



# Review Role of Microorganisms in the Remediation of Wastewater in Floating Treatment Wetlands: A Review

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**Abstract:** This article provides useful information for understanding the specific role of microbes in the pollutant removal process in floating treatment wetlands (FTWs). The current literature is collected and organized to provide an insight into the specific role of microbes toward plants and pollutants. Several aspects are discussed, such as important components of FTWs, common bacterial species, rhizospheric and endophytes bacteria, and their specific role in the pollutant removal process. The roots of plants release oxygen and exudates, which act as a substrate for microbial growth. The bacteria attach themselves to the roots and form biofilms to get nutrients from the plants. Along the plants, the microbial community also influences the performance of FTWs. The bacterial community contributes to the removal of nitrogen, phosphorus, toxic metals, hydrocarbon, and organic compounds. Plant–microbe interaction breaks down complex compounds into simple nutrients, mobilizes metal ions, and increases the uptake of pollutants by plants. The inoculation of the roots of plants with acclimatized microbes may improve the phytoremediation potential of FTWs. The bacteria also encourage plant growth and the bioavailability of toxic pollutants and can alleviate metal toxicity.

Keywords: floating treatment wetlands; water; plants; microbes; pollutants

# 1. Introduction

Constructed wetlands (CWs) are purposely designed and constructed systems, based on the physical, chemical, and biological principles and processes of natural wetlands [1]. The vegetation, soil, and microorganisms are the main components of a CW that contribute to pollutant removal processes from wastewater. The associated environmental and economic benefits have established CWs as a viable option for wastewater treatment [2]. These have been widely applied in the treatment of various

types of wastewater, such as municipal, agricultural runoff, storm runoff, and industrial [3–8]. Floating treatment wetland (FTW) is a novel technology, based on a floating vegetated system, that has unique abilities to remediate wastewater [9,10]. In FTWs, plants are supported by a buoyant mat or raft that floats on the surface of the water [11]. The roots of the plants develop below the floating mat, extending down the water column, and develop an extensive root system beneath the water level [10,12,13]. The development of a widespread and dense root system is necessary for the effective performance of FTWs [14]. FTWs move freely and thus cover a wider area of water than the emergent root system. In a FTW system, the rhizomes and dense root structure develop a special hydraulic flow in the water zone between the mat and the bottom of the water body, and the floating roots act as a filter [15]. This leads to an effective removal of pollutants from the water due to the availability of the increased surface area of roots for adsorption and absorption [16]. The roots and rhizomes provide a habitat for microbial growth and development. The roots and attached biofilms perform different physical and biochemical processes for the removal of pollutants from the contaminated water [17,18]. In FTWs, pollutants are removed by three main processes, namely adsorption, sedimentation, and biodegradation [19].

The benefits associated with FTWs have made it a promising ecological remediation technology in the field of wastewater treatment. These benefits include economic and convenient construction, no digging/earth moving or extra land acquisition, easy operation and maintenance, floating mats that are adjustable with a change in the water level, and excellent treatment performance [10,20,21]. Furthermore, the planted vegetation provides economic and ecological benefits such as the use of vegetation as fodder, providing a habitat for wildlife/aquatic animals, and enhancing the aesthetic value of the pond [10,22]. Globally, FTWs are being applied to remediate various types of wastewater, such as eutrophic water, sewage and domestic, storm water runoff, and industrial [23–29].

Microbes have a fundamental role in the remediation of polluted water by FTWs. The bacteria attached to the roots form biofilms through a repeated proliferation process [30]. The oxygen and exudates released by the plants create a substrate for microbial growth and colonization on the root beneath the water level [31]. Thus, along the vegetation, the performance of FTWs also depends upon the metabolism of the microbial community in water, attached to the roots and floating mats [32–34]. The application of plants in combination with microorganisms in FTWs is an effective and sustainable approach for the treatment of wastewater [35]. The plant–microbe interaction enhances the efficacy of FTWs [36]. Although the plant–bacteria interaction plays an essential role in the removal of contaminants from aquatic ecosystem, the interaction of the plant with bacteria in the FTWs is not well explored [37].

This paper discusses this important component of FTWs and provides a detailed overview of the specific role of microorganisms in FTWs. We have summarized the important species of bacteria that colonize the roots of plants. Furthermore, the specific role of rhizospheric bacteria, endophytes, and algae in the pollutant removal process in FTWs has been elaborated.

## 2. Mechanism of FTWs

In FTWs, pollutants are removed from the wastewater by different mechanisms induced by plants, microbes, and their mutualistic relationships. The presence of a vegetated floating mat in a water body boosts the pollutant removal efficiency of the system by modifying the physicochemical properties of the water [38,39]. The physical characteristics of the plant's roots and the nutrient uptake are interdependent/interlinked. The type of medium in which the roots exit and the nutrients present in the medium specify the root's physical characteristics [9,40]. In general, the roots of plants filter the particulates present in the water. Nutrients are taken up by the plant's roots and accumulated in them, as well as in the parts of the plant above the mat [14]. Most organic pollutants are taken by the plants. The organic pollutants can either be accumulated in the biomass of vegetation or degraded by endophytic bacteria present inside the plants [41,42].

The plants in FTWs contribute to the pollutant removal process by entrapping pollutant particles in the roots [11,43,44]. The roots of plants act as physical filters, and remove suspended particulate matter from the water. For an effective removal, there should be dense roots, so that they can act as a physical filter and a bio-sorbent [15].

The bioactive substances released by the roots have a unique role in the removal of nutrients. These substances balance pH, and increase the humic content in the water, which results in the adsorption and/or precipitation of pollutants in the form of insoluble material [15,21]. The neutral pH induced by the vegetation helps in the settlement of dissolved particulate pollutants [24]. Moreover, these substances alter the physicochemical condition of water, and increase metal and nutrient removal and the sorption characteristics of biofilms [45,46]. For example, plants may remove phosphorus by direct uptake, but the key mechanisms of phosphorus removal are sorption, settlement at the bottom, and physical entrapment in the roots [47]. The FTWs also inhibit the growth of algal communities by removing nutrients from the water, thus reducing their population [48].

Roots act as a suitable surface for the formation of biofilms, which enhance the degradation of organic pollutants and removal of nutrients from wastewater [11]. Root exudates aid in the retention of microbes on the roots by providing them with nutrients [49]. The roots also provide oxygen to rhizospheric bacteria for aerobic degradation of organic matter. The biodegradation of organic matter into simple nutrients occurs when it comes in contact with the biofilm [50,51]. Plants remove these nutrients through direct uptake [52]. Trapping in the biofilm of the roots of macrophytes is an essential mechanism for particulate matter removal. Furthermore, roots let microbial colonies assimilate the carbon compounds and help in the reduction in biological oxygen demand and chemical oxygen demand [26]. Floating wetlands can work under both aerobic and anaerobic conditions. However, the nutrient removal under aerobic conditions is higher than under anaerobic conditions [53]. Other organic compounds are degraded by heterotrophic microorganisms either aerobically or anaerobically, depending upon the oxygen level in water [54].

