Microbial population dynamics in constructed wetlands: Review of recent advancements for wastewater treatment

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ABSTRACT

Constructed wetlands are improvised man-made systems, designed for adopting the principle of natural wetlands for purifying wastewater – the elixir of life. They are used widely as a cost-effective and energy-efficient solution for treating greywater generated from different tertiary treatment sources. It provides an elaborate platform for research activities in an attempt to recycle earth's natural resources. Among the several organic impurities removal mechanisms existing in constructed wetland systems, the earth's active microbial population plays a vital role. This review deals with the recent advancements in constructed wetland systems from a microbiological perspective to (effect/ devise/ formulate) chemical and physical treatment for water impurities. It focuses on microbial diversity studies in constructed wetlands, influence of wetland media on microbial diversity and wetland performance, role of specific microbes in water reuse, removal of trace elements, some heavy metals and antibiotics in constructed wetlands. The impurities removal processes in constructed wetlands is achieved by combined interactive systems such as selected plant species, nature of substrate used for microbial diversity and several biogeochemical effected reaction cycles in wetland systems. Therefore, the correlation studies that have been conducted by earlier researchers in microbial diversity in wetlands are addressed herewith.

Keywords: Constructed wetlands, Denaturing Gradient Gel Electrophoresis (PCR-DGGE), Phytoremediation, Polymerase chain reaction

1. Introduction

Wetlands for water pollution control/remedy in use of wastewater for more than many decades. Many researchers give detailed information of the developments of describe the field, over time, engineering design, process and the control over the process. The scarcity of freshwater resources to meet the increasing demands of water usage and dwindling available resources are pressing problems encountered at a global level. Recovery alternatives such as wastewater treatment, aquifer recharge, rainwater harvesting, etc. are commonly adopted to deal with this situation to conserve water resources [1-5]. Mother Earth / Nature have her own ways of treating wastewater in which wetlands plays a dominant role. Wetlands are one of the natural methods of treating and preserving water resources all through the year in which water covers the soil surface for specific time duration and then percolates into the ground.

Wetlands are hence critical to groundwater recharge. Removal process involved in water treatment in treatment wetland (TW) includes number of many physiological; plant related and microbial related one [5-9].

The main role of constructed wetland is the land designed for vegetation and to treat wastewater. The main issue of constructed wetland requires large land area. 'The researchers may also defined wetland is the receiving water pollution, through design, period on a time on polishing water'. They also classifies TW systems on the basis of vegetation and hydrology, with subsurface systems as horizontal flow and vertical flow, and the hybrid systems having the contribution of both horizontal and vertical flow. Worldwide various types of flows systems existing depend on their suitability, availability and the maintenance. I.e., in America and Australia free water surface flow, rather Europe and their neighboring countries designed the subsurface flow. In general free water surface flow for secondary water treatment, whereas the subsurface flow

for tertiary water treatment [10-15]. At some point at equilibrium of attachment and detachment can occur allowing for study state operates with no bio-clogging. Several important benefits are provided by wetlands; they maintain the diversity of the ecosystem which assists in the treatment of polluted water, by storing, purifying and recycling water resources in leaves, shoots and roots of wetland plants. They control water erosion by reducing storm and flooding damages; they also serve as a wildlife habitat, help sustain growth and maintain the natural equilibrium [16-20].

2. Constructed Wetlands

2.1. General

By adopting the principles of many natural wetlands, man-made/artificial wetlands are selectively designed and purposefully created in an attempt to obtain a fast growing simulated ecosystem using holistic engineered technology treatment to achieve better water quality. Constructed commercial wetlands are serving as an efficient method for brackish water recovery wastewater treatment and control of pollutants for decades. They are simulated water treatment systems that incorporate suitable vegetation and substrate, in addition to the use of a wide variety of microbial flora which play a crucial role in controlling pollution [4-6]. Comparative studies of constructed wetlands with other existing conventional methods have shown that treatment efficiency of horizontal subsurface flow wetland system is comparable (> 75% in total suspended solids) to conventional technologies in water treatment like rotating biological contactor and packed bed filters. Significant reduction in the construction cost and energy requirements makes Constructed Wetlands (CWs) a suitable alternative in wastewater treatment [6-7]. There is sufficient evidence to show that in addition to the treatment of municipal, industrial and agricultural wastewater. Urban storm water and landfill leachate can also be effectively treated by constructed wetlands with considerable reduction in Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) [8]. Additionally, the consistent behavior of constructed wetlands to intermittent operations and adverse conditions like flooding and drought makes it an alternative mitigation strategy with tertiary cycles that makes it a suitable option for organic wastewater treatment [9, 10].

