Chapter 16
Application of Microbial Technology for Waste Removal

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Abstract The continuous rise of waste in the environment becomes a global burden as it decreases the natural balance of waste recycle. It has further accelerated due to quality and amount of waste added in the environment in the last century. Increasing human population, introduction of xenobiotic compounds, overexploitation of natural resources and alarming increased waste generation rate are major threats to environmental safety. Several waste management practices have been implemented to decrease the harmful impacts of waste. Microorganisms are inhabitants of nature that play a major role in biodegradation, bioremediation, nutrient cycling and detoxification to maintain a sustainable environment. Microbial technology utilizes a wide range of selective microorganisms in specific condition for removal of waste from the environment. The utilization of microbes is only limited to culture-dependent method, and the majority of undiscovered microbes has also been explored using culture-independent techniques. Technological advancement has increased the exploration of microbial diversity for their utilization in solid and liquid waste management. Traditional and advanced techniques such as composting, anaerobic digestion and bioremediation techniques have been implemented in solid waste management. Waste from wastewater has been successfully removed using fixed-film processes, activated sludge, biosorption technology and microbial electrochemical technology. Notorious chemicals such as synthetic dyes and oil spillage have been also removed from wastewater using microbial technology. Microbial technology has been magnificently implemented around the world for removal of waste from the environment. This chapter represents traditional and advanced microbial technology in both solid and liquid waste treatments.

Keywords Microbes · Microbial technology · Waste disposal · Aerobic processes · Anaerobic processes Solid and liquid waste management

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16.1 Introduction

Waste management and environmental sustainability are major global concerns of the present society. In the past, there was a balance between the biosphere's natural cycle and mankind activity. The amount and quality of waste were not a burden, hence recycled by natural phenomenon. Human activity always generated waste products either as a by-product of their activity or generation of product which is assumed to be not useful for their life. Despite the generation of waste throughout their life, nature has treated the waste using their own treatment process such as waste degradation, dispersion and dilution. But, the activity of modern society creates disturbance in the equilibrium that continuously accelerates the burden of waste. There are three primary causes that play a synergistic effect on the continuous increase of waste: exponential growth of human population, extensive utilization and diffusion of toxic metals into the environment and tremendous utilization and dispersion of notorious chemicals such as xenobiotic compounds (Gandolla and Aragno 1992). The amounts of different wastes rise in such a way that they create intense damage to the environment and possibility of return to the environment is also reduced. Rapid consumption rate of raw materials leads to an upsurge of huge quantities of physical, chemical and radioactive wastes in the environment, which damage the biosphere rapidly. The accumulation of waste is the core factor for change of environmental consequences and loss of materials (Kumar et al. 2017). There is an urgent interference needed to tackle this critical situation.

Waste is difficult to address, as it becomes useful for someone and not for others. It was also defined as "any material or product which is useless to the producer is considered as waste". According to Dijkema, peoples want to dispose the generated materials even if it requires them to pay for their disposal (Dijkema et al. 2000). Brunner and Rechberger mentioned that despite the fact that waste is always a major concern for the environment and mankind, most human activities generate waste (Brunner and Rechberger 2015). Waste can be classified on the basis of some common characteristics such as physical states, reusable and recyclable potentials, physical properties, source of production, biodegradable potentials and degree of environmental impact (Demirbas 2011). It can be further classified on the basis of source as domestic waste, industrial waste, commercial waste, agriculture waste, mining waste and construction waste. Waste is also classified on the basis of environmental impact as hazardous waste and non-hazardous waste.

Waste has been categorized into various categories in different countries and their treatment is also based on its quality. The reduction and disposal of wastes have become a global concern that needs to be managed. All the waste treatment strategy is based on the return of waste mass to the environment. Disposal of waste into the environment can be done by either concentration of waste at a target site or dispersal of the waste substance into the biosphere. Dispersal is a good strategy but only environmentally acceptable substance can be dispersed. Therefore, waste treatment is aimed to either produce dispersible derivatives or concentrate the harmful ingredients. According to United Nations Environment Programme, waste management programmes should focus on the 3R concept which is expanded as "Reduce, Reuse and Recycle". The proper waste management planning and control is necessary for the prevention of the harmful impacts of waste on the environment (Ghiani et al. 2014).