## 3. Important Components of FTWs

FTW is composed of plants that are vegetated in a floating mat. Different types of material are used as floating mats. The detail of these important components is described below (Figure 1).



Figure 1. Schematic representation of floating treatment wetland and pollutant removal process.

#### 3.1. Growth Media

Different types of growth media have been used to provide support to the plants growing on the floating mat. This growth media can be coconut fiber, peat, soil, bamboo crush, sand, peat rice straw, and compost [55]. The selection of growth media also influences the pollutant removal process. For instance, the use of rice straw as growth media improved the total nitrogen removal process by the formation of thick biofilms, boosting the nitrification/denitrification process [56].

# 3.2. Buoyancy

In FTWs, different materials have been applied with different natural buoyancies. These floating materials serve as a platform to fix the plants. The floating mats are made up of different materials such bamboo sticks, polyester fibers, plastic and foaming sheets [57–59]. The floating material should be hydrophobic, nutrient absorbent, bacterial adhesive, and with no desorption [15].

Some patent floating mats are also available commercially, such as Beemat<sup>®</sup>, and Bioheaven<sup>®</sup>, made up of buoyant material with holes for plantation. The wrapped plastic tubes and pipes manufactured from polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC), and PS (polystyrene) foams are most commonly used for the construction of floating frames and rafts [38]. A natural buoyant material, bamboo, has been found to be a cheap and cost-effective material for the construction of floating rafts [60].

# 3.3. Plants

The selection of plant species has a great influence on the pollutant removal process. The selection of plants depends upon their local availability, the nature of pollutants, and the climate zone. The plants mostly used to develop FTWs are of *Canna, Typha, Phragmites*, and *Cyperus* genera. They have been widely applied in FTWs for the remediation of different types of wastewater [30,56,61–66]. Some species of the *Poaceae* family (*Lollium* sp., *Zizania* sp., and *Chrysopogon* sp.) have been successfully applied in Italy, China, Singapore, and Thailand to develop FTWs. Some plant species are suitable for particular regions and have efficiently removed nutrients and other pollutants in a specific climate. Some other plants such as *Phragmites*, *Carex*, *Acorus*, and *Juncus* were also successfully applied in FTWs, and these effectively adapted in several locations. The selection of macrophytes to develop FTWs is very important for pollutant removal as well as for ecosystem sustainability. The selected plants should be native, easily available, non-invasive species, perennial, able to thrive in a hydroponic environment with an extensive root system and aerenchyma [67]. The application of invasive species in FTWs may result in damage to the ecosystem, and the ultimate cost of habitat restoration may suppress the benefits gained by pollutant removal. [68]. The characteristics that make these macrophytes ideal for FTWs are their robust growth tall shoot length, extensive root system, and large aerenchyma in their roots and rhizomes. Plants with relatively thin fibrous roots have a better performance in total nitrogen removal, and plants with high total root biomass have a better performance in NH<sup>+</sup>-N removal [69]. The root development depends upon various factors such as species, age, type of plant and concentration of nutrients, trophic status of water, nature of pollutants, redox conditions, and use of supporting mats and growth media. A high nutrient load at an earlier plant stage can be harmful to plants and can damage the root system [70].

Similarly, the high load of toxicants can also hinder the growth of the root by permanently damaging young plants. The root development of *P. australis* was constrained up to 40-cm deep after 3 years of plantation due to the toxic effects of digestate liquid fraction. On the other hand, *Typha latifolia* and *Juncus maritimus* did not establish themselves due to the high pollutant load [71].

## 3.4. Bacterial Biofilm

Bacteria have a unique ability to form biofilms, also known as epiphytic microbes. Biofilm formation begins with the attachment of free-floating microbes to gas–liquid and solid–liquid interfaces.

These biofilms have a key role in the assimilation of the biogeochemical cycles and the dynamics of an ecosystem process [72]. In the aquatic ecosystem, aquatic plants are an essential substrate for the establishment, growth, and development of biofilms. Aquatic plants release oxygen, essential for aerobic bacteria attached to roots, and stimulate the nitrogen cycle in the roots' surroundings [73,74]. Biofilms are composed of an extracellular matrix comprised of polysaccharide biopolymers, proteins, and DNA that hold the cell together [75]. The structural integrity of biofilms is obtained by secreted proteins, various types of exopolysaccharides and cell surface adhesions [76]. The development and maintenance of these biofilms rely on small molecules such as homoserine lactones, antibiotics, and secondary metabolites, such as the Staphylococcus aureus matrix, provide proteins for the synthesis of biofilm. The extracellular matrix also facilitates the formation of adhesive protein found anchored to the cell wall of *S. aureus*, holding the cells together within the biofilm by interaction with other proteins [77,78]. The extracellular DNA also strengthens the structural integrity of the biofilms. For example, *Pseudomonas aeruginosa* contains a significant amount of DNA to provide stability to biofilms [79]. The nature of biofilms and associated matrices depends upon the types of substrates, medium, and growth conditions. Bacillus subtilis, a Gram-positive bacterium, can make biofilms via production of two different polymers: polysaccharide extracellular polymeric substances and poly-d-glutamate. Both of these polymers contribute to biofilm formation; however, the contribution of each polymer is determined by strain and prevailing conditions [80]. The plants can also modify the function and structure of the microbial community in their rhizosphere [81]. The biodiversity and species of bacteria determine the functions of the biofilms. The biofilm-forming bacteria have been reported as diverse and host specific. The secretion of macrophytes and growth status can determine the bacterial composition of biofilms in the aquatic ecosystem [82]. Moreover, the bacterial community of biofilms was found to be different than those in the surrounding water column [37].

## 4. Microorganisms

Microbial communities have an essential role in the organic and inorganic pollutant removal process and plant growth promotion in FTWs (Figure 2); however, little has been explored about specific microbial species in roots and their functions in pollutant removal processes from water [83,84]. Some bacteria, such as rhizospheric bacteria, are essential for vigorous plant growth [85]. The bulk soil is the main source of these microbial populations. However, the rhizospheric bacterial population is different from the soil bacterial community [86–88]. Similarly, in FTWs, the microbes can be categorized into biofilm-forming bacteria and water column bacteria.



