microbial regrowth in supply pipelines. Though microbes in drinking water pipelines were not found pathogenic but their presence makes water biologically not stable and unfit for drinking purpose. Cause of microbial regrowth in drinking water supply was due to pertinent micropollutants such as biodegradable organic matter (BOM), NH_4^+ , Fe_2^+ , Mn_2^+ , NO_2^- , dissolved H_2 , reduced form of sulfur by-products and disinfection by-products, etc. These pollutants act as nutrient for microbial growth. Primary role of biofilter is to remove BOM, secondary is to reduce pathogenic load. Removal of pollutants on biofilter impairs microbial growth. Organisms present in biofilm of filter absorb and utilize most organics to fulfill their nutrition and energy requirements (Lewandowski and Boltz, 2010).

3.1 Trickling filter

Ancient Egyptians dug along the Nile to get filtered water for drinking purpose. River sand acted as filter. John Gibb (Scotland, 1804) and James Simpson (England, 1829) prepared first industrial sand filter for drinking water treatment. The system could effectively reduce turbidity and contaminant of cholera in drinking water; however, microbial nature of filter was not known. Current knowledge of indigenous microorganism in drinking water treatment arose 50 years back by several studies done on presence and impact of benign microbial population on filters. First trickling filter was installed in 1922 in Madison, Wisconsin, for large water treatment system. Understanding the microbial community of filter and their role in water treatment is a continuing process, assisted with advent of advance tools for the analysis of microbial diversity and increased understanding of microbial growth in oligotrophic condition and the consideration of potable water as a complex ecosystem (Bureau of Safe Drinking Water, 2016; Daigger and Boltz, 2011; Lewandowski and Boltz, 2010 of safe drinking water).

3.2 Slow sand filtration

Slow sand filtration is a low-cost and simple-to-operate technique for removal of chemical contaminants and pathogens. Essentially an SSF is composed of following vertically arranged layers of components. Topmost layer is the supernatant water which is subjected for filtration. Water column provides enough hydrostatic pressure for its percolation through filtration system. Second is a thick layer of actual filtration medium that is of fine sand (effective size 0.15–0.3 mm). It is a low-cost durable medium for filtration. Because of its smaller particle size (0.15–0.3 mm) fine sand provides large surface area for filtration as well as for the formation of biofilm, however its small voids size decreases flow rate (0.1–0.3 m/h) through SSF. Topmost portion of sand is enriched with microbial growth because of better availability of oxygen as compared to lower portion. Most of the decontamination occurs in this active biological layer, also called as *schmutzdecke*. Microbes form of biofilm on inert sand particles and aid the biofiltration process. Bottom to sand is a layer of gravel which provides free passage to treated water to exit the bed. Gravel supports sand bed and prevents exit hose pipe from clogging. Usually the four layers make up 1 m thick column of the biofilter.

Slow filtration rate of SSF allows longer retention time for supernatant water and water percolating through bed which allows ample filtration and biological activity. Biological activity at the top layer results in *schmutzdecke*, which extends 3 cm above the bed as a slimy matrix. *Schmutzdecke* consists of mineral precipitates and colonized microorganisms comprising bacteria, fungi, protozoa, and even some larger eukaryotes. Intermittently scrapping of

schmutzdecke is essential because its overgrowth blocks percolation process. A gap period of ripening of *schmutzdecke* follows the scrapping to achieve a fully active SSF. A well-developed *schmutzdecke* is necessary for pathogen removal. *Schmutzdecke* is primary contributor to the filtration process while secondary contributors are microbes attached to sand particle in deeper part of SSF (Verma et al., 2017).

SSF efficiently removes various waterborne pathogens including viruses, bacteria, and protozoan cysts of *Giardia* and *Cryptosporidium* enteroparasites. Removal of pathogenic bacteria had been up to 99%–99.9%. However, efficiency is site-specific depending on operating parameters such as temperature, filtration rate, particle size of medium, and bed depth. Log removal for viral population ranges from 2 to 6. Removal efficiency enhances with bed depth but reduces with temperature and rate of filtration. Removal of enteric bacteriophages MS-2 and PED-I was comparatively lesser as they absorb poorly to sand surface. Parasites such as *Giardia* and *Cryptosporidium* were removed efficiently (99.9%) in SSF (Bauer et al., 2011; Lewandowski and Boltz, 2010).

