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BIOREMEDIATION: A GREEN APPROACH TOWARDS THE TREATMENT OF SEWAGE WASTE

ANKITA SAXENA*, VARSHA GUPTA and SONIKA SAXENA[1]
Department of Microbiology, JECRC University, Jaipur-303905, India
[1]Department of Biotechnology, Dr. B. Lal Institute of Biotechnology, Jaipur
*Corresponding author E-mail: ankitasaxena6900@gmail.com

Bioremediation of various waste waters is a new technology that has undergone a lot of deep investigation in recent decades. With growing population, advanced agricultural practices, industrialization, urbanization and multiple use of water has increased the demand for water around the globe. Due to daily human activity and also various agricultural and industrial operations, wastewater is produced in huge quantity. Due to lack of proper management and treatment facilities most of the urban wastewater generated in Indian cities is discharged into natural aquatic environments without any proper treatment. Municipal sewage problems are more complex as the volume of the wastewater is large and it also requires large area for treatment. Several techniques have been recommended for effective wastewater treatment but among all of them bioremediation is the most effective management tool to control the environmental pollution and to improve the contaminated sites.

Keywords: Bioremediation, Biological Organisms, Environmental Pollution, Sewage Waste.

Introduction

Waste water treatment using microorganisms is being exploited worldwide as it is economical, environmental friendly and sustainable. Wastewater treatment is not a single problem for the developing countries however it continues to be the basic sanitation need to protect the surroundings and the water forms that serve as drinking water sources around the globe[1] . Through many research it has been revealed that bioremediation can be an effective solution for water treatment because of the capability of microbes to survive, adapt and thrive within different environments, including wastewater[2-5]. Bioremediation is the "use of microorganisms to convert, modify and utilize toxic pollutants in order to obtain energy and biomass production and destroy the environmental contaminants into less toxic forms"[6,7].

Sewage, or domestic / municipal wastewater, is a type of wastewater that is produced by a community of people. Sewage wastewater is mainly comprising of greywater (from sinks, bathtubs, showers, dishwashers, and clothes washers), blackwater (the water used to flush toilets, combined with the human waste that it flushes away) ; soaps and detergents etc. Wastewaters which are produced by domestic residences may be either collected by a sewerage system and treated at a wastewater treatment plant, or treated and disposed of on- site, where the wastewaters are generated[8].

Worldwide the pollution - associated problems are increasing due to expansion of city and high population[9]. Municipal sewage carries the major load of pollution which eventually is discharged into the nearby rivers. Significant investment has been made into some countries, but in majority of cases, sewage is discharged directly into the river without treatment[9] which is contaminated with various contaminants like faecal pathogens, agricultural nutrients, dissolved and suspended solids etc. which ultimately cause the different health problems [10,11].

If the wastewater is discharged into the nearby rivers or any aquatic environment without any appropriate treatment than the existence of the hazardous contaminants like organics in wastewater severely affects the aquatic life forms as the organics decreases the dissolved oxygen in water bodies and it satisfies its biological oxygen demand (BOD), hence it interrupts the aquatic live forms[12-15]. It can also proceed to the formation of stinking gases, produced from the decomposition of organics anaerobically[16]. Domestic and municipal wastewater generally comprises of pathogenic, or disease- causing microorganisms, thus threatening human health. Such type of wastewater may also have a various types of nutrients, which causes the eutrophication of lakes and other fresh water streams and stimulates the growth of algal blooms[17]. Overall, the treatment of domestic and municipal wastewater must be done before discharging it into natural water bodies[18,19].

Principle of Bioremediation

Environmental biotechnology isn't a new field, composting and wastewater treatments are known examples of old environmental biotechnologies. However, recent studies in biological science and ecology offer various opportunities for more effective biological processes. Remarkable activities of these studies include the clean- up of contaminated water and land areas. After many researches, bioremediation may be define as a process, which depend on biological mechanisms to reduce (degrade, detoxify, mineralize or transform) concentration of pollutants and convert them into harmless forms. According to the site of application, bioremediation methods are categorized into two broad categories: ex situ or in situ. The method for removal of pollutants mainly depends on the type of the pollutant, such as: agrochemicals, chlorinated compounds, dyes, heavy metals, hydrocarbons, plastics, sewage etc. Apart from this, depth and degree of pollution, type of environment, location, cost, and environmental policies are some of the major selection criteria that are considered when choosing any bioremediation technique[20,21].