16.2 Waste Management Practices

There are three categories of waste management practices: physical, chemical and biological. Physical methods comprise of incineration, compacting and sorting for solid waste management, whereas sediment dredging, artificial aeration, mechanical algae removal and water diversion are methods of waste water treatment (Wang et al. 2016). These traditional methods require a lot of time, material, effort and financial sustenance. These methods are able to remove large-size particles but not all pollutants from land or waterbodies competently.

The second technique is chemical method that includes gaseous emissions, gasification and pyrolysis for solid waste treatment. Liquid waste is chemically treated by flocculation, precipitation, chemical oxidation and chemical algae removal (Ma et al. 2015). Chemical methods require the support of a lot of chemical agents that include oxidizing agents, precipitants and coagulant salts. These methods are considered as emergency treatment, as they are not much efficient for the treatment of organic matter. Chemical methods have several other disadvantages such as consumption of high energy, foul odour and generation of toxic methane gas which are hazardous for the ecosystem.

The third approach is biological process that includes anaerobic digestion, composting, vermicomposting, biofiltration, microbial technology, biological-ecological methods, plant purification technology, combinatorial biotechnology, artificial wetland technology and biofilm technology (Xu et al. 2017). Biological methods are environmental-friendly that improve the natural process of waste removal and self-purification process of the polluted water ecosystem (Ravikumar et al. 2017). Technical supports are needed to overcome minor difficulties and cost of waste removal process in the future. Among various biological methods, microbial technology positions its own advantages and is considered as a highly efficient method.

16.3 Microbial Technology

Microorganisms have long since been explored in the food and beverage industry, pharmaceuticals, healthcare products, agriculture and industrial applications and for environment protection. A large number of microbes still need to be discovered and explored for their ecological application. Microbes play an important role in environmental protection and sustainability. Proper selection and effective utilization of microbes are crucial steps for the development of microbial technology. Microbial technology has been utilized for nutrient cycling, biodegradation, bioremediation, environmental detoxification, production of biocatalysts, bio-detergent, biomass fuel production, bioaccumulation, bioleaching, biomonitoring and so on. It has several advantages such as highly competent degradation ability, free from secondary pollution, reduced energy consumption, enduring viability, ease in technical operation and low capital investment.

Microbial technology has been magnificently implemented in several countries for environmental safety and waste management. Microorganisms especially bacteria, fungi, yeasts and their products were effectively used to remove different quality of waste and returned back into the atmosphere. Microbial technology was also used for the production of energy and biofuel from waste; biotreatment of pulp, oil and textiles; as well as production of valuable end products by fermentation process. They were also used for treatment of sewage water using aerobic and anaerobic microbes sequentially. Engineered microbes were recently used as additive for treatment of heavily polluted tidal river in China which shows improvement in treatment up to 70% (Sheng et al. 2012). Advanced microbial technology uses microalgae-bacteria consortium for treatment of nitrogen and phosphorus contamination from surface water (Liu et al. 2017). These photosynthetic bacteria were easily grown, more viable, more environment friendly, economical and highly effective in bioremediation process (Idi et al. 2015). The salient features of application of microbial technology seem to be highly effective in waste removal, and therefore, the present chapter emphasizes primarily on effective microorganisms and recent methods of microbial technology for removal of waste substance from terrestrial and wastewater.