Sand bed retains and inactivates microbes from seeping raw water. Both physical and biological mechanisms are involved in retention and inactivation of microbial retention on sand bed. Physical phenomenon and interactions are involved in straining and adsorption. Size of bacteria (0.01–10 μm) and viruses (0.01–0.1 μm) is much smaller than pore size of media, hence straining is ineffective on top. Organic matrix of *schmutzdecke* and exopolysaccharides (EPS) secreted indigenous bacteria makes adsorption of pathogen effective. Deeper in the bed, other mechanisms of transport (inertia, impaction, sedimentation, interception, hydrodynamic action, and diffusion) become effective. Pathogens carried to the particle are retained on filter media (Hammes et al., 2011).

Biological mechanisms contribute significantly to the removal of pathogens because of slow flow rate. This mechanism is dominant in upper layer of SSF. Slow percolation facilitates enough time for interaction of pathogens with biofilm present on sand particle. Predation is the main activity in mature biofilm responsible for removal and inactivation. For example, bacteria removal in SSF has been attributed to grazing by protozoa and worms. Grazing of adsorbed bacteria in biofilm frees the site for further retention of incoming bacteria. *E. coli* population in the treated water was found to be negatively correlated with the diversity and size flagellate and ciliate populations in the filter which signifies the importance of protozoa in bacteria removal. Meio-faunal species (0.1–1 mm in size) also predate on individual bacterial or algal cells, suspended particles, or other species. Adsorption on sticky biofilm assists the removal of biological particles in deeper region. Moreover, natural death, inactivation, and metabolic breakdown (i.e., reduction of organic carbon) also predominate in lower part (Guchi, 2015).

Natural form of bacterial inhabitant in SSF is oligotroph. They thrive on wide variety of organic substrates present in low concentration. Dense growth of oligotrophic bacteria grown in biofilm is sometimes referred to as zooglea. Zooglea is created and stabilized by sticky secretions from bacteria that are extracellular polymeric substances (EPS), polysaccharides, and proteins. This layer improves adsorption capacity of filter media. Viral particles were demonstrated to be better removal in presence of zooglea. Secreted polymers are proposed to flocculate organisms and destabilize clay and bacteria to facilitate attachment (Guchi, 2015).

Some natural inhabitant bacteria called “autochthonous” bacteria outcompete pathogenic bacteria in oligotrophic condition. Moreover, they may secrete toxic chemicals against pathogenic microbes. The phenomenon is called as “bioantagonism.” For example, survival of *Cryptosporidium* declined in the presence of autochthonous microorganism. Antagonistic mechanism is also hypothesized to be responsible for oocyst decay in SSF by autochthonous bacteria (Guchi, 2015).

Indigenous microorganisms reported in the sand bed are aerobic bacteria, flagellates, ciliates, rotifers, flatworms (Microturbellaria), gastrotriches, nematode (round worms), annelids, and arthropods (haracticids), as well as algae, protozoa, and higher order eukaryotes. However, microbial flora is dominated by Gram-negative pigmented bacteria such as *Pseudomonas* and *Aeromonas*.

Each layer of the sand bed has its own inactivation potential depending on the vertical distribution of biomass. For example, prokaryotes and eukaryotes were active throughout the filter bed in inactivating enteric microorganisms (*E. coli*); however, inactivation potential was highest near the surface of filter bed.

Series operation of SSF enhances pathogen removal efficiency. Also, air saturation of water aids the pathogen removal. Use of lava rock, silica sand, and GAC instead of sand enhanced pathogen removal in greywater. Continuous mode of operation was better for removal of *Escherichia coli* and MS2 than intermittent mode of operation. Few studies on SSF are reported to remove virus also. It was able to remove 1-log_{10} to 43-log_{10} of echovirus-12 and $0\text{--}1.3\text{-log}_{10}$ of bacteriophage, 0.061-log/h of MS2, and 0.053-log/h of PRD-1. Less than 8 h of operation time was found ideal time for removal of infectious viruses. SSF was able to do 3.2-log removal for phages, fecal bacteria, and enteric adenoviruses also. Pathogen removal efficiency of SSF reported in various studies is mentioned in Table 7.2 (Bauer et al., 2011).

All investigated filter configurations achieved substantial mean removal for *E. coli* (≤ 4.7 log), enterococci (≤ 2.4 log), *Clostridium perfringens* spores (≤ 2.1 log), coliphages (≤ 2.8 log), and aerobic bacterial load (Heterotrophic Plate Counts, HPC) (≤ 1.5 log) in secondary effluent (Seeger et al., 2016). HPC is a broad indicator of bacterial load in water. Sometimes it is applied to assess general efficiency of water treatment systems.

