Chapter 10

Bioaugmentation for the treatment of waterborne pathogen contamination water

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1. Introduction

Water is an important natural resource utilized for domestic, industrial, recreational, and agricultural purpose by human society. Quality of water is negatively affected by contamination of various pollutants. Various types of physical, chemical, and biological pollutants emerge from different sources and they deteriorate respective qualities of water. Physical property of water includes electrical conductivity, total dissolved solids, and suspended solids. Chemical property is given by composition of various minerals, carbon content, dissolved oxygen, and nitrogen and phosphorus (Christensen et al., 2015). Biological property refers to presence of various types of microbes and pathogens, especially viruses, bacteria, algae, protozoan, nematodes, insects, and their propagules. Waterborne diseases are any sickness caused by drinking of water polluted with pathogenic microorganisms. There are a variety of pathogenic microorganisms which can cause various types of illness in humans (Table 10.1).

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. (Rippy, 2015). Other examples of raw water are groundwater abstracted through borehole, rivers, natural and man-made lakes, and reservoirs. 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

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TABLE 10.1 Waterborne pathogens and their associated 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-like virus, 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	*Salmonella*	Typhoid and diarrhea	Low	Low
	Shigella, Yersinia enterocolitica	Diarrhea	High	Low
	Campylobacter	Diarrhea-leading cause in foodborne outbreaks	Moderate	Low
	Escherichia coli O157:H7 and other certain strains	Bloody diarrhea (hemorrhagic colitis) and renal failure (hemolytic uremic syndrome) in humans	High	Low
	Legionella pneumophila	Legionnaires' disease (acute purulent pneumonia) and Pontiac fever (a self-limiting nonpneumonic disease)	Moderate	Moderate
	Pseudomonas aeruginosa	Pulmonary disease, skin infection	Low	Low
	Vibrio cholerae	Cholera	Low	Low

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

graywater and blackwater. Graywater is generated from kitchen, laundry, and washrooms, while blackwater includes human excreta, i.e., feces and urine discharged from toilets. Well-engineered wastewater treatment plant and zero-energy constructed wetlands (CWs) are employed to treat domestic wastewater and the treated water can be discharged back to waterbodies (Lee and Scholz, 2006).

Treated or untreated wastewater generated from a treatment plant is known as sewage effluent, which is characterized on the basis of its origin as domestic sewage, hospital sewage, and industrial wastewaters. Domestic sewage is rich in organic content, and thus it contains large number of waterborne pathogens. Hospital sewage is a complex mixture containing sewage and wastewater resulting from the cleaning of laboratories, patient ward, and other facilities. As antibiotics and disinfectants are the major constituents of hospital wastewater, occurrence of antibiotic-resistant bacteria is more prevalent (Pauwels and Verstraete, 2006; Jury et al., 2010). Industrial wastewaters are generated through different industrial activities such as chemical, electrochemical, electronic, petrochemical, and food processing industries. Lack of infrastructure in water distribution system to households also plays an important role in providing clean drinking water. In several cases, drinking water supply pipes are situated close to the blackwater supply pipes. Due to this exceedingly connected piping network, at a time pressure in water supply pipe can be negative or positive relative to the surrounding water. Therefore, in a leaky water distribution system, surrounding chemical and microbial contaminants can easily be mixed with the drinking water supply. A broad range of decentralized and centralized treatment options are available for sewage water. Treated water showed limited growth of microorganisms because of low nutrient levels, and further treatment through disinfectants such as chlorination and ultraviolet (UV) treatment can convert the water in portable form. Although in potable water distribution systems, biofilms are ubiquitously formed and provide a constant source of heterotrophic bacteria (Waluyo, 2017).

Bacteria that require organic carbon rather than carbon dioxide as a carbon source are called heterotrophic bacteria; all human diseases causing bacteria are heterotrophic. While the presence of heterotroph does not in itself constitute a human health hazard, they can serve as a means to assess the microbiological quality of the water and the effectiveness of water treatment (Chen et al., 2015). A large number of process methodologies are in progress for removing or inactivating waterborne pathogens. Chlorination is the most common method of disinfection of potable water, but it is found less effective for *Cryptosporidium* and thus presents a challenge for its removal from water. The emerging number of resistant microbial strains toward the commonly applied physical and chemical disinfection methods and microbial composition of water are key factors to determine the transmission of emerging waterborne diseases. Thus, treatment technologies should be evaluated according to water quality, quantity, and effectiveness because extensive use of chlorine and chloramines promotes biofilm-forming populations including *Mycobacterium*. Increased number of adenovirus was reported in surface water treated with UV light. Waterborne pathogens such as *Naegleria* causing fatal infection showed resistant to chlorine disinfectants and UV light (Horn et al., 2016).