2.2. Types of Wetlands

There are two types of constructed wetlands, namely surface flow wetlands and subsurface flow wetlands. Surface flow wetlands are very similar to natural wetlands and the wastewater flow over the substrate is not very deep. Subsurface flow wetlands are further classified into Vertical Flow (VF) (Fig. 1) and Horizontal Flow (HF) (Fig. 2) systems. Both have proved to be more effective than the surface systems [11-13]. A combination of VF and HF systems are also used which are known as hybrid wetlands (Fig. 3).

2.3. Wetland Plants

Wetland plants come under the category of macrophytes which includes four types namely emergent macrophytes, floating-leaved macrophytes, submerged macrophytes and freely floating
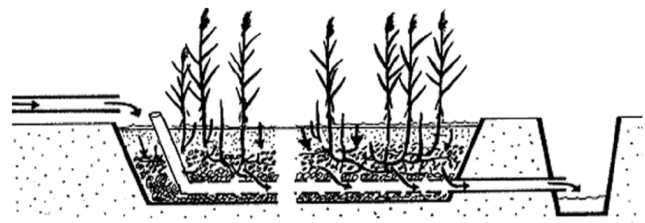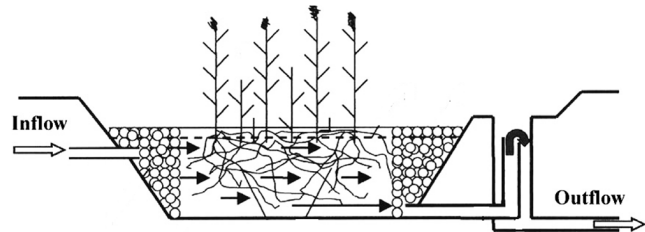

Fig. 1. Vertical flow-constructed wetland.

Fig. 2. Horizontal flow-constructed wetland.

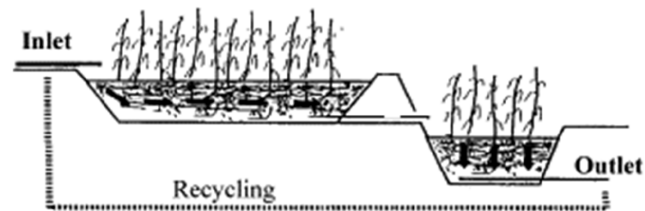**Fig. 3.** Hybrid flow-constructed wetland.

macrophytes. *Phragmites, Typha, Scirpus, Phalaris arundinacea,* and *Iris* are the common species of macrophytes that grow in wetlands [14-16]. The choice of plants has a bearing on the process efficiency. Some plants like *Cirpus grossus* have sustainable living biomass above the soil including stem, stump, branches, bark, seeds and foliage which increases its effectiveness but some others (e.g., *Typha angustifolia*) need to be replaced periodically for better performance [17].

The most important removal mechanism occurring in constructed wetlands is filtration. In addition several microbial-mediated processes, chemical networks, volatilization, sedimentation, sorption, photo-degradation, plant uptake, transpiration flux etc. also contribute to increasing the efficiency of the process either singularly or as a combination of two or more processes [18-19].

