Chapter 14
Microbial Communities in Constructed Wetland Microcosms and Their Role in Treatment of Domestic Wastewater

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Abstract Microbial biomass is the main reducer for majority of organics and nutrients. The aerobic region of constructed wetland microcosms (CWMs) is majorly characterized by presence of *Nitrosomonas* and *Pseudomonas spp.* The diversity of ammonia-oxidizers mainly *Nitrosospira sp.* is higher in CWMs designed to treat domestic wastewater as compared to other bacteria studied. The activity of enzymes within CWMs is a key indicator towards role of microbial community. Rhizospheric region has diverse elements that comprises minerals, sugars, vitamins, organic acids, polysaccharides, phenol and various other organic materials that encourages the microbial groups to degrade wastewater pollutants. The presence of macrophytes has significant effects on microbial richness and community structure. The root exudates liberated by macrophytes are also able to alter the richness and diversity of the microbial population. The decomposition rates of microbes become slow as temperatures drop, which can be optimized by increasing the size of wetlands to accomplish the slower reaction rates. The pH of wastewater has also a strong effect on various microbially mediated reactions and processes. Temperature, hydrologic conditions, macrophytic diversity/richness and biotic succession strongly impact the microbial community structure. A little alteration in the diversity or community structure of the microorganisms directly affects the treatment performance of CWMs.

Keywords Microbial diversity/richness · Constructed wetland microcosms · Removal efficiency · Enzyme activity · Macrophytes

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14.1 Introduction

Water scarcity is the major driver of wastewater reclamation, particularly in arid and semi-arid zones. Reclamation of wastewater has turned out to be an alternate source of water supply for several non-potable requirements in several countries including the USA (US Environmental Protection Agency (US EPA), National Risk Management Research Laboratory, US Agency for International Development 2012; World Bank Group 2013). In the USA, around 7–8% of wastewater after treatment through Constructed Wetlands (CWs) has been reused mostly for agriculture and urban irrigation (US Environmental Protection Agency (US EPA), National Risk Management Research Laboratory, US Agency for International Development 2012). In North Africa, majority of wastewater reused in agriculture and for landscaping entails many undesirable effects on human and ecosystem health (World Bank Group 2013). In the United Arab Emirates (UAE), majority of the wastewater treatment is followed by biological means and utilized to reform the landscape (AlMulla 2016). Around 70% of wastewater generated from household activities in Israel has been reused for several activities (US Environmental Protection Agency (US EPA), National Risk Management Research Laboratory, US Agency for International Development 2012).

Many water reuse programs were initiated in the USA towards expensive nutrient removal mechanisms from secondary wastewater, consequently removing or decreasing wastewater discharge. Climate change and environmental sustenance concerns play a key role in reclamation of wastewater (Tram Vo et al. 2014). During previous decades, policy developers in several nations have expressed plans/guidelines favoring development and implementation of projects linked with reclamation of wastewater. World Health Organization (WHO) provided guiding principles related to human health by the wastewater used for agricultural and aquacultural activities in 1989 which was amended in 2006 for the proper and safe use of wastewater (WHO 2006). Around 30 states and one territory of US till September 2012 have implemented guidelines and 15 states have designed standards that administrate water reuse. China has reformed its water policy to boost the utilization of treated wastewater (Chang et al. 2013).

The Australian council of engineering and innovation has developed wastewater reuse guidelines for metropolitan wastewater improvements (Radcliffe 2006). The Royal Decree (RD) of Spain has recognized the legal charter for reuse of wastewater (Vera et al. 2013; Avila et al. 2015). Constructed wetland microcosm (CWM) is a shallow basin occupied with substrate material, generally soil, gravel, or sand, and planted with macrophytes that can adopt indigenous climatic settings and stand with waterlogged situations (Kumar and Dutta 2019a). A working model of CWM is shown in Fig. 14.1. The microbial biomass is the main known factor for removal of organics and nutrients. Particulate and dissolved organics are actively converted into CO_2 and water by bacteria, fungi and actinomycetes. Macrophytes play a vital role in removal processes by diffusing atmospheric oxygen (O_2) into their root system that are used by the microbes to decompose wastewater contaminants.