**Figure 2.** Role of rhizospheric and endophytic bacteria in plant growth promotion and pollutant removal processes.

In FTWs, the microbial communities mostly originate from ambient water. The amelioration and scrapping specific to the plants' roots perform a central part in the formation of specific rhizosphere microbial communities.

Actinobacteria was found to be a dominant group in the water of FTW systems; however, Proteobacteria was mainly found in the roots and biofilm samples [89]. In Proteobacteria, Alphaproteobacteria was found to be abundant in the rhizoplane of plants vegetated in FTWs, and biofilms were mostly composed of Gammaproteobacteria. The second largest phylum in water and plant root samples was *Cyanobacteria*, but it was not found in biofilm samples. In a comparison of the microbial communities in the roots of *Canna* and *Juncus*, it was found that different plants host different types of microbes in their roots. This difference reveals that plant roots secrete specific exudates and compounds, which attract specific microbial communities [89]. The plant rhizoplane in the water column attracts microbes and develops large microbial mass manifests in the shape of a thick, slimy coat on plant roots.

The presence of autotrophic microbial populations may also depend upon the presence of sunlight, although, in most cases, the floating mat covers the water surface to minimize the availability of sunlight. However, some amount of sunlight may be available under the water to support the Cyanobacterial community. However, the relative abundance of Cyanobacteria in plant root and water samples was found to be similar. In the roots of FTW plants, the genera of Cyanobacteria (*Anabaena* and *Nostochopsis*) that forms a heterocyst was abundantly observed. This indicates the ability of Cyanobacteria to associate with the roots of floating macrophytes and survive in available light conditions. In floating macrophytes, the rhizoplane was found to be enriched with sulfate-reducing bacteria [90]. In FTWs, even in aerobic conditions, anaerobic zones were found in the rhizoplane of the aquatic plants. These anaerobic microorganisms belong to sulfate-reducing bacteria and *Clostridium*. In FTWs, different sulfur oxidizers and sulfate reducers are essential to make out the sulfur cycle, yield, and depletion of hydrogen sulfide within the plant rhizoplane [70]. The sulfur-oxidizing bacteria are essential to protect the plants by the detoxification of reduced sulfides such as hydrogen sulfide.

The FTWs are efficient for nitrogen removal through denitrification by the microbial process. The nitrifiers are augmented in the aquatic root system of FTWs and responsible for ammonia oxidation. The *Nitrosomonas* and *Nitrosovibrio* (*Nitrosospira*) were found only on the plant roots of FTWs plants. The presence of *Rhizobium*, *Bradyrhizobium*, *Azorhizobium* and *Azovibrio* contributes toward nitrogen fixation within the FTWs. Several methanotrophs and methylotrophs were also found on plant roots in the FTWs [91]. These methanotrophs and methylotrophs were also abundant in the rhizosphere of terrestrial plants, and these were not specific to the aquatic plants. However, these bacteria have a key role in the rhizoplane of FTWs plants, predominantly under reduced oxygen levels [92].

Proteobacteria were found in the various rhizosphere systems [91,93–95]. The comparison between FTW plants and terrestrial plants' rhizosphere microbial communities revealed a distinctive mutualistic association of aquatic microbes with aquatic plants. *Bacillus*, a soil bacterial group, was absent in the rhizoplane of FTWs macrophytes. Similarly, Acidobacteria, the major bacterial group in the terrestrial plant, was not found in the rhizoplane of an aquatic plant [94,96]. Cyanobacteria were different in the plant's rhizosphere compared to the aquatic plant's rhizoplane [91,93,96].

*Pseudomonas* has the distinctive capability to degrade several polymers, which are difficult to demean by any other group of bacteria [97]. *Pseudomonas* has a dominant role in the degradation of polyethylene in combination with physical degradation [97]. *Pseudomonas* was found abundantly (95.5%) in a sample of floating foam from FTWs. The development of biofilms on floating mats involves a distinctive mechanism that is different from the formation of biofilm on plant roots and in water samples [97].

Ammonia oxidizing archaea (AOA) and bacteria can attach to the suspended roots in an autotrophic water environment [98]. The ammonia-oxidizing archaea and bacteria were found only on the roots as biofilms. The predominant ammonia oxidizers were ammonia-oxidizing bacteria (AOB) on the rhizoplane of macrophytes. The *Nitrosomonas europaea* and *Nitrosomonas ureae* were well

adapted to NH<sub>4</sub><sup>+</sup>-N rich environments. However, in the terrestrial ecosystem, *Nitrosospira* was found predominantly in AOB communities [98,99].

In a study on three aquatic plants, *N. peltatum*, *M. verticillatum*, *and T. japonica*, the dominant phylum detected was Proteobacteria, ranging from 37% to 83%, followed by Bacteroidetes (8–38%). The other phyla found in root biofilms were Chloroflexi, Firmicutes, and Verrucomicrobia at low frequencies. The dominant bacteria in the phylum Proteobacteria were Alphaproteobacteria, followed by Betaproteobacteria and Gammaproteobacteria. The other bacteria detected at a low frequency were Epsilonproteobacteria and Deltaproteobacteria [74].

The class Epsilonproteobacteria was found to be higher in number in vegetated sediment samples compared to un-vegetated sediments and biofilms [74]. The difference in microbial composition and epiphytic biomass may be the effect of the difference in plant exudates such as polyphenols and allopathically active compounds [100]. The plants can increase the quantity and diversity of bacterial biofilms in the aquatic ecosystem, which ultimately can promote the remediation potential of associated macrophytes [72].

Epiphytic bacterial communities are diverse and host specific. A similar phenomenon was also found in other terrestrial and aquatic plants [82,101]. The biofilms attached to roots exhibit particular niches. The difference in bacterial communities is attributed to the different growth environments such as the difference in water flow, the availability of light, and nutrients conditions [37]. Additionally, plant roots, water characteristics, sediment properties, and aquatic animals also influence the nutrient availability, types, and suitability of the environment for the bacteria. The epiphytic bacteria diversity and species richness were generally greater on roots than those on stems and leaves. Similarly, the bacterial species in vegetated sediments were more diverse than in un-vegetated sediments [74].

Similarly, the bacterial population linked with sea grassroots was different from the adjacent bulk sediment [102]. Thus, the roots of the plant may alter the bacterial community in the surrounding environment. This difference may be due to the influence of root rhizospheric zones on organic matter accumulation, chemical exudates, and oxygen concentration [22,103].

Similarly, the biofilm and sediment's microbial communities were found to be dissimilar from one another. In biofilms, the percentage of class Alphaproteobacteria was higher than in sediments. The class Epsilonproteobacteria and Deltaproteobacteria were mostly detected only in sediment. The parallel findings have been stated by other researchers who investigated the bacterial composition in the sediments of two lakes in China [104].