16.4 Effective Microorganism

Microorganisms are cosmopolitan, present dominantly in soil where they a play vital role for sustainability of the environment. They are also residing in fresh- and marine water, plant, animals as well as air. These microbes are highly diverse, considered to be the largest unexplored reservoir. They perform numerous functions in the biosphere. Effective microorganisms play several roles in the biosphere such as recycling of waste from soil and water, plant growth-promoting activity, inhibition of soil-borne pathogens, enhancement of anti-oxidation capability in soil, nutrient recycling and so on (Shalaby 2011). They have functional diversity; some are known, while most need to be explored. Attention has been paid for the exploration of desired microorganisms from unique and less explored habitats. Selection of effective microorganisms is a fundamental part of microbial technology. It is usually followed by strain improvement using mutagenesis or genetic engineering to make them more capable for desired purposes.

The microbial activity can be further accelerated by application of microbial accelerating agents. Microbial accelerating agents are formulation of microbial growth promoters such as trace elements, amino acids, humic acid enzymes and vitamins in proportionate amount. These agents are always harmless to soil environment. These nutrients can accelerate microbial growth and stimulate them for biological reactions. They promote the proliferation and activity of indigenous microorganisms while inhibiting the anaerobic decomposition of pollutants (Wu and Xie 2012).

16.5 Microbial Technology for Waste Removal

Microbial technology is a scientific technique which utilizes a wide range of microorganisms in meticulous condition without distressing the ecosystem. An eclectic variety of microorganisms have been effectively used for waste removal practices. Microbial technology is fairly used for waste management practices in very effective way. It is eco-friendly, cost-effective and a better substitute compared to expensive physico-chemical remediation processes. The efficient methods adopted for waste removal process using microorganisms are composting, bioremediation, biodegradation, bioaccumulation, biotransformation, anaerobic digestion and wastewater treatment.

16.6 Microbial Technology for Solid Waste Treatment

16.6.1 Composting

Composting is an aerobic microbial decomposition process in which organic matter hydrolyzes into stable residue (Wei et al. 2017). This process is generally used through metabolic activity of microbial consortium to produce safe and stabilized form of organic compost for various agricultural practices. During the process temperature increases spontaneously; it would help to eradicate the pathogenic organisms; hence, finally generated compost becomes safe for usage (Rastogi et al. 2020). Several composting divisions exist at various locations in India such as Mumbai, Bengaluru, Indore, Vadodara, Kanpur and Delhi. These units have 150–-300 tonnes/day as installation capacity (Sharholy et al. 2008).

16.6.1.1 Framework of Composting Process

The following phases are included in compositing process:

1. **Mesophilic phase:** In this phase mesophilic fungi and bacteria degrade the complex compounds such as carbohydrate and amino acids into simple one by rapidly elevating the temperature.

2. **Thermophilic phase:** This is the second phase of composting in which thermophiles degrade the organic matters (lignin, cellulose, fats and hemicellulose). During this phase, thermophilic microbes would utilize the organic carbon content for their feedstock and metabolic activities.
3. **Cooling phase:** In the last phase microbial activity is diminished and temperature has also decreased. During this stage mesophilic microorganisms degrade the residual substances such as cellulose, hemicellulose, sugars and humus (Albrecht et al. 2010).

16.6.1.2 Factors Affecting the Composting Process

Some physiochemical factors such as nutrient balance, C/N ratio, pH, particle size, moisture, temperature, porosity, oxygen, moisture content and nutrient availability play a foremost role in various phases to determine the development of microbial populations during composting (Leow et al. 2018).

16.6.1.2.1 C/N Ratio

As a nutritional parameter, the optimum balance of carbon-nitrogen ratio is essential for the formulation of compost. Ideally, C/N ratio essentially range between 25 and 35, declaring that microbial requisite is around 30 parts carbon to 1 part nitrogen (Kutsanedzie 2015). However, some authors state that C/N value at the range of 20–50 also gives a worthy result (Petric et al. 2015). During the composting process, carbon is transformed to carbon dioxide during organic degradation. Due to higher C/N ratio, there is deficiency in nutrient level to microbiota and composting speed decreases. Lower C/N gives rise to increased nitrogen content compared to degradable carbon and that results in loss of ammonia in soil through leaching or volatilization (Zhang and Sun 2016). Generally, declined C/N ratio indicates higher waste degradation over the mineralization process (Wang et al. 2016).