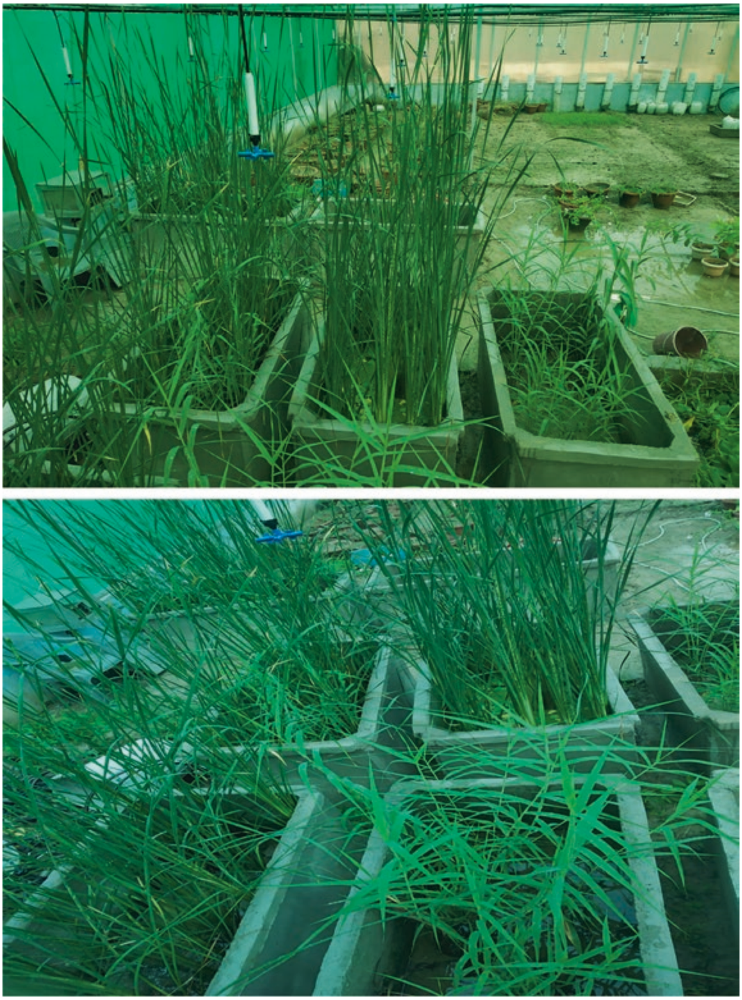

Fig. 14.1 CWM units working successfully for the treatment of domestic wastewater at Babasaheb Bhimrao Ambedkar University, Lucknow

Macrophytes provide several functions such as stabilization of the surface of beds, clogging prevention, offers opportunity for physical filtration, insulation during winters, and provide surface for microbial attachment (Valipour and Ahn 2016).

Basically, the contaminants removal comprises three processes namely biological, physical and chemical methods. Physical processes comprise mainly filtration and sedimentation. Chemical processes include bio-transformation, dividing unionized and ionized acids and bases, cation exchange, solubility of solids and gases. Nitrification, denitrification, photosynthesis, respiration, fermentation, and microbial phosphorus eliminations are biological process (Mitchell and McNevin 2001). The functions of CWMs are extremely dependent on microbial population such as bacteria, fungi and protozoans and their metabolic rate (Wetzel 1993). Pollutants transformation is the major microbial mechanism which directly takes part in treatment of wastewater and alters the redox potential of the substrate (Kumar and Dutta 2019b). In fact, microorganisms self-sustain according to the features of supporting media and remain in dormant stage for several years if ecological conditions are unfavorable (Hilton 1993). It is known that noxious elements like heavy metals and pesticides might have deleterious effects on microbial population present in a CWM. Activity of microbes also gets influenced by the oxygen in the root zone.