TABLE 7.2 Pathogen removal efficiency of slow sand filter reported in various studies.

S. No.	Removal efficiency for microbes
1	45%–60% <i>Escherichia coli</i>
2	4-Log ₁₀ coliform
3	0.3-Log ₁₀ (50%) to 4-log ₁₀ <i>E. coli</i> , 1-log ₁₀ to 43-log ₁₀ echovirus-12, 0 to 1.3-log ₁₀ bacteriophage
4	99.95% Total coliform and fecal coliform, 99.99% fecal streptococcus
5	3.2-Log phages, fecal bacteria, and enteric adenoviruses
6	4-Log total <i>coliform</i> ; 3-log <i>E. coli</i>
7	3.50-Log ₁₀ <i>Salmonella</i> sp., 3.95-log ₁₀ total coliform, 3.68-log ₁₀ <i>E. coli</i>
8	3.71-Log ₁₀ <i>E. coli</i> and 2.25-log ₁₀ MS2 virus by continuous mode of operation and 1.67-log ₁₀ <i>E. coli</i> and 0.85-log ₁₀ MS2 virus by intermittent mode of operation

3.3 Rapid sand filter

Rapid sand filter (RSF) evolved at end of 19th century in the United States of America. It became popular in 1920s because it required lesser necessary facilities with respect to SSF. Unlike slow sand filters, RSF involves only physical process because of absence of biological layer (biofilm) on filter media. Coarse-grained sand and gravels efficiently remove suspended solid by straining and adsorption. RSF must be aided with pretreatment (sedimentation and flocculation) and posttreatment (disinfection) steps to remove pathogens and prevent fouling. It requires lesser area for construction as compared to SSF for treatment of unit volume of water. RSF is constructed in a rectangular tank usually made up of concrete. Three to five layers of graded gravel are installed at the bottom of tank over a network of drainage pipes placed on the floor. Filter media that is coarse sand with a diameter ranging from 0.4 to 0.6 mm is filled over gravel layer. As coarse sand provides larger void as compared with fine sand of SSF, RSF achieves a higher rate of filtration. Gravel layer prevents sand from being drained out during filtration. Also, it facilitates even distribution of water through filtration media during backwash. Top of the RSF is either open for supernatant water (gravity filter) or closed (pressure filter) (O'Connor and O'Connor, 2002).

RSF is not as good as SSF for pathogen removal because pore size of medium is larger and it lacks biofilm. However, RSF removes suspended solid along with biological particles. Prominent biological particles retained by RSF include algal microcolonies (5–20 μm), protozoan cysts (3–10 μm), bacterial cells (0.2–2 μm), and virus particles (0.01–0.1 μm). Rose (1988) reported removal of *Giardia* and *Cryptosporidium*. The deposition of microorganisms and other particles in filters depends on transportation efficiency and retention in surface pore of filter media. Theoretical model for collection of microorganism on anthracite and sand media suggested lowest removal of individual bacterial cells in comparison to free suspended viruses, protozoa, or microbial aggregates and other particulates. In fact, removal of nanoscale particles such as viruses is governed by diffusion while protozoans are removed by cumulative effect of sedimentation and interception. Removal mechanism for suspended bacterial cells involves diffusion, differential sedimentation, and interception. Effective grain size is an important factor of collection of viruses and bacteria on media surface, whereas removal of protozoa and microbial aggregates is chiefly influenced by hydraulic loading rates. Therefore, the model suggests that smaller grain size media is major factor for removal of freely suspended viruses and other nanosized particles, and lower hydraulic loading rates would be improving removal efficacy for protozoan pathogens. Other factors that were not included in the model such as net surface charge on the filter media and microbial surfaces; media properties (type, size, and depth); hydraulic loading rates; upstream chemical use (oxidants and/or coagulants); water quality variables; flow control; and backwashing and post-backwashing practices may also significantly influence pathogen removal efficiency of filter media. Additional factors such as pH, ionic strength, temperature of effluent; concentration, molecular size, and charge density of dissolved organics; and particle characteristics influence removal efficiency. For example, high ionic strength reduces the electric double layer around microorganisms and filter media, thereby increasing attachment efficiency between the two. Backwashing of filter media in RSF may release pathogen from RSF granules. Pathogen removal in water treatment system was observed in many experimental studies. Removal of *Giardia* cysts and *Cryptosporidium* oocysts was shown to be affected by extent of filter maturation and application of coagulant chemicals. Treatment of coagulated primary effluent through RSF demonstrated approximately 1 log unit decrease in fecal coliform, pathogenic bacteria (*Salmonella* and *Pseudomonas aeruginosa*) and enteroviruses, 50%–80% of protozoan (*Giardia* and *Entamoeba histolytica*) cysts, and 90%–99% of helminth ova (Adelman et al., 2012; Hoslett et al., 2018; Jiménez et al., 2009).