A large number of microbial populations have a natural quality to attach on wet surface, multiply, and form a protective slimy layer called biofilm. Biofilm formation in water transport pipes contributes to the occurrence of heterotrophic bacterial populations in water. Prevention of biofilm formation seems almost impossible because of the rapid microbial attachment on moist surfaces and their fast rate of growth. For the removal of established biofilm, harsh treatment methods such as oxidizing biocides can be applied. The increased uses of antimicrobial chemicals result in emergence of resistant bacteria, which requires new treatment strategies for their control. Thus, for removing such diverse categories of waterborne pathogens, some new targeted, cost-effective, and environment-friendly biological solutions such as enzymes, phages, interspecies interactions, and antimicrobial molecules of microbial origin can be used (Fig. 10.1) (Corre et al., 2018; Waluyo, 2017).

Bioaugmentation is a general term describing the addition of organisms or enzymes to a medium so as to remove any undesirable chemicals. It is an eco-friendly and economically viable approach for enhanced degradation of pollutants by addition of pregrown microbe or microbial cocultures in the medium. Microorganisms from different ecological conditions and engineered microbes capable to produce versatile enzymes are added to native microbial population for in situ treatment of contaminated site. Many refractory

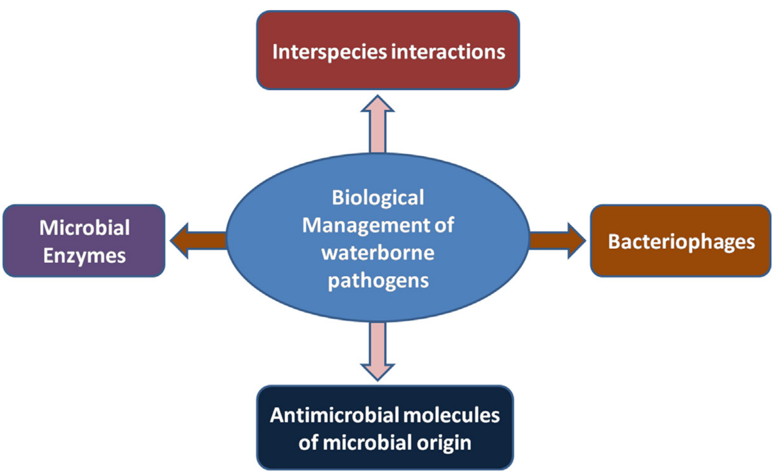

FIGURE 10.1 Biological strategies for removal of waterborne pathogens.

pollutants such as halogenated organics, aromatic and aliphatic hydrocarbons, pesticides, and pharmaceutical products have emerged out in municipal, hospital, industrial, and agricultural effluents in recent time (Herrero and Stuckey, 2015). Presence of pollutants such as chlorinated and fluorinated compounds, lignin, cyanides, synthetic dyes, polyaromatic hydrocarbons, carbamate, chloroacetamide, and benzimidazole limits bioremediation of industrial wastewater because of its toxicity to native microbial community, less solubility, high stability, and poor bioavailability. Augmentation of pollutant-specific microbial cultures intensifies degradation process by circumvention of aforesaid limitations (Herrero and Stuckey, 2015).

Bacteria are the most common organisms for bioaugmentation process. In many wastewater treatment process, different plant species is used to remove excess nutrients, metals, and pathogenic bacteria. Nowadays, wastewater from human and agricultural effluent is treated by means of CWs. CWs are treatment system that uses natural ecological processes to treat wastewaters. It is a kind of green technology to substitute conventional treatment systems, particularly in small communities. Because, it consumes less energy and operational cost, though it requires comparatively large area (Wu et al., 2015).

Disinfectants cannot penetrate the typical biofilm matrix formed on the surface of pipes used in water transport network. For efficient removal of such biofilms, “green chemicals” such as enzyme-based detergents are the favored option. A proteolytic enzyme in combination of surfactants improves the removal of biofilm as surfactant dissolves the biofilm matrix and proteolytic enzymes lyse the microbes staying there (Ben Saad et al., 2019). In every ecosystem, bacterial population is naturally controlled by bacteriophages (phages). Phages are viruses that infect, replicate, and kill bacteria. Phages provide a species targeted way to eradicate biofilms from pipes. Biofilms are composed of a mixture of bacterial species, so the application of phages has many challenges. Introduction of microbial metabolites is also a related approach for the treatment of complex interlinked network of bacterial species in biofilms. This method depends upon antagonism for substrate that can change the equilibrium of species in the biofilm (Jassim et al., 2016).