Rhizospheric region is well known for their rich microbial activities such as macrophyte–microbe interactions and interaction with supportive materials and pollutants. It is the area where majority of biological reactions take place because of close interaction among supportive materials and roots of macrophytes. It is also recognized as a region with diverse elements such as minerals, vitamins, sugars, organic acids, phenol, polysaccharides, carbon compounds and enzymes and various other materials that encourage the microbial groups to degrade organic pollutants (Bertin et al. 2003; Faulwetter et al. 2009; Miersch et al. 2001). A diverse range of microorganism groups perform and influence the performance of CWMs (Dong and Reddy 2010; Ibekwe et al. 2003; Long et al. 2016). Mobilizing of nutrients is facilitated by the rhizodeposition products. Hoffland et al. (1992) evaluated that the solubility of iron and phosphate was increased by organic acid produced by different plants under nutrient limiting conditions; therefore, the nutrient uptake capacity of macrophytes is improved.

Microbial population used organics as substrates and expelled vitamins to encourage growth of microbes that is known as 'rhizosphere effect'. Microorganisms attached with the substratum develop biofilms that are habitually present inside a self-produced medium of extracellular polymeric substance (EPS) (Kumar and Dutta 2019b). Most of nitrogen from wastewater is removed by microbial communities by influencing anammox and nitrification-denitrification mechanisms within CWMs (Kröger et al. 2012; Oehl et al. 2004). However, removal of phosphorus through mineralization and immobilization is also partially affected by microbial activities (Truu et al. 2005).

14.2 Microorganisms in CWMs and Their Role in Treatment Process

Characterization of the microbial groups in CWMs offers valuable information about understanding their role in treatment efficiency (Zhong et al. 2015). The microorganisms require 75–100 days to develop communities in CWMs system with sand filters (Truu et al. 2009; Weber and Legge 2011), in which denitrifying bacteria take 75 days and ammonium- oxidizing bacteria require 95 days for their development (Wang et al. 2016). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) is an advantageous tool for evaluating diversity of microbial community and their structure in CWMs (Ibekwe et al. 2003). This technique was firstly applied by Muyzer et al. (1993) in microbial ecology, and later by Adrados et al. (2014) to evaluate the structure of bacterial community in CWs. Additional significant mechanism is microbial biomass carbon (MBC) that is generally used to measure microbial biomass.

MBC is a subsidiary method to measure the microbial density and has been considered to be a valuable parameter for assessing the contaminant removal effectiveness in CWs (Truu et al. 2009). Therefore, the MBC can offer meaningful information about the microbes in CWs. However, several studies on MBC have been directed toward their relevance to environmental factors, such as land use, macrophytic species and seasons (Calheiros et al. 2009a, b). Earlier findings revealed that the microorganisms show diverse characteristics for the elimination of contaminants (Ahn et al. 2007; Krasnits et al. 2009). These microorganisms have been regarded as the main determining factor for water quality enhancement (Calheiros et al. 2009a, b; Faulwetter et al. 2009). Several other factors such as temperature, macrophytic diversity (Zhang et al. 2010, 2011a, b), hydrologic conditions (Mentzer et al. 2006; Steenwerth et al. 2006), and biotic succession (Kent et al. 2007) impact the structure of microbial community. The quantity of oxygen and root exudates useful for microorganisms in the rhizospheric zone has been found to differ by macrophytic species (Laskov et al. 2006). Majority of bacterial population is found in the rhizosphere region.

Several studies reported that the *Nitrosomonas* species is dominant in the plant roots zone, increasing the removal of nitrogen (Puigagut et al. 2008). Appropriate macrophyte species is vital to determine the microbial structure that is eventually responsible for the removal process. It is reported that the macrophytes which are able to tolerate high salinity (halotolerant), advance the treatment efficiency of CWMs (Wiessner et al. 2005; Wu et al. 2009), through liberating oxygen and making healthier environments for aerobic halotolerant microorganisms (Wu et al. 2012; Xiong et al. 2011). The arrangement of microbial groups in different zones of soil substrate in CWMs designed for the treatment of domestic wastewater was observed by Truu et al. (2005). Later they found that the wetland depth is a key component that affects the activity and community structure (Truu et al. 2009). Several previous investigations have reported microbial diversity in laboratory scale as well as in full-scale CWs (Calheiros et al. 2009a, b; Krasnits et al. 2009; Sleytr et al. 2009; Dong and Reddy 2010; Zhang et al. 2010).