2.4. Wastewater Pollutants

Nitrogen in combined form is one of the principal pollutants present in wastewater; it exists in both organic and inorganic forms. The organic forms of nitrogen include amino acids, urea, uric acids, purines and pyrimidines, while free NH_3, ammonium salts and nitrogen gas (Nitrogen is not a polluting compound) are the forms of inorganic nitrogen [20-24]. Nitrogen content is reduced through a series of treatment pathways such as ammonification, nitrification and denitrification, plant absorption uptake as nitrates, biomass assimilation, nitrate reduction, ammonia volatilization, adsorption, Anammox and Canon processes [25-29]. Several wastewater pollutants and their effects are listed in Table 1. From the table many

Table 1. Common Man-made Wastewater Pollutants and Their Effects [20-33]

Sl No	Category	Name of pollutant	Effects
1	Organic pollutants	Nitrogen (Present as ammonia and organic nitrogen)	Presence of algal blooms in water, increase in the amount of chlorine, increasing risk of cancer, stimulation of harmful microbes like *Pfiesteria* which may lead to eye and respiratory irritation, headache, gastrointestinal complaints and methaemoglobinemia (blue-baby disease) in infants.
2		Phosphorus (Exists in the form of soluble orthophosphate? TSP is insoluble in water ion, organically-bound phosphate, or other phosphorus/oxygen forms)	
3	Hydrocarbons	Petroleum hydrocarbons	Significant damage to body organs (liver and kidney) and body functions, cause reproductive cytotoxicity and childhood cancers.
4	Heavy metals	Zinc	Cause stomach cramps, skin irritations, vomiting, nausea, anemia, damaged pancreas, disturbed protein metabolism, arteriosclerosis, respiratory disorders, danger to infants and unborn and increase in water acidity.
5		Lead	Kidney damage, interrupt hemoglobin synthesis and anemia.
6		Mercury	Toxic to aquatic organisms.
7		Cadmium	Interact with calcium metabolism in animals. In fish, causes larval mortality and temporary reduction in growth.
8		Chromium	Fish are more susceptible to infection. A high concentration of chromium is also known to cause damage in the tissues of several invertebrates, such as snails and worms.
9	Microorganisms	Harmful bacteria	Water-related diseases.
10		Viruses	Viral infections.
11		Protozoans	Leishmaniasis, Amoebiasis, Diarrhoea, Trichomoniasis, Tripanosomiasis, Lambliasis, Toxoplasmosis and Malaria.
13	Emerging Pollutants (Persistent Organic Pollutants)	Pharmaceutical products (antibiotics)	Slow development of aquatic organisms, increased masculinization of different fishes affecting their reproductive rates.
14		Polychlorobiphenyls (PCBs)- A family of 209 chlorinated aromatic compounds	Act as endocrine disruptors.
15		Phthalates (plastics)	Low-molecular-weight phthalates improve motor skills in boys, high-molecular-weight phthalates lower orientation and alertness in girls, risk of endometriosis, early sexual maturation in girls, contribute to the risk of diabetes, lung disorders and disruption of other normal body functions.
16		Polycyclic aromatic hydrocarbons (PAHs)	PAHs may have short-term as well as long-term effects on the normal functioning of the body. Can cause embryotoxic, genotoxic, immunotoxic effects.
17		Bisphenol A (BPA)	Affect puberty and ovulation, lead to infertility, contribute to insulin resistance and therefore Type-2 diabetes, coronary artery heart disease, affect the developing brain during gestation, increase the risk of breast, prostate and other cancers.

of pollutants are very harmful to human community like kidney failure, causes of cancer, gastrointestinal diseases, reproductive system, diabetic, irritation in respiratory organs and eye and even its affects the infants.

So, the need for removal of pollutants is the major issue for researchers' reasonable and possible way. Wastewater BOD & COD, Pollutants are generally removed either aerobically or anaerobically by complex oxidation/methanation processes aided by diverse microorganisms of oxygen or sulphur loving bacteria (associated with plant roots and sediments) [34]. An aerobic organism or aerobe is an organism that can survive and grow in an oxygenated environment. The atmospheric oxygen penetrates into the deeper layers of the substrate with the help of plant roots and creates an aerobic region in the close vicinity of roots. This zone is dominated by Methanotrophs, *Nitrosomonas* and *Pseudomonas aeruginosa* spp. which are responsible for the aerobic degradation of pollutants. Anaerobic regions are created away from the aerobic regions and are dominated by methanogenic and sulfur reducing bacteria. The