For effective bioremediation, microorganisms should attack enzymatically on the contaminants and then convert them into nontoxic products because Enzyme- mediated bioremediation refers to the use of naturally occurring enzymes in microorganisms or plants to degrade or reduce harmful, unwanted environmental contaminants in order to clean the polluted sites[22].

Enzymes are biocatalysts which lower the activation energy and facilitate quick and complete breakdown of substrates. There are several types of enzymes such as oxidoreductases, laccases, hydrolases and peroxidases etc. which are actively involved in bioremediation process[23]. Enzyme - mediated bioremediation can be of two types:

1) Intracellularly 2) Extracellularly

Intracellular enzymes are those enzymes that are present inside their originating cells and Extracellular Enzymes refers to those enzymes that are secreted by the microbes such as white rot fungi. The action of fungi is mainly because of oxidative enzymes (extracellular enzyme), like laccase, manganese peroxidase and lignin peroxidase, which are released from fungal mycelium[24]. Enzymes secreted from white rot fungi have been shown to be effective degraders of pharmaceutical and personal care products (PPCPs). One of the common organic compound which cause the environmental pollution is the xenobiotic organic compounds (XOCs)[25]. Accumulation of XOCs in the greywater commonly results from the usage of personal care products, shampoos, hair conditioners, oils and foodstuffs, moisturising oils and the food additive[26]. The oxidative enzymes are used to treat the grey water having xenobiotic compounds as the oxidative enzymes have the ability to breakdown these xenobiotic compounds into harmless products[27].

Factors affecting Bioremediation

The control of bioremediation procedures is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population, environmental factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

Nutrients

Carbon is known to be the most basic element of living forms and is desired in greater quantities than other elements. Nutrient balancing specially the supply of essential nutrients like N and P can increase the biodegradation efficiency by enhancing the bacterial C: N: P ratio. Various nutrients like carbon, nitrogen, and phosphorous are required by microbes for survival and continuous microbial activities[28,29]. In addition to hydrogen, oxygen, and nitrogen, carbon constitutes about 95% of the total weight. The nutritional necessity of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1.

pH

The optimum pH condition for the effective bioremediation should be in the range of 6.5 - 8.0 as the pH plays the major role in affecting the solubility and biological availability of nutrients, metals, and other constituents; for ideal growth of microorganisms, pH should remain within the tolerance range for the target microorganisms because metabolic processes are extremely susceptible to even small variations in pH[30]. The quantity of pH in soil might show the potential for microbial growth[31].

Temperature directly affects the rate of microbial metabolism and consequently microbial activity in the environment. The biodegradation rate, to an extent rises with increasing temperature and slows with decreasing temperature. The optimum temperature ranges for microbial growth is from 25-45^{o} C [32].

Concentration of oxygen

Some microorganisms like aerobic microbes require oxygen to facilitate their biodegradation rate in a better way on the other hand the anaerobic microbes do not require oxygen for biodegradation process. The existence of oxygen in many cases can increase the metabolism of hydrocarbon[33].

Microbial Populations for Bioremediation Process:

The main objective in bioremediation is to stimulate microorganisms with nutrients and the other chemicals that will permit them to destroy the contamination. Microbes could be isolated from almost all types of environmental conditions and additionally had a wide range of adaptability. They can survive from zero to extremely excessive and desert conditions. In aquatic environment, they can survive in presence and absence of oxygen and also in presence of hazardous compounds or waste stream. According to the [34], microorganisms have the ability to remove heavy metals and hydrocarbons from aqueous solution in large quantities. To survive under water polluted with metal, bacteria have evolved different types of mechanisms which involve the elimination of heavy metals by efflux of metal ions outside the cell, accumulation and formation of complex of the metal ions inside the cell and later decrease the effect of toxic metal ions to a non-toxic state. For successful degradation it is important that microorganisms and the contaminants be in contact. The microorganisms involved in degradation process may belong to bacteria, fungi, yeast and algae[35-38].

The microorganisms can be divided into various groups:

Aerobic: Most of the microbial reactions occurs in the presence of oxygen because the low molecular weight hydrocarbons can be easily readily degraded in oxygen-rich environments, mainly when those hydrocarbons are unsubstituted (i.e., contain only carbon and hydrogen). Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus* and *Mycobacterium.* These organisms have often been testified to degrade pesticides, hydrocarbons, alkanes and polyaromatic compounds. In this process, bacteria use the pollutant as the sole source of carbon and energy[39].