## 4.1. Role of Endophytes

The microorganisms residing in the roots of plants and soil also have a major contribution to the uptake of metals from the contaminated media. These microorganisms boost the breakdown of complex organic and inorganic compounds into simple nutrients, mobilize metal ions, and increase the bioavailability to plants [105–108]. These bacteria, such as rhizobacteria, stimulate the growth of plants and biomass production, and enhance plants' uptake of toxic pollutants, and the their ability to alleviate metal-induced toxicity [109,110]. Endophytic bacteria reside within different tissues of the plant [111,112], increasing the ability of plants to cope with different biotic and abiotic stresses [113]. Broadly, endophytes perform three major roles in the plant which are its protection from biotic stress, relieving abiotic stress, and supporting it by providing nutrients such as the increasing availability of nitrogen, phosphorus, and other essential elements [114]. The prior inoculation of plants with endophytes can reduce the chances of bacterial, fungal, and viral diseases, and even the damage caused by insects and nematodes [113,115]. The relationship of endophytes with host plants may be either as obligate endophytes and or facultative endophytes [112]. In stress conditions, endophytes may help the plant to relieve stress by the combined action of multiple mechanisms [116]. Direct mechanisms include siderophore production [117], antimicrobial metabolites [118], phosphate-solubilizing compounds [119], nitrogen-fixing abilities [120], and phytohormones [42,121,122]. The indirect methods include bioremediation and biocontrol [123]. It is established that certain endophytic bacteria initiate a system

known as induced systematic resistance in their host. This system is effective against different types of pathogenic bacteria, by preventing the induced bacteria from causing any visible disease symptoms in the host plant [113,124]. It is well reported that endophytes stimulate the degradation of xenobiotics and their supplementary compounds by expressing required catabolic genes. The endophytic bacteria have evolved various types of mechanisms to nullify the effect of toxic heavy metals and contaminants, such as the efflux of metal ions, the transformation of pollutants into less toxic forms, and the sequestration of metal ions on the surface of the cell [125]. Endophytes can also mitigate metal stress by promoting photosynthesis, anti-oxidative enzyme activities, modifying translocation, and the storage of heavy metal ions. The inoculation of maize with *Gaeumannomyces cylindrosporus* significantly improved the yield and productivity of maize under lead stress [126]. Similarly, *Pseudomonas aeruginosa* inoculation increases the cadmium tolerance (Cd) of plants and enhances the accumulation and translocation of Cd in inoculated plants [127].

The high concentration of toxic pollutants may cause toxicity to macrophytes, thus decreasing the efficiency of macrophytes to remediate pollutants. The endophytes may overcome this challenge. Endophytes possess plant growth-promoting (PGP) traits and degradation genes that assists the plant in handling with several environmental stresses. The endophytes contribute to the decontamination of mixed contaminants by degradation and heighten the metal translocation by the mutualistic relation of plants and endophytes [128,129]. A few studies have highlighted the application of endophytes in the macrophytes of FTWs for the treatment of sewage effluent, textile effluent, polluted river water and potentially toxic metals [25,130,131]. The major advantage of using endophytes to improve xenobiotic remediation is that it is easier to genetically modify the microorganisms for maximum pollutant degradation than the plants. Furthermore, the efficiency of the remediation process can be easily tracked by the estimation of the abundance and expression of pollutant catabolic genes in soil and plant tissues. The unique environment of plants facilitates the endophytic bacteria to make large population sizes due to the minimal competition. The pollutant is degraded by endophyte bacteria in planta, and eliminates the toxic effect on the plant [113,132].

The application of endophytes in a FTWs system, vegetated with *P. australis*, improved the remediation potential of the plant and successfully removed the toxic metals such as iron, nickel, manganese, lead and chromium from the polluted river water. These inoculated endophytes were tracked in the root/shoot interior of *P. australis*, proving their potential role in pollutant removal [131]. The specific strains of endophytic bacteria inoculated to *T. domingensis* enhanced the remediation of textile effluent [133]. Similarly, the inoculation of *Leptochloa fusca* with a consortium of three endophyte bacteria strains in CWs boosted the efficiency of plants to remediate tannery effluent. This endophytic inoculation also enhanced the growth of *L. fusca*, increased the removal of pollutants and decreased the toxicity of treated wastewater [49].

#### 4.2. Role of Rhizospheric Bacteria

The rhizospheric bacteria in FTWs have a prominent role in the degradation of organic matter, [134,135], and the translocation of potentially toxic metals [81,136,137]. This bacterial population differs qualitatively and quantitatively from those found in the bulk soil [138–140]. The microbial species in soil biota may pathologically infect the roots and rhizosphere biota [141,142]. The plant roots secrete exudates and metabolites, which chemotactically attract bacteria [143]. The rhizospheric bacteria of macrophytes in wetlands have a prominent role in the removal of pollutants [144]. The roots of the plants actually control the microbial colonies in the rhizosphere with the exchange of oxygen, CO<sub>2</sub>, nutrients, and bio-chemicals [145,146]. The iron and ammonia can be oxidized by the oxygen released from the roots [81,147]. The roots' microbial populations also have an impact on the emission of methane, as well as other gases from the wetland system [148,149]. The enzymes and organic acids released by rhizophytes modify the nutrients and make them available to roots [135].

The roots of wetland plants secrete bioactive chemicals, which favor the development of microbial communities on roots [150]. The roots can also oxidize and reduce the sulfide present in their

rhizosphere by regulating oxygen concentration, redox potential, and the release of low-nitrogen exudates such as sugar [151].