However, the information about how the diversity of microorganisms changed in the long-term operations is still inadequate (Adrados et al. 2014). The roots of macrophytes play a central role in developing structure of bacterial community (Faulwetter et al. 2013). Zhang et al. (2011a, b). It is confirmed that root exudates of macrophytes such as *Thalia dealbata* inhibited the growth and development of the Cyanobacteria. Presence of excess nutrients and other noxious substances in a wetland system distresses the biofilms and their arrangements (Calheiros et al. 2009a, b). The study carried out by Llanos-Lizcano et al. (2019) characterized a total of 180 (65 anaerobic and 115 aerobic) heterotrophic bacteria from rhizosphere of *C. articulatus* and *Thalia geniculata* together with non-planted constructed wetland, in which water flows horizontally.

Peralta et al. (2013) also described parallel outcomes about the bacterial communities in different types of constructed and natural wetlands; they also found that the α-Proteobacteria was the most dominant class, followed by γ-Proteobacteria and β-Proteobacteria. CWMs with different phosphorus loading rates were characterized for microbial populations by Ahn et al. (2007) using length heterogeneity PCR (LH-PCR) relay on the 16S rRNA gene. The outcome of this study was that the sediment has abundant α-Proteobacteria (about 48–60%) and then Actinobacteria and Firmicutes and remains constant throughout the study. Several other researchers have evaluated numerous connections between bacterial diversity, pollutants loading rates and removal efficiencies in CWMs (Wu et al. 2016; Zhi et al. 2015; Zhang et al. 2016).

14.3 Microbially Mediated Reactions in CWMs

The filtration is the most significant removal mechanism working in CWMs, whereas some microorganism-mediated procedures, biochemical networks, sedimentation, volatilization, photodegradation, sorption, transpiration flux and plant uptake also enhance the performance (Bitton 2005; Morvannou et al. 2014). It is reported that some bacterial populations in animal waste actively function for decomposition of several organic compounds and to suppress pathogens. One such bacterial group namely chemolithotrophic ammonia-oxidizing bacteria take part in several chemical and biological breakdown of ammonium. They are responsible for conversion of ammonium (NH_4) to nitrate (NO_3) through nitrogen cycling (Behrends et al. 2001; Ansola et al. 2014). Oved et al. (2001) reported the influence of urban wastewater on the function and community structure of these bacteria in soil substrate via denaturing gradient gel electrophoresis (DGGE).

The result showed a substantial and constant change in the structure of communities of these bacteria with *Nitrosomonas* dominated effluent. It is also reported that the diversity of these ammonia-oxidizing bacteria mainly *Nitrosospira sp.* was higher in CWMs as compared to other bacteria studied. Therefore, it is evaluated that the diversity of ammonia-oxidizing bacteria inside a constructed wetland system play key role on its stability (Rowan et al. 2003). Basically, there are six key biological processes employed in the treatment of wastewater; these are microbial phosphorus elimination, nitrification, denitrification, fermentation, respiration and photosynthesis (Mitchell and McNevin 2001). Wastewater that is heavily loaded with biological oxygen demand (BOD) and chemical oxygen demand (COD) is usually undergo oxidation and methanation process supported by oxygen and sulfur loving bacteria (Chan et al. 2008).