Anaerobic: In the absence of oxygen. Anaerobic bacteria are not as commonly used as aerobic bacteria in degradation process because the degradation rates pollutants become very slow in anoxic environments. These types of bacteria are used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, de-chlorination of the solvent trichloroethylene (TCE), and chloroform[40].

Ligninolytic fungi: Using fungal mycelium or fungal species to neutralize the toxic material from contaminated sites is named myco – remediation. Some fungi species such as *Phanaerochaete chrysosporium* and *Polyporus* sp. have the capability to reduce the effect of toxic environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, polychlorinated biphenyls (PCBs), and organochlorine pesticides.

Algae: "Use of algae to treat wastes or wastewaters" is known as the Phycoremediation. The algae are distributed widely throughout the earth and have adapted to a diversity of habitats. Phycoremediation is the use of macroalgae, microalgae and cyanobacteria for the removal of nutrients and xenobiotics from wastewater and carbon dioxide from the air[41]. Examples such as *Chlorella, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas, Spirulina, Oscillatoria, Desmodesmus, Arthrospira, Nodularia, Nostoc, Cyanothece* etc. found to be the most effective algae spp. for the treatment of wastewaters[42,43]. Cyanobacteria showed the excellent ability for bioaccumulation and biosorption because they are present ubiquitously in water and have a flexible metabolism[44].

Types of Bioremediation

On the basis of removal and transport of wastes for treatment there are basically two methods

- Ex situ bioremediation.
- In situ bioremediation

Ex situ bioremediation: Ex- situ techniques are useful to treat soil and groundwater by means of excavation (soil) or pumping (water) respectively from contaminated places and then transferring them to another site for treatment. Ex situ bioremediation methods are usually dependent on: the cost of treatment, intensity or degree of pollution, type of pollutant and mainly the location of the contaminated place[45].

Biopile

Biopiling is an ex situ technique that is also known as the bioheap or compost piles technique. Piling - mediated bioremediation includes above- ground piling of excavated contaminated soil, followed by addition of nutrient and sometimes aeration to improve bioremediation basically by enhancing the microbial activities. In this process, air is supplied to the biopile system through piping or pumps that either forces air into the pile under positive pressure or draws air through the pile under negative pressure[46]. The microbial activity is enhanced through microbial respiration which results in degradation of the adsorbed pollutant[47]. This technique is most effective in treating soil contaminated with pollutants such as petroleum hydrocarbons, BTEX, phenols, PAHs with up to 4 aromatic rings and explosives such as TNT and RDX.

Land farming

Land farming is also a very powerful and effective method to treat contaminated soil. During this method the contaminated soil is excavated and spread over a prepared bed and periodically tilled until the degradation of the pollutant[48]. The main objective is to encourage indigenous bio-degradative microorganisms and to facilitate their aerobic degradation of pollutants [45,49,50,51].

Just like other techniques, this technique also has some limitations because of which it is not widely used to treat pollutants, which include: large operating space, reduction in microbial activities due to unfavourable environmental conditions, additional cost due to excavation and reduced efficacy in inorganic pollutant removal etc. make land farming based bioremediation time consuming and less efficient compared to other ex situ bioremediation techniques[52,53].

Bioreactors

Nowadays the use of Membrane bioreactors for wastewater treatment is the rapidly developing technique as it reduces the load at municipal water supply and sewage systems[54]. The treatment of wastewater has become essential by government guidelines in many parts of the world due to the importance of maintaining the hygienic nature of freshwater. Bioreactors like rotating biological contactor, biological fluidized bed reactor, membrane bioreactor, continuous stirred tank bioreactor, upflow anaerobic sludge blanket reactor etc., are the most commonly used bioreactors for the treatment of different types of wastewater[55].

In situ bioremediation:

Bioremediation technologies that are used "in place" without removal of the contaminated matrix. In situ remediation includes techniques such as bioventing, biosparging, bioslurping and phytoremediation along with physical, chemical and thermal processes. Both intrinsic and engineered bioremediation technologies can be used in situ.

In situ bioremediation techniques have been successfully used to treat chlorinated solvents, dyes, heavy metals and hydrocarbons polluted sites [56,57,58,59].