# 5. Role of Bacteria in Pollutant Removal Process

# 5.1. Nitrogen Fixation

The nitrogen fixation by microbes is a critical natural source of reactive nitrogen in the wetland ecosystem [152]. The oxygen and organic matter supply from the roots favor the enrichment of nitrogen-metabolizing microorganisms in the rhizosphere [40,153]. In the rhizosphere of wetland plants, bacteria transform the nitrogen by ammonification, nitrification, denitrification, uptake, and the anaerobic oxidation of ammonia by nitrate and nitrogen fixation [154]. The metabolic energy required for this process is obtained from the oxidation of organic matter and lithotrophy. In wetland plants, most of the nitrogen metabolism occurs at or near the roots [155,156]. The roots either take up the produced ammonia or they oxidize it into nitrites and nitrates. That oxidized nitrogen diffuses to the roots or to denitrifiers, which reduces the nitrate to  $N_2$  gas in the absence of oxygen [157]. Microbes perform an N-fixation of non-reactive  $N_2$ , and nitrogen is produced [158]. The heterotroph and autotroph prokaryotes contribute toward the production of a large amount of reactive nitrogen by nitrogen fixation [152]. The nitrogen fixation by cyanobacteria in wetlands depends upon the availability of light [152]. The important N-fixing bacterial genera are Enterobacter, Azospirillum, Pseudomonas, Klebsiella, and Vibrio in wetlands [153,159]. The heterotrophic nitrogen fixer usually makes mutual symbiosis with the roots and exchanges the sugars from the roots for ammonia that bacteria produce [152,160]. The nitrogen fixation process took place several times in the planted area of wetlands relative to the non-planted area, especially in the oxygen-deprived area of wetlands [153,161]. The same bacteria also influence nitrogen fixation and denitrification. Often, these processes take place concurrently near the roots of macrophytes [162]. The nitrogen-fixing bacteria dwell on the roots or in the rhizosphere of most of the aquatic macrophytes such as P. australis, J. effusus, J. balticus, Sagittaria triflolia, Zostera marina [163–165]. Roots also contribute to nitrogen fixation by reducing nitrogen from their rhizosphere, adjusting the pH level and redox potential [151]. Nitrogen-fixing microorganisms, such as *Azospirillum*, reside in the rhizosphere; these stimulate hormones, such as auxins, to influence the pH and redox potential and boost the nitrogen fixation process [161].

# 5.2. Degradation of Organic Pollutants

Microbes are known as bio-remediators due to their capability to break down virtually all classes of organic pollutants [166–168]. Microbes degrade the organic pollutants by a process of co-metabolism. In this process, microbes in the rhizospheric zone of aquatic and terrestrial plants degrade the complex carbon-based compounds in order to obtain organic carbon and electron acceptors [169]. In natural water, the biodegradation rate depends upon the microbial population and amount of xenobiotics [170], and the numbers of the microbes are heavily influenced by the macrophyte species [171]. Plants give organic carbon to microbes present in the rhizosphere that assist them to degrade complex organic compounds [172], such as hydrocarbons and aromatic hydrocarbons [173,174]. Bacteria also release indole acetic acid (IAA) to improve plant growth [175]. Many bacteria isolated from aquatic plants also showed pollutant degradation and plant growth-promoting activities [176,177]. The biofilms attached to aquatic plants are capable of degrading organics such as phenolics, amines, and aliphatic aldehydes [178]. Additionally, these biofilms are capable of degrading dissolved organic matter such as polychlorinated biphenyls (PCBs) and atrazine [54,179,180]. The aquatic plant rhizosphere is also enriched with methanotrophs containing a collection of Proteobacteria, which utilize methane for obtaining carbon and energy [181]. Methanotrophs can degrade numerous types of harmful organic complexes [182,183] such as chlorinated ethenes by enzymatic reactions. The Eichhornia crassipes can remediate eutrophic water by influencing the production of gaseous nitrogen [184,185].

## 5.3. Removal of Heavy Metals

The rhizospheric and endophytic bacteria have been reported to play a prominent part in the removal of heavy metals (Table 1). Bacteria promote the removal of metals by their ability to sorb the metallic ion into their cell walls [186]. Metal uptake by plants can be enhanced by bacteria, which increase the bioavailability of metals to plants [187,188]. The microorganisms can accumulate heavy metals with the help of specific metal-binding proteins and peptides such as metallothionein and phytochelatins [189]. The transcription factors of metal-binding proteins facilitate the hormone and redox signaling process upon exposure to toxic metals in the context of toxic metal exposure [190]. Cyanobacteria decrease the metal toxicity by the production of proteins that can bind metals [191]. The genetically modified *Ralstonia eutropha* can reduce the harmful Cd (II) by the production of metallothionein on the surface of the cell [192]. Likewise, *Escherichia coli* regulates the accumulated Cd toxicity by the production of many proteins and peptides [193]. The production of metallo-regulatory protein is a natural resistant method against arsenic (As) and mercury (Hg) in microorganisms [46].

The metal toxicity affects the performance of the phytoremediation process [194]. Microorganisms augment and facilitate plants to make heavy metals and antibiotic-resistant proteins [195]. The antibiotic-resistant proteins can reduce the abiotic and biotic stress induced by metals. Some of the *Bacillus* sp. strains have the ability to devise a mechanism to alleviate the metal stress by an active transport efflux pump [194]. The endophytic bacteria also influence the functional and phenotypic characteristics of the plants in which they reside [196]. Moreover, these bacteria influence the activity of plant antioxidant enzymes and lipid peroxidation, which support the plant resistance system, particularly resisting the oxidative stress in the plants caused by heavy metals [197,198]. Methylation can also be used by a few endophytic bacteria to induce the defense and detoxification of metals. Few gram-negative bacteria possess the specific mercury-resistant (*Mer*) operon gene for the degradation of organic mercurials and reductions in Hg<sup>+2</sup> [199].

Bacteria	Metal	Reference
Lactobacillus delbrueckii and Streptococcus thermophillus	Fe, Zn	[200]
Acinetobacter sp., Bacillus megaterium and Sphingobacterium sp.	Fe, Mn	[201]
Anoxybacillus flavithermus	Fe, Cu	[202]
Leptothrix, Pseudomonas, Hyphomicrobium and Planctomyces	Mn	[203]
Methylobacterium organophilum	Cu, Pb	[204]
Herminiimonas arsenicoxydans	As	[205]
Enterobacter cloaceae	Cd, Cu, Cr	[206]
Acetobacter	Pb, Cu, Mn, Zn, Co	[207]
Chryseomonas luteola	Cd, Co, Cu, Ni	[208]
Ochrobactrum anthropi	Cr, Cu	[209]
Anabaena spiroides	Mn	[210]
Ralstonia solanacearum	Pb	[211]
Proteobacteria and Bacteroidetes	Cu	[212]
Bacillus cereus	Cu	[213]
Bacillus licheniformis	Pb	[214]
Ralstonia solanacearum	Pb	[211]
Enterobacter aerogenes	Cd	[215]
SPseudomonas azotoformans	Cd, Cu, Pb	[216]

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#### 5.4. Metal Biosorption and Bioaccumulation

Generally, bacteria perform metal ion biosorption into their cell wall by two processes, which are passive and active [217]. Passive biosorption takes place in the cell walls of living and dead/inactive bacterial cells, supported by multiple metabolism processes [218]. The reaction between the functional groups (e.g., amine, amide, carbonyl, hydroxyl, sulfonate, etc.) of the cell wall and metal ions causes the adsorption of metal ions to the cell surface [106]. In the metal ion binding process, different mechanisms (e.g., ion exchange, sorption, complexation, chelation and micro-precipitation) may be involved independently or synergistically [219].