2. Biological control of *Legionella pneumophila*—a most tracked waterborne pathogens in man-made water systems

Clean drinking water is considered as an essential component for human health. In current scenario, owing to overgrowing population and rapid industrialization, most of our drinking water sources get contaminated with various types of organic and inorganic pollutants. Because of the increasing load of organic and inorganic pollutant in drinking water sources, various types of pathogen and nonpathogenic microorganisms arise in water system.

The main sources of waterborne pathogenic disease through drinking water are fecal—water—oral route of transmission (Ashbolt, 2015). However, there are many pathogenic bacteria of nonfecal origin such as *Legionella* sp., *Pseudomonas aeruginosa*, nontuberculous mycobacteria, or free-living amebae, which may colonize engineered water systems primarily within biofilms and sediments (Wang et al., 2017). This type of pathogenic microorganisms cannot be controlled only by chemical water treatment in engineered water systems before water distribution. *Legionella pneumophila* is a thin, aerobic, flagellated, non—spore-forming, Gram-negative bacterium recognized as a pathogen causing Legionnaires' disease (LD), a severe pneumonia, and a mild form of illness named Pontiac fever (Diederen, 2008). *L. pneumophila*, mostly growing inside free-living protozoa feeding on biofilms, results competitive advantage for its survival. Living inside the protozoan host is advantageous for bacteria in many ways like it can defend against phagocytic destruction and get protected from adverse environmental conditions, nutrient-rich growing condition, and water disinfection procedures (Boamah et al., 2017). For controlling such type of resistant waterborne pathogenic bacteria such as *L. pneumophila*, use of a new natural active antibacterial agent proved to be a promising strategy. In spite of the following survival strategy, growth of *L. pneumophila* in its habitat is also influenced by interactions with bacterial inhabitants found in mixed community biofilms. Interaction of *L. pneumophila* with the other microbial community in biofilm raises competition for nutrients (Abu Khweek and Amer, 2018). A study showed that among 80 aquatic bacterial isolates, 55 display antagonistic activity against *L. pneumophila*. Thus, it is practical to hypothesize that active molecules are locally produced by bacterial competitors which inhibit the growth of *L. pneumophila* (Guerrieri et al., 2008).

2.1 Selection of anti-*Legionella* compounds producers

Environmental waterborne bacterial species function as *L. pneumophila* competitors can be isolated from natural and anthropogenic waterbodies such as pond, swimming pool, river, well, and tap water. As studied by Corre et al. (2018), bacterial strains were isolated from above-discussed water samples using R2A agar plates within a maximum of 5 h after the original sampling. Isolated strains were purified for morphological and molecular identification. Molecular identification of bacterial isolates was performed by 16S rRNA gene sequencing. The isolated bacterial strains were screened for anti-*Legionella* activity. A reference strain *L. pneumophila* serogroup 1 Lens CIP 108286 (Cazalet et al., 2008) was used for anti-*Legionella* strain selection. Spot-on-lawn assay was used for strain selection described previously by Loiseau et al. (2015). Various inhibition zones of different diameters were observed, and stain showing maximum diameter was identified as most potent *L. pneumophila* competitor. Among bacterial isolates of four phyla, that is, Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria, highest anti-*Legionella* activity was shown by Firmicutes phylum and the Gammaproteobacteria class (Corre et al., 2018).

2.2 Biological molecules showing anti-*Legionella* activity

There is limited number of reports available regarding the chemical nature of diffusible molecules responsible for anti-*Legionella* activity. Surfactin (lipopeptide), a well-characterized biosurfactant acquired from *Bacillus subtilis*, was found active toward various *Legionella* species including the Lens strain (CIP 108286) (Loiseau et al., 2015). According to previous reports, *L. pneumophila* was found to be sensitive to a variety of membrane-active biomolecules (Berjeaud et al., 2016). Lipopeptides and rhamnolipids derived from *Bacillus* and *Pseudomonas*, respectively, might be a potent anti-*Legionella* compound. In addition, many antimicrobial peptides were found capable of killing *Legionella* sp. (Berjeaud et al., 2016). It is clear from above studies that the sensitivity of *L. pneumophila* to a variety of biomolecules is inadequately understood because of the lack of mechanistic data. Some *Pseudomonas* strains releasing volatile organic compound (VOC) showed anti-*Legionella* properties. These strains inhibited the growth of *L. pneumophila* through VOC. For the first time, Corre et al. (2018) reported a long-range aerial intervention between *Pseudomonas* and *Legionella*. However, the function of this VOC remains completely unexplored in natural aquatic environment.