16.6.1.2.2 Temperature

Composting is an exothermic technique, which includes organic matter degradation by bio-oxidative microbial activity. In this process a huge magnitude of energy is produced. But microorganisms utilize only 40–50% of the generated energy for the synthesis of ATP; the rest of the energy is lost in the form of heat. The huge quantity of heat is the basic reason for increased temperature during the process and it can reach up to 70–90 °C. This process was named as "microbial suicide" by Finstein (Waszkielis et al. 2013). This high temperature inhibits the microbial growth and slows down the biodegradation process. The organic matter decomposition rate is dependent on the temperature of the raw material. In the higher temperature (above 70 °C), only few thermophilic bacteria can survive and show metabolic activity. So, for the destruction of pathogens, weed seeds and fly larvae, maximum temperature would be maintained at least 3–4 days (Garg and Tothill 2009). Increased microbial diversity will be necessary to obtain higher biodegradation rate and the required temperature should be in the range of 30–45 °C (Finstein et al. 1983). The set point for feedback temperature control ranged between 30 and 45 °C during the process of composting.

16.6.1.2.3 Aeration

For the aerobic decomposition of raw material, sufficient oxygen supply is required. Anaerobic condition could be developed in the deficiency of oxygen in the environment that will create pungent odour of methane (CH_4) gas. Oxygen would be refilled in the waste materials by using perforated pipes (Garg and Tothill 2009).

16.6.1.2.4 Moisture Content

Water is the most essential part of composting cycle and microbial activity. The maintenance of optimal moisture content is dependent on particle size, physical state of the initial material and the type of composting system. Usually, 60% of moisture content is considered as suitable for starting material. Because different materials have different water-holding capacities, an exact generalization of moisture levels cannot be made. If the moisture content is too low, early dehydration occurs which arrests the microbial activity, giving physically stable but biologically unstable compost. In modern composting systems, water can be added during the process. In newly designed plant system having a capacity to remove a large amount of water, evaporative cooling system and high rate of heat generation, this dried material required the addition of water for sustainability of microbial activity. This will be only possible in conjunction with mechanical turning. The biological activity of stabilized end material can be prevented by lowering (about 30%) the moisture content of composting process (Diaz and Savage 2007).

16.6.1.2.5 Time

The period of active composting: dairy waste can be converted into compost around 1–14 weeks; this is followed by 3–4 weeks of curing period (Garg and Tothill 2009).

16.6.1.2.6 pH

The estimated pH range for the decomposition of organic matter is 5.5–8.0 (Zhang and Sun 2016). If the pH is more than 8.0, lime or bleaching powder is used at the collection/storage points. If the pH decreases less than 5.5, then microbial nitrification and volatilization occur, which results in the production of enormous quantity of acids and CO_2 (Wang et al. 2016). Volatilization of ammonia leads to disappearance of protein mineralization and slowdown of nitrogen during consequent stages of composting (Guo et al. 2012). Alkalization of compost is associated with preeminent pH (more than 9). It may obstruct the survivability of pH-sensitive microbes that have plentiful contribution in compost sanitation (Hachicha et al. 2009). Herbocel or sanitreat process is applied to control flies and foul odour (Raza and Ahmad 2016).

16.6.1.3 Composting Methods

There are numerous composting methods that suit the goal of the researchers and the nature of waste materials to be composted can be adopted for their purpose of utilization. Some of the composting methods are enumerated below.