3.4 Stormwater biofilter

Stormwater biofilter refers to bioretention system, bioinfiltration swale, and rain garden. It is trending as part of urban landscape because of its

multifaceted implications such as filtration, groundwater recharge, evapotranspiration, mitigation of urban heat island, and esthetic value. The system is prepared by filling soil, sand, and gravel media in low-lying area of landscape and vegetated to slow down urban runoff for better percolation while reducing the contamination load in downstream receiving zone. Usually bioretention systems consist of five vertically arranged zones. Ponding zone or detention area constitutes the topmost layer where received water is kept before filtration. An overflow drain is installed to avoid flooding. Ponding zone is followed by vegetation-rich biological zone performing pollutant uptake from water. Biological zone rests over filter media (sand, sandy loam, or loamy sand) which facilitates high infiltration rate and pollutant removal capacity. Zone of filter media is followed by a lower transition stratum of coarse sand and the lowest drainage layer of fine gravel. Bottom of bioretention system can be pervious or impervious. Besides aforesaid five layers, a biofilter system may also be configured with a submerged zone (SZ) covering the basal portion. SZ is also called internal water storage zone as it remains saturated with water between storms.

3.4.1 Submerged zone

SZ can be prepared by connecting a pipe to the drainage exit and rising the pipe opening to the transition level so that water leaves the exit only when SZ is fully saturated. Level of transition can be moved up or down by maintaining the drainage exit at respective level. SZ creates anaerobic microenvironment where denitrifying bacteria act to reduce nitrate content in water. However, this zone also affects removal of pathogenic bacteria (Rippy, 2015).

3.4.2 Removal of pathogenic bacteria in stormwater biofilter

A comparative metaanalysis was done to evaluate pathogen removal efficiency of stormwater biofilters with or without SZ. Studies performed on 358 biofilters including 89 designs with SZs and 269 designs without SZs were compiled to compare log removal of *E. coli* (EC), fecal coliforms (FC), and enterococci (ENT) in laboratory mesocosm and in field conditions. Stormwater monitoring usually focuses these three groups of bacteria as indicator of pathogen load (Table 7.3). Fecal indicator bacteria (FIB) includes *Klebsiella* and *Escherichia* genera. EC is a subgroup of FC that comprises both nonpathogenic and pathogenic members and ENT include 36 species of Gram-positive diplococoid bacteria, some of which are human pathogens. FIB is taken as proxy for pathogen load in stormwater. Pathogens exhibit high infectivity even at their low abundance in water. Low abundance poses difficulty in detection; hence proxy like FIB is essential. FIB concentration in stormwater remains high, sometimes several order higher than standards for potable and recreational purpose of water. FIB contamination in stormwater occurs by human fecal (sewage), nonfecal, and animal sources. It poses serious

TABLE 7.3 Pathogen removal efficiency of stormwater biofilter.

	Bacterial source	Log EC Removal		Log ENT Removal		Log FC Removal	
		NSZ	SZ				
Laboratory mesocosm	Horse manure					1.70 (0.19) ^a	
	EC O1:K1:H7 (with/without stormwater sediments)	0.75 (0.16) ^a					
	Natural stormwater + EC (unreported strain)	1.42 (0.12) ^a	2.63				
	Natural stormwater	1.78 (0.58) ^a	2.56 (0.43) ^a			1.07 (0.64) ^a	1.72 (0.36) ^a
	EC strain O1:K1:H7 and stormwater sediments	1.47 (0.07)	1.83 (0.08)				
Field systems	Natural stormwater	0.54				0.51	
	EC ATCC 13706	1.63 (0.09) ^a					
	Natural stormwater						1.06 (0.24) ^a
	Natural stormwater	0.09 (0.49) ^a		0.43 (0.49) ^a			
	Natural stormwater		3				2.5
	Raw sewage + synthetic stormwater	0.76	1.07	0.7	1.07		
	EC K-12	0.55 (0.14) ^a					
	Natural stormwater	0.00 (0.24) ^a				0.11 (0.20) ^a	
Overall average		0.86 (0.2)	2.22 (0.3)	0.57 (0.14)	1.07	0.85 (0.35)	1.76 (0.4)

DST, defined substrate technology; *MF*, membrane filtration; *NSZ*, no submerged zone; *SZ*, submerged zone.