3. Antagonistic microbial strains as biological pesticides for lethal pathogenic microbes

Domestic wastewater pollution is one of the major causes of various infectious diseases because it contaminates our nearby water sources such as river, lake, and other public water bodies, as well as acts as source of disease transmission by waterborne pathogens. Therefore, a proper domestic wastewater treatment system must be applied to avoid negative impact on public health. There are many physical and chemical methods available for domestic wastewater treatment, but use of biological control methods should be preferred because of its eco-friendly nature (Feichtmayer et al., 2017).

Domestic wastewater is generated through household activities and recreational areas commonly discarded from kitchen, shower, wash basin, toilet, and laundry. Water used in homes except from toilet is known as graywater, whereas the water coming out from toilet is known as blackwater. It contains human faces and urine and can be a cause of waterborne diseases if not treated properly. In domestic wastewater, the solid content is only 0.1% and remaining 99.9% is water. The solids can be present in suspended (30%) as well as in dissolved (70%) state (Waluyo, 2017). The suspended solids can be removed through physical precipitation, whereas dissolved solid can be treated through biological and chemical methods. Chemically domestic wastewater consists of organic (70%), inorganic (30%), and various dissolved gases. Organic substances present in wastewater are protein 40%−60%, carbohydrate 25%−30%, and oil or fat (Wind, 2007). Other pollutants usually found in the domestic wastewater are detergent, soaps, shampoo, dishwashing liquid, and disinfectants. To solve the domestic wastewater problem, potential indigenous microbial strains can be utilized for pollutant degradation and removal of pathogens. This can be done through isolation, screening, and identification of the indigenous heterotrophic strains which are detergent-tolerant and antagonistic toward pathogens. These heterotrophic strains either individually or in consortium formula candidates were used to degrade pollutant and eliminate waterborne pathogens (Waluyo, 2017).

3.1 Microbial consortium for optimum pollutant and pathogen removal

The potential heterotrophic detergent-tolerant strains of bacteria were examined by laboratory methods. These bacterial strains were screened through antagonistic test against waterborne pathogenic bacteria (*Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae*, and *Escherichia coli*). A paper disk method was used to find potential heterotrophic detergent-tolerant strains against pathogenic bacteria. Heterotrophic bacteria produce certain compounds, found lethal against pathogenic bacteria. These compounds can remove the pathogens by different ways such as affecting cell wall, disturbing cell membrane function, and inhibiting protein and nucleic acid synthesis. Waluyo (2017) has identified four potent bacterial species for detergent-tolerant and antagonistic toward pathogen. These isolates are *Bacillus thuringiensis* strain MSS-2, *Bacillus cereus* strain BQAR-01, *Bacillus cereus* strain JDA-1, and *Bacillus* sp. B31. These bacterial strains could potentially be used as the decomposer bacterial consortium for removing domestic wastewater-borne pathogens.

4. Bacteriophage for pathogen reduction in wastewater

In every ecosystem, bacterial population is naturally controlled by bacteriophages (phages). Phages are viruses that infect, replicate, and kill bacteria. In current scenario, broad-spectrum antibiotics are used for killing pathogenic bacteria, in different sectors such as health, agriculture, and waste treatment, etc. (Jassim et al., 2016). The action of these broad-spectrum antibiotics is not specific; they kill other nonpathogenic bacterial populations and their repeated application develops antibiotic-resistant bacterial strains. On the other hand, phages target specific bacteria without harming surrounding normal microflora. Phages are widely distributed on earth, particularly the places colonized by bacterial hosts, such as hospitals, animal farms, slaughterhouses, wastewater, soil waste, and aquatic environments (Jassim and Limoges, 2014). Phages for various bacterial pathogens can be isolated from the samples collected from different ecological habitats; for example, phages for human pathogenic bacteria such as *Salmonella serotypes*, *E. coli*, and *Bacteroides fragilis* have been isolated from human feces. Phages also inhabit a broad variety of raw food products (e.g., meat, chicken, fishes, pulses, biscuit dough, cheese, yoghurt, mushrooms). This indicates occurrence of phages in the same environments inhabited by their bacterial hosts and consumed daily along with food by humans (Calci et al., 1998; Breitbart et al., 2003).