Intrinsic bioremediation

In situ bioremediation technique also include the intrinsic bioremediation also known as natural attenuation or bio - attenuation, which involves the remediation of polluted sites without any involvement of external force because this process is totally depend upon the aerobic and anaerobic microbial process to biodegrade the polluted substances. The main advantage of intrinsic bioremediation is that this technique is less expensive compared to other in situ techniques due to the absence of external force [60,61]. One of the major disadvantage of intrinsic bioremediation is that it may take longer time to reduce the pollutant concentration because there is no external force is applied to speed up the remediation process[62].

Bioventing

Bioventing is the most common *in situ* treatment which is widely used to treat the contaminated site. This process involves providing air and nutrients through wells to contaminated area to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides the amount of oxygen which is necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. The advantage of using this bioremediation technique is that it can be used to treat the contamination which is deep under the surface [63].

Biosparging

This bioremediation technique is very similar to bioventing which involves the injection of air and nutrients (if needed) under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. However, just like bioventing, air is injected at the saturated zone, due to which upward movement of volatile organic compounds occur to the unsaturated zone which ultimately promotes biodegradation[64]. This technique is can be used to reduce the concentration of petroleum constituents that are dissolved in groundwater or adsorbed to soil below the water table via indigenous microorganisms. The ease and low cost of installing small - diameter air injection points allows considerable flexibility in the design and construction of the system.

Bioaugmentation

Bioaugmentation is the process of adding group of natural microbial strains or a genetically engineered microbe (which act as a bioremediators) to treat the soil or water contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene. The chlorinated compounds are completely degraded to ethylene and chloride by *in situ* microorganisms[65]. Generally, in this process genetically engineered microorganisms are widely used as they break down the pollutants and convert them into nontoxic forms at much faster rate than natural microbial strain[66,67,68].

Genetically engineered microorganisms have shown potential for bioremediation of soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a broad coverage of chemical and physical pollutants[69,70].

Phycoremediation

Phycoremediation is defined as the "use of algae to treat wastes or wastewaters". The algae comprise both the microalgae as well as the marine macroalgae more commonly known as the seaweeds. The algae are distributed broadly throughout the earth and have adapted to a diversity of habitats. This has also allowed the algae to develop wide tolerance to environmental conditions including nutrient levels[41]. In addition, microalgae have the capability of eliminating environmental pollutants such as heavy metals, hydrocarbons and pesticides via various mechanisms, ranging from bio sorption, bio concentration, bioaccumulation[71].

The major advantage of using algae in bioremediation of wastes, resulting in treated waters as well as the production of a useful biomass due to its high nutritive value which can serve as feedstock for a diversity of valuable products, including food, feed, fertiliser, biofuel[43,72,73]. During the phycoremediation process, algae remove carbon dioxide through photosynthesis and have the potential to be a carbon reducing system when combined with production of biofuel and integrated with waste remediation[74].

Advantages and Disadvantages of Bioremediation

Table 1. Advantages and disadvantages of bioremediation[74]

Advantages	Disadvantages
Natural Process and Safe for the environment	Bioremediation is limited to biodegradable compounds
Cost Effective Process as it eliminates the transportation costs and operating	There are some concerns regarding the degradation products, which can be more toxic than the parent compound
Microorganisms have the ability to degrade a large number of contaminants, and the result remained after their work is harmless to the environment.	Bioremediation is not going well on clay soils, compact, where oxygen or nutrients are difficult to insert in the treaty
Does not generate waste	Process of bioremediation lasts much longer than other treatments, such as excavation, soil and incineration can be necessary to provide a control institutionalized for long- term protection
Can be made directly on the site, ecosystem disruption is minimal	Biological processes are often highly specific.
It may be combined with other treatment technologies	

Case studies related to bioremediation of sewage wastewater

According to[75], at a global level, around 80% of wastewater is formed which is then discharged into the environment untreated, causing extensive water pollution[75].

Wastewater treatment is not a single problem for the developing countries however it continues to be the basic sanitation need to protect the surroundings and the water forms that serve as drinking water sources around the globe[1]. Wastewater is a by-product of domestic, industrial, commercial or agricultural activities[76].

Through many research it has been shown that bioremediation can be a useful technique to treat waste water due to the ability of microorganisms to survive, adapt, and flourish into various types of environments, including wastewater[2-5].