Table 2. List of Microbes with Efficiency

Sl No	Microorganisms studied	Treatment efficiency (% removal)	Wastewater studied	Type of Wetland	Reference
1	Total coliform, *Escherichia coli* (*E. coli*) and Helminth eggs	90%	Domestic wastewater	Hybrid systems (train with vertical and horizontal systems)	[102]
2	Fecal coliforms and total coliforms	99%	Septic wastewater effluent	Pilot-scale combined CW system	[103]
3	*Salmonella*, fecal coliforms and *E. coli*	96, 98 and 99%, respectively	Swine wastewater	Two-cell field-scale surface flow constructed wetland	[104]
4	Fecal coliforms and enterococci, *Salmonella*, *Giardia cysts*, *Cryptosporidium oocysts*	> 99%, 93-96%, 88% and 69%, respectively	Domestic wastewater	Horizontal flow constructed wetland	[105]
5	*E. coli*, *Salmonella* and helminth eggs	99.5% for *E. coli*, *Salmonella* and helminth eggs - 100% in all samples	Domestic wastewater	Horizontal subsurface flow constructed wetland	[106]
6	Coliphage, total coliforms, fecal coliforms, *Giardia* and *Cryptosporidium*	*Giardia* and *Cryptosporidium* (duckweed pond at 98 and 89%), coliphage, total and fecal coliforms SSF wetland (95, 99, and 98 percent, respectively	Secondary unchlorinated wastewater	Three types: duckweed-covered pond, a multi-species subsurface flow (SSF) and a multi-species surface flow (SF) wetland were studied	[107]

anaerobic degradation is a two-step process in which the first step is fermentation and the second step is called methanogenesis [35-37].

The hydraulic retention time (HRT) also plays a pivotal role in specific pollutant removal. Studies have proved that suitable adoption of HRT results (Anaerobic behaves differently for proteins, carbs and fats in food – please check) in better removal efficiencies. For instance fecal coliform and N-removal efficiencies can be increased by increasing the HRT, while considerable P removal can be achieved with longer HRT probably greater than 15 d [38-39]. Thus a series of factors as discussed above along with modified wetland designs and active management are to be considered for the efficient working of CWs in order to improve the effluent water quality [40-42].

The presence of bacteria, fungi, and algae are abundant in wetlands and they enhance the organic pollutant removal in subsurface flow wetland systems. Microorganisms play a key role in the biogeochemical cycles of wetland systems and each species of microbes has its own contribution towards the treatment of wastewater from different sources having varying pollutant loads. The clear understanding of effluent interactions with the wetland systems and the characteristics of the improved water thus obtained after treatment will pave the way for customized design of CW units depending on the nature of the feed it is receiving [43-45].

This review is focused on the microbiological advances in constructed wetlands area. The understanding about the role of microorganisms is important because the efficiency achieved as a result of the treatment is due the combined interaction between the plants in the wetland, wetland media, type of wastewater used and the pollutants associated with it as well as the characteristics such pH, temperature, humidity and other environmental conditions.

This paper therefore addresses several perspectives that contribute to the microbiological efficiency of constructed wetlands. It includes the microbial diversity studies in constructed wetlands, the correlation of microorganisms in several other phenomena occurring in the system, the contribution of different substrates to the microbial diversity and richness and the contribution of microorganisms in ensuring treatment efficiency. Compare the process of anaerobic is efficient than aerobic. And the list of microbes is given in Table 2.

3. Recent Microbiological and Molecular Biological Studies

3.1. Microbial Diversity Studies in Constructed Wetlands

Microorganisms are of critical importance to the symbiotic health of earth's inhabitants and play a vital role in the removal/conversion of pollutants in constructed wetland systems [46]. The microbial fauna is present in the soil matrix, which is further divided into aerobic and anaerobic zones. The aerobic zones are rich in microbial diversity which assists in metal oxidation (bacteria fixes inorganics by reacting only with its organic content) while the latter are rich in sulphate-reducing bacteria [47-48]. The concentration of ammoniacal nitrogen, nitrate, nitrogen and carbonaceous materials contributes to the abundance of microorganisms in the wetland system. The decay products of plant litter also play a part in the growth of microorganisms in the surface layer [49]. Numerous studies have been undertaken/done for determining the role of various parameters influencing the microbial efficiency of contaminant removal in constructed wetlands. Any research on basic living

organisms, such as indigenous microorganisms demands a vast arena of traditional techniques ranging from isolation, culturing and identification to modern techniques of gene sequencing, PCR-DGGE and a lot more besides [12, 50].