^aAverage removal (standard error) from different system designs: e.g., column length, vegetation type, and media formulation.

challenge for public health and therefore to stormwater management system like biofilter. Average FIB removal efficiency of biofilter with SZ was higher (approximately eightfold) than the one without SZ (Peng et al., 2016).

3.5 Biofilter design consideration for removal of microbial contaminants

3.5.1 Filter media

Physicochemical nature of filter media strongly affects filtration of microorganisms. Traditional filtration media, represented by a mixture of fine and coarse sand, compost, and an overlying layer of mulch, shows poor microbe removal ability under field conditions despite sufficient hydraulic conductivity, good removal efficiencies for many pollutants, and acceptable support for vegetative growth. Many reasons might be responsible for poor efficiency such as operating conditions, maintenance issue including clogging and short circuiting, overloading of stormwater, and microbial regrowth. Laboratory-scale sand biofilters removed indicator bacteria in range of $0.45 \log_{10} - 0.5 \log_{10}$. \log_{10} removal capacity for protozoan indicator (*C. parvum*) and viral indicators (F-RNA coliphages) was 3.2 and 3.9, respectively, as reported in another study. Removal efficiency can be enhanced by altering physical and chemical properties of filter media. Smaller grain size and inclusion of secondary geomeia, e.g., activated carbon, zeolite, improve filtration rates while chemical modification of media enhances transport, attachment, and die-off process (Martin et al., 2002).

3.5.2 Amendments of filter media

Microbial removal of GAC-amended biofilters shows $0.02 \log_{10}$ net leaching to more than $3 \log_{10}$ EC removal. High surface area and exceptional sorption capacity make GAC a better medium for contaminant removal. GAC-amended biofilters facilitate microbial growth and potentiate leaching in the course of intermittent flow, thereby encouraging formation of biofilm in pore spaces on the collector grains. Moreover, biofilm adds further heterogeneity to the surface which may influence infiltration by altering hydrodynamics of the flow through the porous media. Extracellular polymeric substances secreted on biofilm alter roughness, hydrophobicity, and electrokinetic properties of the collector surface. Another geomeia studied for amendment is zeolite. Zeolites are porous aluminosilicate minerals commonly used as adsorbent because of its exceptional sorption and ion-exchange characteristics. It is an effective adsorbent for chemical compounds owing to high specific surface area of porous surface. Nevertheless, zeolite is useful for microbial removal. Zeolite-modified stormwater biofilter studies explored the effect of zeolite particle size, surface modification, and the presence of vegetation on *E. coli* removal. Another common amendment for sand media is biochar. Similar to GAC,

biochar is prepared by pyrolysis of organic material under oxygen-limited condition. Unlike GAC, preactivation of biochar is not necessary for its use. Physicochemical properties such as hydrophobicity and adsorption characteristics of biochar depend on variable of preparation method, e.g., feedstock, pyrolysis duration, and temperature, etc. Biochar modified sand show reduction of the remobilization of *E. coli* under intermittent flow conditions. Smaller particle sizes are better for *E. coli* removal. Physically weathered (by wet–dry or freeze–thaw cycles) biochar-amended biofilter exhibited improved *E. coli* removal capacity. Despite of abovementioned three amendments, expanded shale and red cedar wood chips (RC) have also been applied with sand media. They exhibited 0.2–0.9 and 0.1–1.0 log₁₀ removal efficiencies respectively for *E. coli* depending upon *E. coli* concentrations (from 10² to 10⁶ CFU/100 mL) in the influent water (Schifman et al., 2015, 2016; Torkelson et al., 2012).