As phages are natural killer of bacteria, they can be used as an alternative of antibiotics. According to recent data, 70% of the existing bacterial population of the world showed resistance to antibiotics because of its extensive use in fields and livestock. As a consequence of this, many bacterial infections and surgeries become more dangerous because of the spread of antibiotic-resistant bacteria. Therefore, phages emerge as a potential biocontrol agent against many multidrug-resistant strains of bacteria (Jassim et al., 2016; Mulani et al., 2015).

4.1 Role of phage to control waterborne bacterial pathogens

Water covers 78% of earth surface out of which approximately 1% is freshwater. According to WEF (2015) announcement, water crisis is the number one global risk, based on impact to society, and is number eight based on likelihood of occurring within 10 years (Weinbauer, 2004). Throughout the world, there are 750 million people who lack access to safe water. In developing countries, more than 1.5 million children died because of waterborne bacterial infections each year (Fenwick, 2006). Hunter (2003) reported that in Asian and African countries, the mortality due to contaminated drinking water exceeds 5 million people per year; and children under 5 years of age are the most affected by microbial diseases transmitted through water (Seas et al., 2000). More than 50% of drinking water–associated diseases are microbial intestinal infections, among which cholera is the most occurring. Sewage coming from human populations or from livestock is the primary source of pathogenic microorganisms in drinking and recreational water. Therefore, maximum risk for pathogenic microorganism contamination in drinking water is associated with the sewage mixing; it can occur through mixing of drinking water supply with sewage transport system (Jassim and Limoges, 2014). In recent years because of the extensive increase in antibiotic-resistant bacteria stains exploitation of phages as biocontrol agent attracts considerable attention. Phages have many features that make them a suitable biocontrol agent. These consist of their effectiveness in killing their target bacteria, specificity, adaptivity, ability to survive in harsh environmental conditions, and the fact that they are self-replicating and self-limiting (Jassim et al., 2016).

4.2 Phage in wastewater treatment

Many studies reported that the presence of phages acts as a bioindicator for the presence of bacteria in waterbodies. This method could be useful for designing the treatment approach for removing pathogenic bacteria from wastewater. Strategy of wastewater treatment through phages requires the initial characterization for occurrence of pathogenic bacteria. Therefore, the first treatment approach should be identification of pathogenic bacteria from wastewater and isolation and enrichment of lytic phages against these species. This phage-mediated elimination of bacteria has the competence to control treatment performance by regulating the abundance of pathogenic bacteria (Periasamy and Sundaram, 2013). Many workers suggested the application of phages directly in wastewater treatment system. In this regard, many studies projected the use of specific phage as an eco-friendly approach for removing filamentous foaming bacteria in activated sludge process system. Several phages showing lytic cycle can potentially be exploited as biocontrol agent for pathogenic bacteria. These studies have concluded that treatment of sewage with phages provides a cost-effective and sustainable method for killing pathogenic bacteria (e.g., *E. coli* and *Salmonella*) (Jassim et al., 2016).

5. Pathogen bacteria removal in constructed wetlands

CWs are treatment system that uses natural ecological processes to treat wastewaters. Construction of this engineered system requires wetland plants, soil, rocks, and their microbial populations. These CWs function through biotic and abiotic interactions among different living and nonliving components present there to remove contaminants in surface water, groundwater, or waste streams (Vymazal, 2014). Wastewater emerging from different sources such as industrial, agricultural, and municipal can potentially be treated with CWs. Most of the earlier studies on CWs have determined on the elimination of organic and inorganic pollutants, but an increasing number of investigations have reported the capability of CWs to eliminate human pathogenic bacteria (Wang et al., 2016).

In accordance with the hydrology of wetlands, CWs are usually classified into two types: free water surface (such as natural wetlands having saturated substrate with shallow flowing water) and subsurface flow (water moving through substrate, supporting plants growth). Based on the direction of water movement, subsurface flow could be further divided into vertical flow and horizontal flow CWs (Ben Saad et al., 2019).