In another study the performance of consortia involving *Bacillus pumilus, Brevibacterium sp,* and *Pseudomonas aeruginosa* was examined for the sewage wastewater treatment in terms of reduction in COD (chemical oxygen demand), BOD (biochemical oxygen demand) MLSS (mixed liquor suspended solids), and TSS (total suspended solids). During this experiment, various parameters were adjusted including inoculum size, agitation, and temperature to attain the effective results in less time. As a result, it was obtained that the consortium in the ratio of 1: 2 (effluent: biomass) at 200 rpm, 35°C is capable of efficiently decreasing the concentration of COD, BOD, TSS, and MLSS within the ideal discharge limits, that is, 32 mg/L, 8 mg/L, 162 mg/L, and 190 mg/L of the sewage wastewaters. The use of such specific consortia can conquer the inefficiencies of the conventional biological treatment facilities presently working in sewage treatment plants[77].

[78] investigate the potential of bacteria for the treatment of municipal wastewater. In this study, total eight bacterial isolates were used. These bacterial isolates were known on the basis of morphological and biochemical characterization and identified as *Bacillus licheniformis* NW16, *Pseudomonas aeruginosa* NS19, *Pseudomonas sp.* NS20, *Planococcus salinarum* NS23, *Stenotrophomonas maltophilia* NS21, *Paenibacillus sp.* NW9, *Paenibacillus borealis* NS3 and *Aeromonas hydrophilia* NS17 out of which the bacterial isolate known as *B. licheniformis* showed highest remediation potential than other isolates. *B. licheniformis* and *Aeromonas hydrophilia* showed highest reduction (42.86%) in BOD level each. *B. licheniformis* and *Paenibacillus sp*. showed 82.76% and 81.61% reduction in COD respectively. From this study it was also found that all the bacterial isolates have potential to reduced phosphate from 17.55% -72.3%. Apart from this, *B. licheniformis*, *Ps. aeruginosa*, *Pseudomonas sp*., *Paenibacillus sp*. and *Aeromonas hydrophilia* displayed reduction in TSS and TDS ranging from 42.69%-79.94% and 14%-81.4%. respectively.

The performance studies of bioreactors carried out by many researchers have also been reviewed. Out of many bioreactors, Upflow anaerobic sludge blanket bioreactor is widely used for the treatment of sewage wastewater. Upflow anaerobic sludge blanket technology, normally referred to as UASB reactor, is a form of anaerobic digester that is used for treatment of various wastewater. [79] treat the raw domestic wastewater through an integrated UASB sludge digester system in temperate climates. To increase the working of an upflow anaerobic sludge blanket (UASB) reactor the addition of a sludge digester to the process was investigated for curing of raw domestic wastewater under temperate climates conditions. For the UASB reactor operating alone at a hydraulic retention time of 6 hrs, the COD removal efficiency was decreased from 78% to 42% when the temperature was decreased from 28^{o} C to 10 ^{o}C

Recently, new technologies concerning water and wastewater treatment have been established and among these methods, the fixed bed biofilm reactor along with membrane bioreactor is the latest substitute to conventional technologies. In this research, a combined fixed bed membrane bioreactor (FBMBR) through a hydraulic retention time (HRT) of 36 h was established to eliminate contaminants from real paper recycling wastewater. The removal efficiencies of chemical oxygen demand (COD), ammonium, nitrite, nitrate and overall nitrogen (TN) for permeate and supernatant existed in the range of 92–99%, 59–97%, 78–97%, 59–98% and 68–92%, respectively. Through this research study it was concluded that the FBMBR can be effectively used for the elimination of contaminants from wastewater[80].

Apart from these bioremediation techniques phycoremediation is also a biological treatment which are considered as an ecological and environmental friendly technique to remove contamination in wastewater. According to the research study conducted by[73], phycoremediation is one of the helpful method that have a high ability to lower-down the extreme contaminants in wastewater photosynthetically.

According to many scientists like[42,43] there are many species being used for the treatment of various wastewater through phycoremediation technique like *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas*, *Spirulina*, *Oscillatoria*, *Desmodesmus*, *Arthrospira*, *Nodularia*, *Nostoc*, *Cyanothece* etc.

It is an alternative technology which is nowadays widely used for the wastewater treatment compare to conventional treatment process in economical and sustainable way. For example, for the treatment of sewage wastewater Chlorella minutissima, Scendesmus spp & BGA (Nostoc) and their consortium was used and the results showed that these algal species were very effective in removal of BOD, COD, NO_3, NH_4, phosphate and TDS in sewage wastewater.