3.5.3 Surface modification of filter media

Surface property of sand filter influences transport and filtration mechanism of pollutant and microbe removal. Surface coating with metal, metal oxides, metal hydroxides, and chemicals with antimicrobial properties are reported to increase microbial removal during sand filtration in drinking water systems. Metal and metal oxide coating imparts positive charge and coating of hydroxide or polymeric modification reduces negative charge to filter media surface. Both kinds of coatings facilitate microbial attachment to filter media wooing to net negative charge on cell-membrane. Iron oxide–coated sand media significantly removed *E. coli* and enterococci from urban stormwater as compared to uncoated counterpart (Mohanty et al., 2013; Zhang et al., 2010). Amendment of sand with surface-modified GAC with metals (Cu, Zn) or metal oxides (CuO, TiO₂) and metal hydroxides (Cu(OH)₂, Zn(OH)₂) shows improved removal of indicator bacteria. Addition of surface-modified zeolite with metal, metal oxide, or metal hydroxide enhanced *E. coli* removal. Some nanometallic or polycationic coatings utilized for coating of collector surface exhibit antimicrobial properties. Such coating enhances die-off of microbes transported to filter. EC and RC media when coated with antimicrobial agents that is 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride or silver nanoparticle enhanced *E. coli* log₁₀ removal in amended biofilters.

3.5.4 Biofilm

Biofilm plays significant role in removal of microbes following similar mechanism as described for SSF.

3.5.5 Infauna

Macrofauna: Earthworms, potworms, springtails, mites, fly larvae, beetles, millipedes, centipedes, isopods, ants, spiders, and snails constitute common

taxa of stormwater biofilter. Earthworms make most considerable biomass contributing 80% of total invertebrate abundance together with potworms, springtails, and mites. Macrofauna is associated with fragmentation and decomposition organic matter, plant nutrient uptake and growth, and infiltration in biofilter. Indirect role of macrofauna in microbial removal is suggested to play by impacting biofilter residence time and/or plant root architecture. For example, earthworm's burrowing activity increases soil infiltration activity 2–15-fold. Earthworm's activity is also proposed to increase plant growth and expansion of plant roots and has significant effects on pathogen when used for composting of biosolids or sludge. The activity was found associated with increased microbial diversity, better transport of pathogen, and removal of fecal coliforms, *Salmonella*, enteric viruses, and parasitic worm eggs.

Micro/mesofauna: Protozoans (2–50 μm) and nematodes (30 μm –1 mm) are grazing organisms of the biofilter composing micro- and mesofauna. Protozoa are specialized grazers, that is, they capture and graze on targeted population of specific suspended or attached microbe. Conversely, nematodes are generalized grazers ingesting all kinds of suspended microbes including protozoa within a limited size range. Primarily, protozoa influences structure of microbial community while nematode alters community size. Protozoa–*E. coli* interactions experiment for stormwater biofilter performed in glass column demonstrated higher bacterial removal by protozoa-supplemented sediment as compared to sterile sediment or sediment with microbial community deficient of protozoa or biologically immature sediment.

3.5.6 Vegetation

Plantation of grasses, sages, and small shrubs, etc., is done in bioretention system for esthetic purpose. Plant roots procure nutrition from organic and inorganic nutrients from the system and simultaneously influence soil processes such as nutrient cycling, nutrient availability, soil structure, moisture content, and stability. Besides, vegetation affects soil microbiome, biofilm growth, porosity, and hydraulic retention time by creating preferential flow paths, which in turn influences pathogen removal. Rhizosphere exudates influence diversity and abundance of soil infauna (macro-, meso-, and microfauna). Infauna directly impacts pathogen removal process. Biofilter planted with shrubs (*Melaleuca incana*, *Leptospermum continentale*) and grasses (*Paspalum conjugatum* and *Buchloe dactyloides*) showed improved *E. coli* removal. The removal was enhanced due to reduced infiltration rates in vegetated biofilter system. Plants selected for biofilter must have morphological and physiological plasticity toward changing microclimatic condition (Peng et al., 2016).