Microorganisms play a very important role in degradation of different chemical pollutants in CWs. They were recognized as primary contributor for removal of the majority pollutants in CWs. Different microbial species containing specific functional group is associated with the degradation of each pollutant; therefore, optimized operational conditions that enhance the activity of respective fictional group associated with the degradation of different pollutants will improve the functioning of CWs. There are many naturally occurring rhizospheric bacteria and fungi which facilitates plant growth by promoting the nutrient uptake. These microorganisms also secrete some chemicals which act antagonistically toward plant pathogens. For example, plant growth−promoting rhizobacteria promote plant growth and development by fixing nitrogen and producing phytohormones and induced systemic resistance (Ben Saad et al., 2016). By introducing antagonistic bacteria in the rhizospheric region of CWs vegetation, waterborne pathogens can be reduced. Ben Saad et al. (2019) have reported the inactivation of pathogenic bacteria *Salmonella typhi* ATCC 560 in a horizontal subsurface flow−CW system planted by *Phragmites australis* by means of antagonistic bacteria.

5.1 Selection of antagonistic bacteria for removal of waterborne pathogens

Different points of CWs form a natural habitat for the existence and growth of heterotrophic bacterial population. Bacterial species found in the rhizospheric region of CWs have the potential to show antagonism against human pathogenic bacteria. Functioning of CWs in terms of waterborne pathogen removal depends upon the potential antagonistic heterotrophic bacterial species. These types of bacterial species can be isolated from different ecological niches and screened to lab scale and then inoculated in CWs to improve the functioning of these engineered systems. Ben Saad et al. (2019) reported control of *S. typhi* ATCC 560 by antagonistic bacteria inhabited in rhizosphere of *P. australis* planted in horizontal subsurface flow CW system. In another study, water and rhizospheric soil samples were collected from different components in the CWs (Ben Saad et al., 2016). After sampling, isolation, and purification, strains were screened for specific functions such as plant growth promoting by siderophore production, bacterial motility, biofilm production, and antagonist activity against waterborne human pathogenic bacteria. For colonization on plant roots, biofilm formation is important along epidermal tissue. Antagonist test of isolated strains was performed against human pathogenic bacteria *S. typhi.* The microorganism producing broad range of lytic agents, antimicrobial compounds, antibiotics, bacteriocins, exotoxin proteins, and secondary metabolites showed accomplishment in antagonistic activities. Therefore, these selected bacterial strains have been used to control the density of pathogenic bacteria and improve the water treatment in CWs. This study showed that specific bioinoculation can potentially enhance the pathogenic bacteria removal process. It is an eco-friendly approach to improve water treatment process by CWs, and it highlighted the fact that bioaugmentation can provide a better pathogen removal efficiency (Ben Saad et al., 2019).

6. Conclusion

Man-made water systems provide appropriate environment for growth and multiplication of waterborne pathogens such as *L. pneumophila*, causing LD. A large number of anti-*Legionella* strains were identified in freshwater environment creating an unfavorable growth condition for *L. pneumophila.* In contrast, presence of biofilms and protists in water systems protects this pathogen from natural competitors and harsh treatment conditions. Thus, this pathogen could be biologically controlled by strains acting as *L. pneumophila* competitors. The mode of action and chemical nature of biomolecules showing anti-*Legionella* effect requires further research.

Detergent-tolerant, heterotrophic bacteria play important role in domestic wastewater treatment and pathogen removal. Superior bacterial strains were identified based on detergent tolerance and pathogen antagonistic. These bacterial species in different combinations could potentially act as decomposer bacteria formula for domestic wastewater treatment.

Phages have the potential to treat wastewater and drinking water generated from different sources. The success of the phage-mediated biocontrol depends on the complete knowledge of wastewater microbial community dynamics and interactions. Phages isolated from relevant natural environmental conditions can be applied according to need for targeting any unwanted pathogenic bacteria such as *Shigella*, *Salmonella*, *E. coli*, *V. cholerae*, etc.

Removal of waterborne pathogens through CWs has confirmed to be an efficient wastewater treatment process. In CWs, pathogens are removed through different mechanisms such as natural die-off, sedimentation, inactivation or death due to temperature, predation, oxidation, mechanical filtration, exposure to biocides, and UV radiation. Few current studies confirmed that bioinoculation of appropriate heterotrophic bacterial strains has a clear potential to improve the process of pathogenic bacteria removal. The antagonist substances produced by the inoculated strains are the compounds that inactivate pathogenic bacteria. These antagonist substances immobilized on some natural polymers can be potentially applied in field conditions to inactivate pathogenic bacteria.

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