Studies on microbial diversity of natural and constructed wetlands are accomplished by characterization of the soil bacteria community structure and composition [51]. This can be done by several microbiological techniques, one of which is pyrosequencing of 16S ribosomal DNA [52]. Pyrosequencing of 16S ribosomal DNA from soil samples collected from different locations of constructed wetlands when compared to the results of samples from natural wetlands, revealed that natural wetlands harbor a wider variety of microorganisms than those in CWs. Studies have proved that the diversity of bacterial populations living inside plant tissues are shaped as a result of several physiochemical and biological processes that occur in wetlands [53]. The amount of oxygen present at different periods as well as the organic load contributes to the bacterial diversity of constructed wetland systems [12]. The availability of nutrients and their characteristics also influences microbial diversity in CWs. Oxygen regulates nutrient biogeochemistry in constructed wetlands and the improved sediment quality is due to the presence of deep rooted macrophytes [54].

The microbial diversity is found to be a function of configuration and design of the CWs. The performance and microbial diversity of two pilot-scale multi-stage sub-surface flow constructed wetland systems and the results indicated a linkage between microbial community, treatment performance and design of the CWs; an increased bacterial diversity was observed in the last stage of multi-stage CWs. This study also pointed to a link between nutrient removal and community composition [55].

The prevailing pattern of water flow over a given time, specifically, the duration and timing at which surface water is present in the CW unit is one important factor that determines the structure of the bacterial communities in the wetland complex soils and sediments [56]. The bacterial community structure and the specific bacterial consortia within wetlands determine the denitrification potential. The extent of denitrification occurring in CWs is linked to bacterial communities [43, 57-58]. The study, conducted on riparian plant species, revealed that biological diversity is strongly related to steep environmental gradients in hydrology and soil redox status [59]. To understand the mechanism behind the source of microbial organisms from the CW, Ibekwe carried out / undertook characterization of microbial composition of soil samples from two constructed wetlands treating dairy wash-water using PCR-DGGE. The results from this study revealed that the source of bacterial communities present in soil samples was from the gastrointestinal tracts of animals. It also showed higher percentage of *Nitrosospira*-like sequences and *Nitrosomonas*-like sequences which confirmed/ showed/ revealed that wetland systems are highly dependent upon the microbial activities and diversities for optimal wastewater treatment and the nature of wastewater to be treated [44].

Another recent study suggested that each plant favors growth of specific microbial communities and plant diversity helps to harbor different species of microorganisms which respond differently to factors such as nutrient availability, contaminant loads, specific plant species and combinations selected thus increasing microbial based wastewater treatment capacity [60-61].

Contrary to the (above-mentioned research findings/observations stated earlier), certain studies reported that plants do not have any (significant impact) on the structure of the microbial communities [62].

3.2. Correlation Studies in Wetlands

The contamination removal procedure in CWs is rarely achieved by individual processes (BOD reduction only needs photosynthesis oxygen from cyanobacteria), but by the combined interaction of plant species present in the system, nature of substrate used in the system, microbial diversity and several biogeochemical cycles of wetland systems. Each component contributes at its own level to the overall efficiency of the wetland system and hence correlation studies play an important role in understanding the ecological and biophysical processes in wetlands. For example, studies have proved that in the filtration process, the filter materials used and the resident plants exert considerable effect on the establishment of microbial community in wetland systems [63]. As stated earlier, plant diversity helps to harbor a huge variety of microbial species which means that the microbial activities in planted wetlands are usually more than that in unplanted wetlands. Depending upon the different C:N:P ratios provided by wastewater from various sources, the plant growth is affected and in due time produces a more stable ecosystem within it [64]. River water purification methods involving CWs, the microorganisms present in the soil, especially the species and their number, the enzyme activities of the soil and the rate of pollutant removal maintained significant correlations [65]. Studies on fumigation extraction and ecoplate techniques showed that plant diversity increases the plant biomass production and is well correlated with the size and structure of soil microbial community patterns in constructed wetlands [66].

Experiments conducted in two-stage CWs treating tannery wastewater, planted with *Typha latifolia* and *Phragmites australis* in expanded clay aggregates for 31 months and analyzed the diversity of bacterial communities by enumeration techniques and DGGE. They came to the conclusion that there is no clear relation between the sample collection time, hydraulic loading applied and the bacterial diversity [22, 67].