On the other hand, in the active biosorption process, metal ions are up taken by living cells. The fate of metals that enter the inside of living cells depends upon the organisms and specific elements. The elements can be bound, stored, precipitated, and sequestered in some specific intracellular organelles and may be transported to a particular structure [106,220].

The endophytic bacteria exhibited outstanding heavy metal bioaccumulation and detoxification abilities [59,221]. The plant–bacteria symbiotic relation improves the phytoremediation potential of plants by the increased uptake of heavy metals due to the secretion of organic acid by bacteria. These organic acids secrete, by bacterial influence, the pH of the system and increase the bioavailability of the metal ions to plants [222]. For example, the application of endophytic bacteria, *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16, on *Brassica napus* increased the Pb accumulation in plant shoots [223]. *Saccharomyces cerevisiae*, commonly known as baker's yeast, is a successful bio-sorbent for the removal of Zn and Cd due to its ion exchange mechanism [224,225]. Similarly, *Cunninghamella elegans* has been proven an efficient sorbent for the remediation of textile effluent enriched with heavy metals [226].

Bacteria also produce biosurfactants and release them as root exudates. These biosurfactants enhance the bioavailability of metals in the soil and aquatic medium by their interaction and complexation with insoluble metals [227]. On the other hand, the extracellular polymeric substances, mainly composed of proteins, polysaccharides, nucleic acid, and lipids, perform a key part in the complexation of metals and reduce their bioavailability [125]. For example, *Azobacter* sp. formed complexes with chromium and cadmium by the formation of extracellular polymeric substances (EPS) and decreased the uptake of metals by *Triticum aestivum* [228]. The secretion of different metabolites such as siderophores and organic acids (including citric acids, oxalic acid, and acetic acid) influences heavy metals' bioavailability and their translocation in plants [229,230]. In an earlier study, the inoculation of the endophytic bacterium (*Pseudomonas* sp.) improved the plant's growth and increased the nickel (Ni) accumulation in the plant [220].

# 6. Role of Fungi

Fungi perform a potential role in the remediation of heavy metals by increasing their bioavailability and transformation into less toxic forms [231–233]. Some fungi, such as *Klebsiella oxytoca*, *Allescheriella* sp., *Stachybotrys* sp., *Phlebia* sp. *Pleurotus pulmonarius* and *Botryosphaeria rhodina*, have the capacity to bind metals [234]. Fungal species like *Aspergillus parasitica* and *Cephalosporium aphidicola* can remediate lead-contaminated soil by their biosorption process [235,236]. The fungi *Hymenoscyphus ericae*, *Neocosmospora vasinfecta* and *Verticillum terrestre* showed resistance to Hg and the ability to transform the toxic state of Hg (II) to a non-toxic form [237]. Fungi of the genera *Penicillium*, *Aspergillus*, and *Rhizopus*, have proven efficient in heavy metal removal from polluted water [238,239].

Fungi link closely with the roots in wetland plants and have a significant influence on wetland functioning [240,241]. Root exudates attract fungi toward the rhizosphere. The roots and fungi in wetland plants make multilevel physical, chemical, hormonal, and genetic interactions, which may be species specific [242,243]. The rhizospheric fungi community is different than soil communities. The types and interactions of the fungal community with the rhizosphere may be influenced by plant species, soil characteristics, climate, type of water, and other microorganisms [244]. The plant–fungi association in wetland plants performs different key functions such as the emission of metal-chelating siderophores, denitrification and metal detoxification [245,246]. Bacteria can easily stick to the surface

of the substrate compared to algae due to their smaller size [247]. The other reason for the high ratio of attachment of epiphytic bacteria to aquatic plants compared to algae is the specific metabolites released from the plants [184,248].

## 7. Role of Inoculated Bacteria

It is well established that plant-bacteria synergism is essential to enhance the phytoremediation potential of plants and ultimately FTWs (Table 2) [49,249,250]. The inoculation of FTWs by immobilized denitrifiers greatly improved the nitrogen removal from wastewater [61]. Endophytes can be isolated from and within various plant tissues that include roots, stems, leaves, flower, fruit, and seed [112]. The root is the main source of endophytes, and legume root nodules have a large diversity of endophytes [251]. Some plants have an underground stem, so, in these plants, stem and root endophytes may be similar [252]. Bacterial endophytes that were obtained from the shoot of sugarcane promoted fixation as well as acetylene reduction activities [253]. The inoculation method affects bacterial colonization, and inoculation should be performed appropriately [254]. Nonetheless, no standard method is defined for the inoculation of plant roots in FTWs. The two common methods of inoculation are the inoculation of seeds and the inoculation of soil [252,255,256]. In seed inoculation, the inoculum is introduced into host plants directly when they are in the seed or seedling stage. The soil inoculation is done directly in root media or the pot in which the plant is growing. In FTWs, the roots of the plant are inoculated directly by pouring the inoculum in the water near the root of the plant. For example, Shahid et al. (2019a) prepared the inoculum of five different rhizospheric and endophytic bacterial strains and inoculated the roots of plants by directly adding a specific amount of inoculum into the water [20]. Previously, many attempts have been performed to create an effective partnership between plant and metal-resistant bacteria in order to effectively treat water contaminated with heavy metals [250,257,258]. FTWs vegetated with *Brachia mutica* and inoculated with bacteria were used to treat sewage effluent and it was found that the concentration of heavy metals, including Cd, Fe, Cu, Cr, Mn, Co and Pb, decreased significantly from the effluent. The removal of iron was significant (79 to 85%) [259]. Similarly, in another study, a consortium of hydrocarbon-degrading bacteria was added into the hydrocarbon-enriched water for its remediation by FTWs [260]. The inoculation of these rhizospheric and endophytic bacteria was reported to enhance the degradation of hydrocarbons, and also improved the efficiency of the FTWs.