Oxygen enters into the deposits of the supportive materials with the assistance of macrophytic roots and produce an aerobic zone nearby roots. This region is characterized by presence of *Pseudomonas aeruginosa spp.* and *Nitrosomonas* that are accountable for the aerobic breakdown of contaminants. The anaerobic zone is characterized by the dominance of methanogens and sulfur degrading bacteria. Degradation of contaminants anaerobically involves mainly two-steps, first one is fermentation and the other one is methanogenesis (Cui et al. 2010; Pedescoll et al. 2011). An ultimate characteristic of CWMs is that their roles are controlled mainly through microbial population and their metabolic rate (Wetzel 1993). Microbial population chiefly includes bacteria, fungi, protozoa, rind algae and yeasts. The biomass of microorganisms is a main reducer for majority of organics and nutrients. The activity of microorganisms converts several organic and inorganic materials into innocuous insoluble materials, and, changes the redox reaction (reduction/oxidation) of the media material.

The transformations are either aerobic or anaerobic. Aerobic settings are commonly described to be more efficient for the removal of maximum organic contaminants. It is reported that the rate of nitrification-denitrification with two macrophytic species are much higher than CWMs with one or without macrophyte (Hua et al. 2017). The macrophytic type, density and their distribution can affect contaminants removal due to alteration in bacterial communities and consecutive aerobic and anaerobic settings (Calheiros et al. 2010; Zhu et al. 2017). It is known that the lower concentrations of nitrogen can also affect the removal efficiency.

14.4 Seasonal Variability Among Microbial Communities with Respect to Macrophytes

The structure of microbial population may vary widely according to the change in environmental conditions. In summer season, *Phragmites australis* contained dominant microbial groups (e.g., actinobacteria, bacteroidetes, and proteobacteria). However, unplanted units possess maximum photosynthetic bacteria and cyanobacteria (Wang et al. 2016). The richness of proteobacteria with *Phragmites australis* during winter season was >40% and intensely declined in unplanted units. Moreover, the abundance of cyanobacteria and photosynthetic bacteria declined in unplanted units. In both periods, bacteria remained more dominant in root zone as compared to supportive material. The occurrence of macrophytes has positive effects on richness of microorganisms (Wang et al. 2016). Yovo et al. (2016) demonstrate that *T. geniculata* are able to enhance dissolved oxygen through the well-developed aerenchyma. Maximum root diameter enables it to diffuse more oxygen into the system (Longstreth and Borkhsenious 2000).

Various species of emergent macrophyte *Cyperus* excreted bactericidal exudates within rhizosphere (Alufasi et al. 2017), and evidence toward *C. articulatus* bacterial exudates has been also documented (Caselles-Osorio et al. 2017). *C. articulatus* is able to allocate oxygen in excess to the rhizospheric region and offers an efficient elimination of nitrogen (>75%). Nevertheless, the outcomes with the similar species can differ according to season and operational conditions (Shelef et al. 2013). In CWMs, microorganisms are crucial agents for biogeochemical reactions that enhance water quality during the treatment process (Mitsch and Gosselink 2007; Saunders et al. 2013). A little alteration in the diversity or community structure of the microorganisms might directly affect the treatment performance of CWMs. During the winter, the activity of microbes produces sufficient warmness to prevent the bottom films from cold. The decomposition rates of microbes become slow as temperatures drop, thus, this can be optimized by increasing the size of wetlands to accomplish the slower reaction rates.

Tian et al. (2017) reported that the bacterial diversity is negatively correlated with the organics and nutrients removal, particularly in summer season when the population size and diversity are high. Some bacterial populations are dominated by anaerobic facultative that can work actively under both anaerobic and aerobic situations with respect to unstable ecological environments. During unfavorable conditions, several microbial populations become inactive and can remain in this state for years (Hilton 1993). Faulwetter et al. (2009) reported that the specific microbial group is responsible for the removal of particular contaminant. Hence, expanding the understanding about the organization of microorganism's community is useful to disclose the contaminants removal mechanisms in CWMs. Most common anthropogenic wastewater contaminants and their effects on human and aquatic organisms are given in Table 14.1.