1. *Vessel Composting*

   This method depends on the variety of forced aeration and mechanical turning techniques for the enhancement of the composting process. A modified version of in-vessel composting refers to confining the materials within an enclosed area such as a container, building or vessel (Gonawala and Jardosh 2018). This method is too costly and labour-intensive.
2. *Windrow Composting*

   In this composting process, raw materials are employed in a long narrow piles or windrows that curved frequently. Aeration is utilized for the mixing of materials into the setup. In the windrow composting process, system arrangement should be started from 3 ft. height for manures (dense materials) and 12 ft. height for fluffy materials such as leaves. It is a rapid process due to the materials' heat holding capacity and expensiveness in nature (Ayilara et al. 2020).
3. *Vermicomposting*

   Vermicomposting is a fundamentally accomplished process for digesting organic matter by combined action of earthworms and microorganisms to transform the organic waste material into a compost for soil amendment. Earthworms are insatiable consumers that biodegrade matter as vermi-castings or excreta. Earthworms are key players in vermicomposting but they also stimulate microbial activity through fragmentation of organic matter and aeration, and they increase the surface area for microbes. During composting, microbial colonization begins that leads to succession of microbial community composition (Rogayan et al. 2010). Vermicompost provides growth-promoting hormones and nutrients to plants and also plays a responsive role in improving the soil structure by increasing nutrient capacities and moisture content of the soil. With the usages of vermicompost, fruits, flowers, vegetables and other plant products are grown in better quality (Arumugam et al. 2017).

16.6.1.4 Advantages and Disadvantages of Composting

Despite the advantages of compost, some disadvantages also occur (Beffa 2002).

Advantages

- It is cost-effective and very sophisticated.
- Humus and plant litter nutrients can be recycled into the soil.
- It increased the microbial diversity into the soil.
- Compost can be used as biofertilizer as well as biocontrol agent, as they compete with phytopathogens.
- It would be beneficial for biodegradation of toxic compounds and pollutants.

Disadvantages

- Pungent odour nuisances.
- Propagation and dispersal of potential pathogens or allergenic microorganisms.
- If heavy metal content is too high in compost, it could affect the soil.
- This process could be used only for sewage sludge that originate from non-industrial sources.

16.6.2 Aerobic Digestion

In the aerobic biological treatment system, organic matter in waste material is digested under humid and warm condition present. Activated sludge, trickling filters and oxidation ponds are the foremost categories of aerobic systems. Aerobic digestion system works by employing the basic biochemical reaction applied for all the microorganisms (McKinney 1957).

$$\begin{aligned}\text{Food} + \text{Microorganisms} + \text{Oxygen} \rightarrow\ & \text{Increased Microorganisms} \\ & + \text{Carbon dioxide} + \text{Water} \\ & + \text{Waste products } (+\text{Energy})\end{aligned}$$

All the digestion system operates on the fundamentals of biochemical ethics, but some vary from each other by oxygen transfer method. Compressed air is utilized in activated sludge for mixing of oxygen source. Generally, in trickling filter microorganisms are attached to the stones and attained their oxygen from the diffused air. Details of aerobic digestion treatment process have been included in wastewater treatment process in this chapter.

16.6.3 Anaerobic Digestion

Anaerobic absorption is a fermentative decomposition technique which converts the organic waste into composites that can be utilized as biofertilizers and soil conditioners (Rastogi et al. 2020; D'Annibale et al. 2006). Anaerobic absorption is defined as a sequence of organic developments, where microbes can catalyze organic waste material in anaerobic condition. Stages included in anaerobic digestion are acidogenesis, hydrolysis, methanogenesis and acetogenesis.

16.6.3.1 Hydrolysis

Hydrolysis is the preliminary juncture of anaerobic absorption, in which insoluble organic substances such as polymers of amino acids, carbohydrates and fats are converted into soluble matter such as long chain of fatty acids, amino acid and sugar molecules (carbohydrates). Hydrolase enzymes are supported in the hydrolysis reactions. The microbes which synthesize these enzymes are termed as hydrolytic microorganisms such as *Bacteroides*, *Clostridium*, *Bacillus*, *Proteus vulgaris*, *vibrio* and *Micrococcus* and *Staphylococcus* bacteria (Amani et al. 2010). Some hydrolase enzymes are utilized in the form of peptidases, esterase and glycosidases. During anaerobic digestion process, hydrolysis is considered as a rate-limiting stage where insoluble complex organic substance is slowly depolymerized.