3.3. Influence of Wetland Media

Different unconventional wetland media are used for treating different types of wastewaters and their removal efficiencies are monitored in various studies. They eventually accelerate nutrient removal and foster microbial diversity.

The removal efficiencies of different substrates vary; the maximum removal efficiency of a component in question can be attributed to one substrate rather than the other one, which will offer the same for another component (Fig. 4 and 5). These differences can be explained in part by the fact that each substrate is different from the other with reference to physical and chemical characteristics [68-69]. The type of substrate and the presence of suitable plant species assist in pollutant removal. It promotes the richness and diversity of suitable species of microbes that can contribute to better treatment efficiency [70]. Substances like carbohydrates are degraded in the portions of the substrates where dense roots are present. Microorganisms harbored by the substrates also serve as a predefined measure used to track the treatment efficiency

of CW units [71]. Studies showed that there is a dominance of *Nitrosomonas* species in the vicinity of plant roots, enhancing the nitrogen removal when compared to the non-vegetated residues [72]. Also, when compared to substrate, higher bacterial diversity is found in the rhizosphere region.

A comparative study of various substrates in removing phosphorus (P) showed that the choice of adsorbing substrate greatly influences the lifetime of constructed wetlands [73]. Wetlands can be effectively used in the removal of non-biodegradable pollutants. The important factors to be considered while selecting the substrate materials are high sorption capacity, efficiency, easy availability and economical factor/aspect [74-76]. Some of the widely used substrates in CW and their applications are stated.

Maerl (calcified seaweed) is a highly potential constructed wetland substrate, with a proven reported phosphorus removal rate of 98% [77]. Compost was used as a substrate for treating wastewater in vertical flow wetlands from refineries where total suspended solids, COD, BOD and heavy metals were removed with significant removal percentages [78]. The use of coal slag bed to treat domestic wastewater showed 60% removal efficiency for carbonaceous matters, 50% removal of ammonia nitrogen, 40% for phosphorus and 80% efficiency in removing total suspended solids [79]. Gravel and Zeolite were also experimented as bedding material and the average removal efficiencies for ammonia nitrogen, COD, Phosperous and Fe(III) were compared for units operating in horizontal and vertical modes. Ammonia nitrogen, Fe(III) and Phosperous showed more efficiency in vertical flow CW units while the COD removal was high in horizontal flow CW units [80].

The substrates such as blast furnace artificial slag, coal burn artificial slag, and midsized sand artificial slag were also analyzed for the removal of total P (TP), total N (TN) and ammonium N. The results indicated that the percentage removal of TN was high in coal burn artificial slag while the others were high in blast furnace artificial slag [81]. Complete total oxidized nitrogen removal was observed in rice husk media [82]. High efficiency of ammonia removal and highest total nitrogen removal efficiency of 59.5% was observed in CW systems in which lightweight aggregates of fly ash were used as substrate [17].
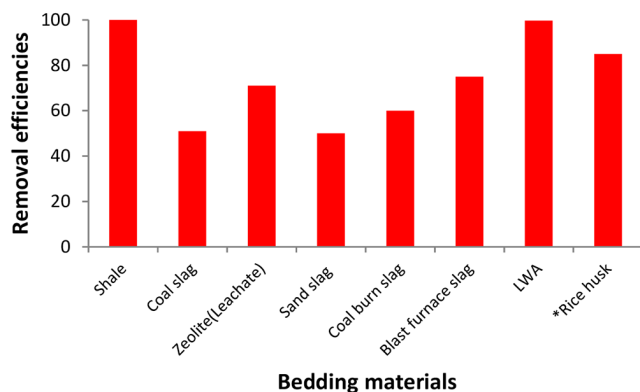
* The removal efficiency of rice husk was monitored for different HLR and the results showed that systems with longer HRT had maximum efficiency.