14.5 Enzyme Activity

The action of enzymes within CWMs has been observed as a key indicator towards the role of microbial populations involved in wastewater treatment (Mentzer et al. 2006; Zhang et al. 2011a, b). The community structure of microbial populations has been correlated to specific enzyme activities (Zhang et al. 2006). Enzyme activity within soil substrate of a CWM (Kang et al. 1998; Martens et al. 1992) is considered as a significant factor for improving water quality (Freeman et al. 1997; Shackle et al. 2000). Functions of several enzymes within CWMs get influenced by various aspects such as biological factors (fauna, higher taxa and microbial communities), edaphic factors (nutrient composition, texture, pH, depth, organic matter content, etc.) and environmental factors (Zaman et al. 1999; Duarte et al. 2008; Reboreda and Caçador 2008).

The activity of enzymes might be altered by modifying the carbon supply either qualitatively or quantitatively to enhance the removal efficiency of CWMs (Shackle et al. 2000). They also described that supply of exogenous enzymes can improve the biodegradation processes. The actions of numerous enzymes including urease, phosphatase, protease and cellulase exhibited great dissimilarities over time. It is stated that the activity of urease was always advanced in CWMs developed with *Phragmites australis* as compared to other wetlands planted with several macrophytes. Activity of enzymes is inversely proportional to depth of soil (Aon and Colaneri 2001; Niemi et al. 2005) with maximum in the upper layer of soil substrate nearly for all wetlands. Root activity within rhizosphere presented a stronger association with enzyme activity.

Table 14.1 Most common anthropogenic wastewater contaminants and their effects on human and aquatic organisms

S. No.	Category	Contaminant	Effects	Reference
1	Organic	Nitrogen	Algal blooms in water bodies, possibly increased cancer risk, encourage infectious microorganisms such as *Pfiesteria* that can cause irritation to eye and methemoglobinemia (blue-baby syndrome) in babies	Bojcevska and Tonderski (2007)
		Phosphorus	Eutrophication, headache, gastrointestinal illnesses	
2	Heavy metals	Zn	Acidification of water, skin irritations, stomach contractions, vomiting, nausea, anemia, damage to pancreas, disturbance in protein metabolic rate, respiratory maladies, prone to infants and unborn	Calheiros et al. (2009a)
3		Pb	Kidney damage, anemia and interference with hemoglobin synthesis	Calheiros et al. (2009b)
4		Hg	Toxic to aquatic organisms like fishe, neurotoxic	Calheiros et al. (2010)
5		Cd	Temporary reduction in growth of fishe by larval mortality, in animals interacts with calcium metabolism	Truu et al. (2005)
6		Cr	Fish are more susceptible to infection, damaging the tissues of a number of invertebrates such as worms and snails	Calheiros et al. (2017)
7	Hydrocarbons	Petroleum hydrocarbons	Can damage liver and kidney severely, reproductive cytotoxicity and juvenile cancers	Calheiros et al. (2009a)
8	Microorganisms	Harmful bacteria	Several water-borne diseases	Calheiros et al. (2008a)
9		Viruses	Viral infections	
10		Protozoans	Amoebiasis, Trypanosomiasis, Diarrhea, Leishmaniasis, Trichomoniasis, Lambliasis, Toxoplasmosis and Malaria	

(continued)

Table 14.1 (continued)

S. No.	Category	Contaminant	Effects	Reference
11	Persistent organic pollutants	PCBs	Act as endocrine disrupting substances	Calheiros et al. (2008b)
12		Phthalates (plastics)	Early sexual maturation, lower orientation and attentiveness in girls, lung disorders, menace of diabetes, endometriosis and disorder in normal body functions	Calheiros et al. (2014)
13		Bisphenol A (BPA)	Lead to infertility, affect puberty and ovulation, can cause type-2 diabetes by insulin resistance, heart disease, inhibit the development of brain at gestation and enhance the risk all types of cancers	Carballeira et al. (2017)
14		Polycyclic aromatic hydrocarbons (PAHs)	Inhibits the normal working of the body. Prone to immunotoxicity, genotoxicity and embryotoxicity	Carvalho et al. (2012)
15	Emerging pollutants	Pharmaceutical products	Reduction in growth of aquatic organisms, affecting the reproductive rates of fish by masculinization	Caselles-Osorio et al. (2011)

Due to this stronger correlation, the activity of enzymes can get affected by macrophytes root activity that significantly affects the pollutants removal efficiency (Kong et al. 2009). Macrophytes can also impact soil enzyme activity via altering species composition and diversity of microbes through excreting exogenous enzymes and liberating oxygen and exudates. They are also taking part in reactivation of free enzymes that possibly get deactivated and conserved by tannin compound with several other substances in anaerobic region, through oxygenation via intensifying root structure (Neori et al. 2000).