16.6.3.2 Acidogenesis

The second stage of anaerobic digestion process involves acidogenesis, where acid-gas-producing bacteria can hydrolyze the soluble molecules to alcohols, lactate, volatile fatty acids and carbon dioxide. Numerous fermentation pathways are involved in this process. Different bacterial genera are responsible to carry out acidogenesis. Some bacterial species that perform fermentation under anaerobic digestion are as follows: for the production of alcohol, *Saccharomyces* is used; for lactate fermentation *Lactobacillus* and *Streptococcus* were utilized; *Butyribacterium* species are employed for butyrate fermentation; and *Clostridium* is used for propionate fermentation. The group of bacteria such as *Clostridium*, *Sporomusa*, *Acetobacterium* and *Eubacterium* is utilized for the production of acetate. These acetate-forming fermentative bacteria are distinctive species for acidogenesis (Amani et al. 2010).

16.6.3.3 Acetogenesis

Acetogenesis is a process involved in the third stage of anaerobic digestion method. Acetogenic bacteria called acetogens can transform the alcohols and volatile acids into acetate in the form of by-product hydrogen and carbon dioxide. Acetogenic bacteria such as *Syntrophobacter wolinii* and *Smithella propionica* are acetate-producing microbes that abrade the butyrate and propionate, respectively. On the other hand, some bacterial species such as *Pelotomaculum thermopropionicum* and *Syntrophobacter fumaroxidans* convert volatile fatty acid into formic acid with the liberation of CO_2 and H_2. *Clostridium aceticum* has the ability to produce acetate from H_2 and CO_2 (Amani et al. 2010).

16.6.3.4 Methanogenesis

Methanogenesis is the ultimate step of anaerobic digestion. In this phase methanogens play a significant role in the consumption of acetogenesis products. It can convert the acetate and molecular hydrogen to methane gas and terminate the activity of acetate-forming bacteria. Few pathways are involved for the production of methane from acetate:

- Acetoclastic methanogenic pathway: In this pathway bacterial species *Methanothrix concilii* and *Methanosaeta soehngenii* are able to transform acetic acid to carbon dioxide and methane.
- Hydrogenotrophic methanogenic pathway: Hydrogen and carbon dioxide are used for the production of methane by employing *Methanobacterium bryantii*, *Methanobrevibacter arboriphilus* and *Methanobacterium thermoautotrophicum.*

Some another bacterial species such as *Methanococcus voltae*, *Methanobacterium formicicum* and *Methanobrevibacter smithii* are applied for the production of methane from methanoate, hydrogen and carbon dioxide (Amani et al. 2010).

16.6.3.5 Factors That Affect the Anaerobic Digestion Process

Temperature: Temperature is the most significant cause for the continued existence and microbial growth in anaerobic digestion. There are two ranges of temperature for the growth of microorganism such as mesophilic (30–40 °C) and thermophilic (50–60 °C) bacteria that optimize the digestion of organic matter. In mesophilic environment (35 °C), the organic part of municipal solid waste can easily be assimilated in the atmospheric condition. Maximum microbial growth rate, microbial activity and production of methane are accomplished by anaerobic digestion under thermophilic conditions (Mata-Alvarez 2002).

Moisture: Moisture content is necessary for controlling the cell turgidity, transport of nutrients, enzymes and hydrolysis of complex organic matters (Khalid et al. 2011). The methanogenic activity increased in high-solid sludge at 90–96% moisture content of mesophilic anaerobic digestion process (Jiunn-Jyi et al. 1997).