Bacteria/Bacterial Biofilm	Nature of Bacteria	Plant	Plant-Bacteria Interaction	Summary	Reference
Bacterial Biofilm	_	Ipomoea aquatic and Corbicula fluminea	_	The removal efficiencies of TN, NH <sub>4</sub> <sup>+</sup> -N, TP, total organic carbon (TOC), Chl- <i>a</i> , total microcystin-LR and extracellular microcystin-LR were $52.7\%$ , $33.7\%$ , $54.5\%$ , $49.2\%$ , $80.2\%$ , $77.4\%$ and $68.0\%$ , respectively.	[261]
Proteobacteria	Nitrosomonadaceae	<i>Canna Indica</i> and Iris pseudacorus	Bacteria were mainly attached on the fiber filling of floating mat and plant roots	The average removal efficiencies of chemical oxygen demand (COD), TN, NH <sub>3</sub> -N and TP for <i>Canna indica</i> set-up were 23.1%, 15.3%, 18.1% and 19.4% higher, respectively, than that of the setup with only substrate, and 14.2%, 12.8%, 7.9% and 11.9% higher than <i>Iris pseudacorus</i> . FTWs.	[262]
Nitrifying and Denitrifying	Carrying <i>nirS</i> , <i>nirK</i> and <i>amoA</i> genes	Unplanted	Specific microbial communities were visualized with denaturing gradient gel electrophores (DGGE)	COD was efficiently removed in all systems examined (>90% removal). Ammonia was efficiently removed by nitrification. Removal of total dissolved nitrogen was ~50% by day 28	[22]
Biofilms	_	Carex virgate, Cyperus ustulatus, Juncus edgariae, and Schoenoplectus tabemaemontani	Biofilm performed a key role in the removal of Cu, P and FSS. Plant roots and biofilm interaction enhanced metal speciation	The presence of a planted floating mat with biofilms improved removal of copper (>six-fold), fine suspended particles (~threefold reduction in turbidity) and dissolved reactive P compared to the control.	[11]
Ammonifying bacterial strains	Engineering bacterial strain	Cymbidium faberi	The ammonifying bacteria adhered to plants roots enhanced oxygen supply to microorganism involved in nitrification process and increased capacity of plants roots to absorb ammonia nitrogen.	The organic nitrogen decomposition rate was up to 86.50% by adding the strain agent while it was 75.66% without them in the control test group in FTWs	[263]
Adsorptive biofilm	Natural	Thalia dealbata	Combined action of plant and biofilms	The average removal rates for TN, $NH_4^+$ -N, $NO_3^-$ -N, $NO_2^-$ -N, TP and chlorophyll-a in summer–autumn season were 36.9%, 44.8%, 25.6%, 53.2%, 43.3% and 64.5%, respectively, effectively reduced the concentrations of total suspended solids (TSS), <i>Escherichia coli</i> and heavy metals.	[55]
Photosynthetic bacteria	_	Vetiveria zizanioids	Combined action of plant and inoculated bacteria improved purifying effect of FTWs	Efficiently removed TN and TP	[264]
Biofilm Reactor	Protozoa and Metazoa	Bambusoideae	In the batch reactor, COD was mainly removed by the biofilm on the filamentous bamboo	The removal rate of the COD, NH4 <sup>+</sup> –N, turbidity, and total bacteria were 11.2–74.3%, 2.2–56.1%, 20–100%	[265]

Table 2. Application of bacteria to enhance phytoremediation potential of floating treatment wetland	nds.
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Bacteria/Bacterial Biofilm	Nature of Bacteria	Plant	Plant–Bacteria Interaction	Summary	Reference
Acinetobacter sp.	Perchlorate reducing bacterium	Pistia stratiotes	Phyto-accumulation and rhizo-degradation were key mechanisms involved in perchlorate removal	<i>Pistia</i> showed 63.8 ± 4% (w/v) removal of 5 mg/L level perchlorate in 7 days	[266]
Denitrifying polyphosphate accumulating microorganisms	_	Festuca arundinacea	Improved the growth of plant and biomass	The average removal rates were 86.32%, 93.60%, 90.12%, 72.09%, and 84.29%, respectively, for NH4 <sup>+</sup> -N, NO <sub>3</sub> <sup>-</sup> -N, TN, TP, and ortho-P.	[267]
Acinetobacter, Bacillus cereus and Bacillus licheniformis	Endophytic bacteria	Brachiaria mutica	The inoculated bacteria showed persistence in water as well as successfully colonized the root and shoots of the plants	Maximum reduction in COD, biological oxygen demand (BOD <sub>5</sub> ), TN, and PO <sub>4</sub> was achieved by the combined use of plants and bacteria.	[259]
Biofilms	Natural	Juncus effuses Carex riparia	Metals were found in the root biofilm, probably due to microbial respiration activity	Analysis showed Ni concentration in leaves were between 23 and 31 $\mu$ g/g dry matter, and between 113 and 131 $\mu$ g/g in roots. Accumulation of Zn was 45-80 $\mu$ g/g in leaves and 168–210 $\mu$ g/g in roots.	[14]
Klebsiella sp., Pseudomonas sp. and Acinetobacter sp.	Endophytic Bacteria	Typha domingensis	Possessed pollutant-degrading and plant growth-promoting abilities and successful survival of bacteria was found in plant tissues	The average reduction in COD and $BOD_5$ was 87% and 87.5%, and significantly removed heavy metals.	[26]
Biofilm	Nitrifying and denitrifying bacteria	Canna indica	Improved nitrification and denitrification process and overall high removal of total nitrogen	Significantly higher removal rates of ammonia nitrogen (85.2%), total phosphorus (82.7%), and orthophosphate (82.5%) were observed	[18]
The community was mainly composed of Cyanobacteria, Proteobacteria, Bacteroidetes, Planctomycetes, Firmicutes, Actinobacteria, Chlorobi and Acidobacteria.	Periphyton	_	Improved its nutrient removal capacity	Successfully maintained TN and TP concentration in the river water at less than 2.0 and 0.02 mg $\rm L^{-1}$ respectively	[268]
Dechloromonas, Thiobacillus and Nitrospira	Heterotrophic and autotrophic	_	Mixotrophic denitrification occurred in auto and heterotrophic bacteria	About 89.4% of the TN was removed from autotrophic coupled floating wetlands, and 88.5% from heterotrophic enhanced floating wetlands	[39]
Bacillus subtilis, Klebsiella sp., Acinetobacter Junii and Acinetobacter sp.	Hydrocarbon degrading bacteria	Brachiara mutica and Phragmites australis	Alkane-degrading gene ( <i>alkB</i> ) abundance confirmed microbial growth in plant's root and shoot and in water.	Reduced oil content (97%), COD (93%), and BOD (97%), in wastewater	[260]
Acinetobacter Iwofii, Bacillus cereus, and Pseudomonas sp.	Phenol-degrading bacteria	Typha domingensis	The inoculated bacteria showed successful colonization and survival in the rhizosphere, root interior and shoot interior of the plant and enhanced plant growth and biomass	Bacterial augmentation enhanced the removal potential significantly, i.e., 0.146 g/m²/day vs. 0.166 g/m²/day without bacterial inoculation	[269]