Moreover, it has been experimentally proved that Chlorella spp. was having best phycoremediation potential as well as manure production among all microalgae[3].

Autotrophs play an important role in wastewater treatment mainly domestic waste water by its photosynthetic ability. To examine the role of algae, [81]collected the samples of wastewater from sewage wastewater treatment plant. Than the samples were used to isolate the potent algal species like *C. vulgaris* and *S. quadricauda* cultures and used them for the treatment. To examine the role of the microalgae in wastewater treatment, the wastewater was treated into two different patterns (i) when wastewater was treated with *C. vulgaris* and *S. quadricauda* culture; and (ii) when wastewater was treated without *C. vulgaris* and *S. quadricauda* culture (Control). During the experiment, all physico-chemical parameters like pH, phosphate, nitrate, BOD and COD using standard methods were quantified for 0th, 5th, 10th, 15th and 20th days, respectively. The results showed that the removal efficiencies of COD, BOD, nitrate and phosphate of wastewater were 80.64%, 70.91%, 78.08% and 62.73%, respectively when wastewater was treated with *C. vulgaris* upto 15th days. On the other hand, when wastewater was treated with *S. quadricauda* the removal efficiencies of COD, BOD, nitrate and phosphate of wastewater were 70.97%, 89.21%, 70.32% and 81.34%, respectively upto 15th days.

[82]used the phycoremediation technique for the treatment of sewage wastewater as well and industrial flue gases for biomass generation from Chlorella vulgaris microalgae. In this study the sewage waste water (SWW) and industrial flue gas was provided in batch and continuous mode for cultivation of *Chlorella vulgaris* (microalgae). The phycoremediation and microalgae cultivation via sewage with industrial flue gases is known to be a favourable concept for removal of pollutants along with biomass generation to control the environmental pollution. Results showed that the Chlorella vulgaris reduces the concentration of COD up to 78% in batch mode and 42% in continuous mode. The other nutrients like NO_3, SO_4, and PO_4 from the sewage wastewater were eliminated up to 75% in batch mode whereas, 55% with continuous mode 55%. Concerning the flue gases, *Chlorella vulgaris* was very effective in reduction of CO_2 up to 64% in batch mode and 72 % in

continuous mode whereas the concentration of SOx and NOx were decreased up to 62% and 63% respectively in batch mode and 59% and 55% in continuous mode. From the results the scientists concluded that the *C. vulgaris* proved to be very effective in the treatment of SWW and industrial flue gas.

Phytoremediation

The blending of two latin words —plant and —remedy, forms the term phytoremediation. Phytoremediation is an inexpensive and feasible sustainable method for the removal of contaminants. At the same time, it is environmental friendly and further it does not affect people living and working in the surrounding as it uses plants for cleaning environment[83]. Phytoremediation is vast, developing term which has been used in recent decades for a group of green eco-friendly technologies that fundamentally based on plants. Phytoremediation is a yet another developing technology with worthy efficiency for treating effluents and this technology should be encouraged, so that it can be applied practically to restore the water and soil resources in situ. It is an eco- friendly and green technology which uses plants for remediation and thus would prove to be a safe technology for restoring environment[84].

Nowadays apart from microbial degradation phytoremediation technologies are widely used to remediate the substances in contaminated soil, sludge, sediment, groundwater, surface water and wastewater[85,86]. Sometimes water contaminated with heavy metals such as cadmium and lead are not easily absorbed by microorganisms., in such case, phytoremediation proves a better treatment tool for bio-treatment because natural plants or transgenic plants are able to bio accumulate these toxins as the aquatic plants have excellent capacity to reduce the level of toxic metals, BOD and total solids from the wastewater[87].

Phytoremediation technique includes several techniques which are applicable in treatment of wastewater (surface water and groundwater), in the removal of unwanted nutritive substances from water reservoirs[88].