3.6 Microbial-earthworm ecofilters

Microbial-earthworm ecofilters (MEEs) was developed for the first time in 1992 at the University of Chile by Professor Jose Thoa. It is a low-cost

wastewater treatment system suitable for developing countries, especially rural area. It is a natural engineered system referring to a passive engineered wastewater treatment system in which traditional vermicomposting system is inoculated with potentials of earthworms. Earthworms and microorganisms function symbiotically to remove pollutant and pathogen from wastewater. Different kinds of pollutant removal processes, that is, sedimentation, filtration, adsorption, precipitation, volatilization, and uptake by earthworm and microbe, occur in MEE. Primarily, microorganisms are responsible for biochemical degradation of pollutants, while earthworms regulate microbial population and activity. The latter degrade and homogenize ingested material by muscular actions of their foregut and add mucus to it. Thus, the activity of earthworm conditioning of the filter media results in improved microbiological activity. Besides, earthworms regulate microbial biomass directly and/or indirectly via three main mechanisms: comminution, burrowing, and casting; grazing; and dispersal (Samal et al., 2017). Of the various filter media (river bed material, wood coal, glass balls, and mud balls) evaluated for performance of MEEs, river bed material showed the best activity for removal of pollutants and pathogens. Pathogen removal efficiency of MEE was studied at a pilot scale for treatment of domestic wastewater (Arora et al., 2014a,b). A 4-month study revealed that MEEs could proficiently remove BOD, COD along with total coliform, fecal coliforms, fecal streptococci, and other pathogens. In addition, study also suggested that antibacterial activity of the isolated microorganisms might be responsible for the removal of pathogens. Another pilot-scale study found that MEE is dominated by *Proteobacteria*. However, major turnover of microbial biomass was chiefly affected by earthworm-associated bacterial groups including γ -*Proteobacteria*, *Bdellovibrio*, *Lyso-bacter*, and *Myxococcales*. Sinha et al. (2012) also reported pathogen removal in a small-scale plant for treatment of toxic wastewaters from the petroleum industry.

Earthworm species and filter media types are key factors for MEEs performance because they are the chief vital components of MEEs which directly or indirectly affect removal processes of contaminants over time. Other important factors are worm load, chemical factors, hydraulic loading rate, and seasonal temperature and operating parameters, such as hydraulic loading rate, nutrient load, packing bed height, and design of setup which can alter removal process. In addition, biological activity of worm and microbe is sensitive to temperatures, pH, ammonia, and sodium.

Filter media makes external environment and affects structure and function of the earthworm's body wall. Earthworm respire through its body wall and hence filter media is a regulatory factor for metabolic and physical activity of earthworm. For example, quartz sand media caused less injury to earthworm's body wall as compared to ceramsite media. River bed material was found best as compared with other natural filter media (wood coal, glass balls, mud balls, slag-coal cinder ceramsite, and quartz sand) for pathogen removal through

MEE (Xing et al., 2011; Kumar et al., 2015). This filter media showed maximum removal of pathogen such as total coliform, fecal coliform, fecal streptococci, and *E. coli*. Arora et al. (2014a,b) also reported river bed material as the best media for removal of the aforementioned pathogens.

Vermicompost and manure aids to buffering capacity (pH rang = 6.2–9.7) of filter media and brings pH in tolerance range for earthworm's survival. Ammonium with ammonium sulfate had no influence on mortality at 2 g/kg, although ammonium chloride exhibits comparatively low toxicity (LC50 for ammonium of 1.49 g/kg) to the worm. Other chemical factor is salt stress of NaCl. Earthworms showed ability to detoxify moderate concentrations of NaCl if exposed for a long time.

Seasonal variation in ambient temperature was demonstrated to significantly affect removal of pathogen and indicator organisms, earthworm population, and bacterial and actinomycetes number. The optimum temperature range for earthworm (*Eisenia fetida*) is 25–27°C in which the organisms show best activity, growth, and reproduction. However, slightly higher temperature positively influences pathogen removal efficacy of MEEs (Jiang et al., 2016; Sinha et al., 2012).

4. Conclusion

Biofilter is promising and an economic aid or alternative to physical and chemical disinfection of wastewater. Based on the solid media, source of wastewater and flow rate biofilter can be slow sand filter, RSF, MEE, etc. Indigenous, oligotrophic microbes of biofilm pose competition to pathogen, release antimicrobial agents, feed on pathogen, and assist in transportation and filtration processes. Variation of biofilter with additional macrofauna like earthworm and/or plants further improves removal efficiency of indicator organisms. Solid sand filter, RSF, MEE, and stormwater biofilter were able to efficiently remove indicator organisms such as *Giardia*, *Cryptosporidium*, *Salmonella*, *E. coli*, total coliform, and fecal coliform, etc. Application of synthetic and natural biodegradable filter media, analysis of indigenous flora and fauna of biofilter, and optimization of operating condition are the areas of current research in this field.

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