Fig. 4. Comparison of removal efficiency of NH$_4$-N in different Bed materials.
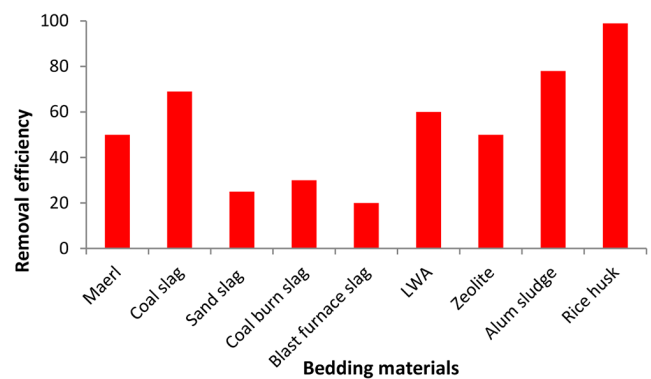

Fig. 5. Comparison of removal efficiency of TN in different Bed materials.

3.4. Role of Microbes in Water Reuse, Removal of Trace Elements, Heavy Metals and Antibiotics

Research studies on the reuse of water after CW treatment requires knowledge of the origin of (septage and sewage) wastewater as well as the type of indicator microorganisms such as total coliforms, fecal coliforms and *E. coli* [83-86].

Treated wastewater is widely used for watering, landscaping and aquifer recharging purposes. It is therefore recommended that cost-effective and energy-efficient techniques be adopted for wastewater reuse [87] without precipitating chemicals. Experimental evidences suggest that constructed wetlands play an efficient role in the removal of wastewater containing xenobiotic compounds, illicit drugs, transformation products, antibiotics, etc. [88-89].

It was reported that the use of halotolerant microorganisms improves the efficiency of treatment [90-91]. Halotolerant varieties of vegetation and their associated sediments improve the exposure of air in the system and provide better conditions for the growth of aerobic halotolerant microorganisms, which in turn improves the removal efficiencies of the CW unit [92-93]. The study of presence of pathogens and the effectiveness of connected bacteria is necessary to ensure the reuse of treated water [94]. On the other hand, some studies show that increase in microbial activity need not always/necessarily contribute to the water quality after treatment [54].

Heavy metals, one of the highly toxic environmental contaminants are produced by a variety of industries and are treated with conventional methods like reverse osmosis (RO) and nanofiltration (NF) technologies [95]. Constructed wetlands planted with *Phragmites australis* have proved to be efficient in the removal of heavy metals such as arsenic and zinc through principal processes such as sorption, precipitation and co-precipitation the system showed highest microbial diversity and richness with a dominating Proteobacteria [96-97]. The degradation of antibiotics is also a feature which is accomplished by microbial pathways, regulated by the presence or absence of plants, the flow type, temperature, artificial aeration redox potential and most importantly the CW design characteristics, though the removal efficiency is proved to be a little low in cold climatic conditions [98-99]. Studies showed 90% removal of trace elements such as Cd, Cr, Pb, As, Zn and BOD from sewage with higher treatment efficiency in summer than in winter [100-101].

4. Conclusions

Recommendations Regarding the Sustainable Application of Constructed Wetlands

Constructed wetlands offer an economic, self-maintained and cost-effective alternative for the conventional treatment of different types of wastewater. This review is focused mainly on the microbial prospects which form the basis of the removal mechanisms in constructed wetland systems such as ammonification, nitrification, denitrification, plant uptake, biomass assimilation, etc.

Moreover, microbial efficiency of constructed wetlands is also required to be studied to decide the prospects of further treatment of wastewater before its release to the environment. Not much literature is available regarding the percentage removal of pathogenic microorganisms existing in wastewater from different sources and such areas of research need to be encouraged for the optimization of the microbial parameters which may aid in the sustainable application of constructed wetlands.

The availability of nutrients and the mode of utilization, oxygen solubility/ conditions, organic matter load, design and configuration of the CWs, type of water regime and plant diversity are the other parameters that need to be addressed. Influence of wetland media is another important arena, as the type of substrate along with the presence of suitable plant species is crucial in determining the bacterial composition which is ultimately responsible for the removal efficiency of the system. Though there are certain experimental results existing in these areas, many basic contradictions are also present. These exceptions and contradictions of treatment technologies need to be analyzed carefully and the specific conditions required for maximum output have to be optimized before the design, construction and execution of every constructed wetland project.

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