14.6 Effect of Temperature on Microbial Activity

Numerous biogeochemical reactions that regulate the pollutants removal efficiency are greatly affected by temperature, consequently altering the overall performance of CWMs (Lee et al. 2009). Various environmental aspects showed annual cycles that facilitate the performance of whole system. Out of which, temperature of wastewater is most significant. Atmospheric effects, such as rain, water reaeration and evapotranspiration also follow seasonal patterns (Kadlec 1995). Several distinct wetland processes, for example, microbially mediated reactions and decomposition of organic matter are greatly inhibited by temperature. It also has significant influence on nitrogen transforming processes such as nitrification, mineralization and denitrification.

The reactions for phosphorus sorption have lower temperature effect. The removal of nitrogen, phosphorus and particulate carbon by physical processes are not much affected by temperature. The water and temperature of surface soil within wetland system characteristically show both annual and diurnal cycles (Tanner and Headley 2011). The diurnal variations in wastewater temperature are around 5 °C for free water surface wetlands system (Kadlec and Reddy 2001). During warm periods, the average daily wastewater temperature is almost equivalent to the average daily air temperature (García et al. 2003).

14.7 Effect of DO on Microbial Activity

The oxygen limited conditions favor methanogenesis and bacterial groups existing in humid and hot atmospheres, and consume more oxygen than it diffuses from atmosphere (Hamilton et al. 1995). Therefore, CWMs are the perfect models for fermentation process with anaerobic conditions allowing easier breakdown of organics. The metabolic action of microbes in the rhizospheric region gets influenced by the availability of oxygen (Chen et al. 2011; Saxena et al. 2019). The pH of wastewater in CWMs exerts a strong effect towards various reactions and processes, together with biological conversion, separating ionized and unionized acids and bases, cation exchange and solvability of gases and solids (Niveditha 2019).

14.8 Conclusion

One of the major characteristics of CWMs is that their roles are measured primarily by microbial population and their associated metabolic rate. The microbial biomass is the main reducer for majority of organics and nutrients. The activity of enzymes within CWMs has been observed as a key indicator towards role of microbial community in removing contaminants during the treatment process. The aerobic region of CWMs is characterized by presence of *Nitrosomonas* and *Pseudomonas aeruginosa spp.* It is also reported that the population of ammonia-oxidizing bacteria mainly *Nitrosospira sp.* is higher in CWMs designed to treat domestic wastewater as compared to other bacteria studied. The presence of these bacterial populations inside a treatment wetland has a major effect on its stability. Microbial cells attached with substratum develop biofilms that are habitually present inside a self-produced medium of extracellular polymeric substance (EPS). Microbial population use organics as nutrients and expel vitamins and several metabolites to encourage growth of additional microbes that is known as rhizosphere effect. Rhizospheric region has diverse elements that comprises minerals, organic acids, sugars, vitamins, polysaccharides, phenol and various other carbon-based materials that encourage the microbial groups to degrade organic pollutants.

The presence of macrophytes has encouraging effects on richness and community arrangement of microorganisms. Several organic materials expelled by the macrophytes in the root zone alter the microbial population dynamics. The pH of wastewater in CWMs has a strong effect toward various reactions and processes. The decomposition rates of microbes become slow as temperatures drop, thus, this can be optimized by increasing the size of wetlands to accomplish the slower reaction rates. A little alteration in the diversity or community structure of the microorganisms might directly affect the overall treatment performance of CWMs. Hence, expanding the knowledge about the organization of microbial community structure is instrumental in understanding about the contaminants removal mechanisms of CWMs.

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