# Table 2. Cont.

Bacteria/Bacterial Biofilm	Nature of Bacteria	Plant	Plant–Bacteria Interaction	Summary	Reference
Acinetobacter Iwofii, Bacillus cereus, and Pseudomonas sp.	Phenol degrading bacteria	Phragmite australis	Improved plant biomass and high rate of inoculated bacteria survival observed in plant roots, shoot and water	Plant-bacteria synergism significantly improved the phenol degradation and removal. Highest reduction in COD, BOD, and TOC was achieved by bacterial augmentation	[270]
Acinetobacter, Acinetobacter sp., and Bacillus niabensis	Hydrocarbons degrading bacteria	Leptochloa fusca	Achieved successful degradation of Hexadecane The Inoculated bacteria displayed highest persistence in the roots followed by shoots and then in the wastewater and improved plant growth promoting (PGP) activities	Hydrocarbons degradation was recorded up to 92%, COD was reduced up to 95%, BOD up to 84%, and TDS up to 47% and alleviated the toxicity	[41]
Archaea, anaerobic ammonium oxidation (Anammox) bacteria	Natural	Oenanthe javanica	High abundance and diversity of bacteria in planted floating wetland	The average removal rates of $NH_4^+$ -N, $NO_3^-$ -N and total nitrogen were 78.3, 44.4 and 49.7% respectively	[44]
Proteobacteria Actinobacteria Cyanobacteria, and <i>Rhizorhapis</i>	_	Eichhornia crassipes	Bacteria were involved in pollutant degradation and nutrients removal	Suspended solids, TN, TP, $NO_3^-N$ and COD was 86%, 75%, 80%, 95% and 84%, respectively.	[271]
Bacillus subtilis, Klebsiella sp., Acinetobacter Junii, and Acinetobacter sp.	Hydrocarbon degrading bacteria	Typha domingensis and Leptochloa fusca	Persistence of bacteria and expression of the alkB gene in the rhizoplane of inoculated plants	Reduction in hydrocarbon (95%), COD (90%), and BOD content (93%)	[272]
Acinetobacter junii, Pseudomonas indoloxydans, and Rhodococcus sp.	Rhizospheric and endophytes	Phragmites australis and Typha domingensis	Removal efficiency was further enhanced by augmentation with bacteria and promoted plant growth	Color, COD and BOD after an 8-day period were 97, 87 and 92%, respectively, 87–99% reduction in heavy metals	[273]
Consortium of five strains namely Aeromonas salmonicida, Bacillus cerus, Pseudomonas indoloxydans, Pseudomonas gessardii, and Rhodococcus sp.	Rhizospheric and endophytes	Phragmites australis and Brachia mutica	Persistence and survival of inoculated bacteria in roots and shoots, and inoculated bacteria improved the plant growth and biomass production	Reduced COD, BOD <sub>5</sub> , and TOC up to 85.9%, 83.3%, and 86.6% in 96 h, respectively. TN was reduced from 37.5 to 2.07 mg l <sup>-1</sup> , N from 33.3 to 1.23 mg l <sup>-1</sup> , and TP from 2.63 to 0.53 mg l <sup>-1</sup> . Trace metals were also reduced up to 79.5% for iron, 91.4% for nickel, 91.8% for manganese, 36.14% for lead, and 85.19% for chromium.	[20]
Acinetobacter juniistrain, Rhodococcus sp. strain, and Pseudomonas indoloxydans	Dye degrading bacteria	Phragmites australis	The inoculated bacteria showed persistence in water, roots and shoots of inoculated plants of FTWs	The COD was reduced to 92%, BOD to 91%, color to 86%, and trace metals to approximately 87% in the treated wastewater.	[274]
Bacillus cerus, Cyperus laevigatus, Aeromonas salmonicida and Pseudomonas gessardii,	Rhizospheric and endophytes	Typha domingensis and Leptochloa fusca	Improved remediation performance of inoculated plants, inoculated bacteria were found in root and shoots of inoculated plants	The TN, $NO_3^{-1}$ and TP contents decreased to 1.77 mg l <sup>-1</sup> , 0.80 mg l <sup>-1</sup> and 0.60 mg l <sup>-1</sup> , respectively. Additionally, the concentration of iron, nickel, manganese, lead, and chromium in the water lowered to 0.41, 0.16, 0.10, 0.25, and 0.08 mg l <sup>-1</sup> ,	[131]
These strains were Ochrobactrum intermedium, Microbacterium oryzae, Pseudomonas, Acinetobacter sp., Klebsiella sp., Acinetobacter sp., P. aeruginosa, Bacillus subtilus, and Acinetobacter junii	Bacteria possessing capabilities of hydrocarbon degradation, rhamnolipid production, and plant growth promotion.	Phragmites australis, Typha domingensis, Leptochloa fusca, and Brachiaria mutica	Produced biosurfactants and promoted plant growth. Bacteria showed persistent in the rhizoplane, roots and shoots of plants	Reduced COD, BOD, TDS, hydrocarbon content, and heavy metals by 97.4%, 98.9%, 82.4%, 99.1%, and 80%, respectively, within 18 months.	[25]

## 8. Conclusions

Microbes, bacteria and algae are the major components of epiphytic microbes, which colonize the lower surface of floating plants. Bacterial biofilm has a crucial role in the removal of organics, inorganics and metals in FTW systems. The plant species and pollutant concentration in wastewater influence the nature and diversity of bacteria. Furthermore, the availability of nutrients influences the metabolism of bacteria and the pollutant removal efficiency. The rhizosphere and endophytes both have a prominent role in the pollutant removal process. The rhizospheric bacteria mostly remove the pollutants near the root system, whereas the endophytes mostly remove the pollutants inside the roots and shoots. The rhizospheric and endophytic bacterial community also enhances the pollutant removal process by alleviating the pollutant stress, increasing tolerance towards environmental changes, and regulating plant growth by direct and indirect mechanisms. The inoculation of plant roots with specific strains of bacteria also boosts the pollutant removal process.

It is clear from this information that plant–microbe interaction is vital for the pollutant removal process in FTWs. There is a need to conduct further research to gain a better understanding of specific microbe and plant interactions and their beneficial role in the pollutant removal process in the aquatic ecosystem. Environmental factors such as temperature, pH, and the availability of nutrients have a profound effect on the pollutant removal abilities of microorganisms. These factors need further investigation to achieve the optimal performance of microorganisms in FTWs. The nature of pollutants affects the persistence and survival of bacteria and may determine the type of bacterial communities in a wetland system. Bacteria specific to the removal of particular types of pollutants need to be identified and isolated for their future application in FTWs. Bacteria that are easy to culture in the lab with minimal prerequisites, which possess the potential to treat a diverse range of pollutants and can be augmented with diverse macrophytes in FTWs, need to be widely explored for their use in FTWs.

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