- **Phyto-degradation/phyto-transformation**: Contaminants are taken up into the plant tissues where they are metabolized, or bio transformed. Where the transformation takes place depends on the type of plant and can occur in roots, stems, or leaves.
- **Phyto-stimulation/rhizo-stimulation**: Phytostimulation is the process where root released compounds enhance microbial activity in the rhizosphere. This process is critical for the applied technology of rhizoremediation that combines phytoremediation and bioaugmentation.
- **Phyto-volatilization**: Plants take up water and organic contaminants through the roots, transport them to the leaves, and release the contaminants as a reduced or detoxified vapour into the atmosphere.
- **Rhizofiltration**: This process takes place in the soil or groundwater immediately surrounding the plant roots. Exudates (excretions) from plants stimulate rhizosphere bacteria to enhance biodegradation of contaminants.
- **Phytostabilisation**: Plants prevent contaminants from migrating by reducing runoff, surface erosion, and ground water flow rates. Hydraulic pumping can occur when tree roots reach ground water, take up large amounts of water, control the hydraulic gradient, and prevent lateral migration of contaminants within a ground water zone.

Some major plant species having phytoremediation ability include: *Brassica napus* L. subsp. *napus* and *Festuca ovinia* L.[89], *Ludwigia octovalvis* [90], *Sesbania cannabina* [91] etc. for example [92]examine the effect of phytoremediation with mixture of plants on municipal wastewater (MWW). In this study, a phytoremediation garden was prepared by using different aquatic plants species namely *Pistia stratiotes*, *Eichhornia crassipess*, *Hydrocotyle umbellatta*, *Lemna minor*, *Tyhpa latifolia*, and *Scirpus acutus*. The physico-chemical study of MWW was carried out before and after phytoremediation technique. As a result, it was obtained that the pH, EC and turbidity of MWW were reduced by 5.5%, 33.7%, and 93.1%, respectively along with the reduction in total dissolved solids (TDS) by 35.2%, Cl by 61%, HCO_3 by 29.2%, hardness by 45.7%, Ca by 32.3% and Mg by 55.9%. From this research it was concluded the phytoremediation with a mixture of plants was more effective than that relying only on a single plant species.

Fareed et al. 1993, evaluated the phytoremediation potential of water hyacinth (*Eichornia crassipes*) and water lettuce (*Pistia stratiotes L.*) for the removal of nitrogen and phosphorus (nutrients) from domestic sewage contaminated pond (approximately 10500 m^2 in area, average 2.5 m in depth) by using self-designed experimental devices.[93] In this study all the physicochemical properties of water and plant samples as well as N and P mass balance were examined. After examination the range of physicochemical parameters of influent were demonstrated as follows: water temperature (WT: 24.5 $^{\circ C}$ –31.0 $^{\circ C}$), pH (6.94–8.25), DO (4.58 mg L^{-1}–15.73 mg L^{-1}), COD_{Mn} (5.00 mg L^{-1}–13.15 mg L^{-1}), TN (1.60 mg L^{-1}–5.60 mg L^{-1}) and TP (0.16 mg L^{-1}–0.73 mg L^{-1}). It was found that the water hyacinth is more suitable than water lettuce for the profound purification of water contaminated with domestic sewage with high nitrogen concentrations as water hyacinth exhibited hyperactive accumulating capacity for nitrogen (58.64% of total reductions), than water lettuce. These results showed that the water hyacinth has the larger total root surface area (0.97 m^2 g^{-1}–1.10 m^2 g^{-1} fresh weight), active absorption area (0.31 m^2 g^{-1}–0.36 m^2 g^{-1} fresh weight), and leaf area and higher root activity (71.79 µg g^{-1} h^{-1}–98.34 µg g^{-1} h^{-1}), root biomass (kg m^{-2}), and net photosynthetic rate (20.28 mol CO_2 m^{-2} S^{-1}) than those of water lettuce. On comparison water hyacinth showed the high Total Nitrogen (TN) removal efficiency (47.42%) than water lettuce (46.56%) whereas water lettuce showed a higher Total Phosphorus (TP) removal efficiency (58.27%) in domestic sewage than water hyacinth (53.44%). From this study it was concluded that in future, the combination of both macrophytes was recommended for the phytoremediation of most domestic sewages containing dual contaminants (N and P).

Conclusion and Future Aspect

Bioremediation is a very productive and attractive option to remediating, cleaning, managing and recovering technique for solving polluted environmental problems through microbial activity. This technique has the ability to decontaminate the environments inexpensively yet effectively. A wide range of microbes with remediating abilities is waiting to be explored but due to the lack of sufficient knowledge about microorganisms and their role in the environment might alter the acceptability of their uses. The proper information regarding the diversity of microbial population in environment is necessary to get a better insight into potential bioremediators that will result in emerging a suitable bioremediation technique, thus, conserving the long-term sustainability of water ecosystems.

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