Retention Time: In mesophilic condition, 8–40 days of retention time is necessary for anaerobic digestion. According to the report of Fdez, thermophilic anaerobic digestion process of solid organic matter is also accomplished in the range of 8–40 days (Fdez-Güelfo et al. 2011).

pH: The ideal pH range for anaerobic digestion process is 6.8–7.2. Hydrolysis and acidogenesis process enhanced at pH 5.5–6.5, whereas methanogenesis requires pH 7.0 for optimum activity (Ward et al. 2008).

***Carbon and Nitrogen Content*:** Carbon is the energy source for the growth of microbial community, whereas nitrogen assists in the enhancement of microbial growth. The requirement of carbon source by microorganisms is 25–30 times more greater than that of nitrogen, chiefly at a ratio of 20–30:1 (Ward et al. 2008). Elsewhere, for methanization process, the required elements C/N/P/S (carbon/nitrogen/phosphorus/sulphur) have a nutrient ratio of 600:15:5:3 for microbial growth (Khalid et al. 2011).

16.6.4 Bioremediation

Bioremediation is distinct as a technique involved in the degradation of organic matter primarily by microorganisms that can utilize for the transformation of less toxic substances. In this process, naturally occurring microorganisms such as bacteria and fungi and chlorophyll-containing plants can detoxify hazardous substances which are harmful for human health and the environment. The microorganisms are isolated from the contaminated site by the physiological factors that influence the optimization of bioremediation method. Some of the environment factors such as type of soil, temperature, pH, aeration and nutrients are incorporated for the availability of contaminants to the microbial inhabitants. Based on the utilization of microbes, it can subcategorize microorganisms into various groups:

Aerobic bacteria: Microorganisms which survive in the existence of oxygen and utilize the contaminant carbon as the energy source for their growth. Some of the aerobic bacterial species such as *Alcaligenes* sp., *Mycobacterium* sp., *Rhodococcus* sp. and *Pseudomonas* species have the abilities to degrade under aerobic condition. Aerobic bacterial species can also degrade the chemical constituents such as hydrocarbons and pesticides, both poly-aromatic compounds and alkanes.

Anaerobic bacteria: For the degradation of polychlorinated biphenyls (PCBs), anaerobic bacteria can be employed for the bioremediation process of river sediments. Dechlorination process is involved in the bioremediation by anaerobic bacteria. In this process, solvent trichloroethylene (TCE) and chloroform are degraded in the absence of oxygen molecule.

Ligninolytic fungi: Fungi such as *Phanerochaete chrysosporium* are involved in the process of degradation of the extremely miscellaneous range of toxic environmental pollutants. They utilize some easily available substrates including straw, sawdust or corn cobs for their growth and survival.

Methylotrophs: Methylotrophs are a group of aerobic bacteria that utilizes methane and converts it to carbon as energy source for their augmentation and survival. In this pathway the initial enzyme, i.e. methane monooxygenase, is used as a catalyst to produce chemical compounds such as chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

16.6.4.1 Bioremediation Strategies

Various methods are involved in the process of bioremediation and are employed depending on the degree of saturation and aeration of an area as follows.

16.6.4.1.1 Culture-Dependent Approach

In the process of culture-dependent method, microorganisms are cultured into the nutrient growth media and further processed maintaining an objective. This technique involves microbes for the degradation of poisonous composites and manufacture of by-products (Gupta et al. 2019). There are two categories of bioremediation process.

In Situ Remediation

In this technique, generally site-specific microflora plays a significant role in the bioremediation process of the polluted site, which involves without any excavation and transport of contaminants. This method is eco-friendly and cost-effective (Singh 2014). Generally, three methods are used for in situ remediation: bioventing, biosparging and bioaugmentation. These methods are favourable for less polluted sites (Gupta et al. 2019).

1. *Biosparging*

   Petroleum hydrocarbons containing benzene, toluene, ethylbenzene, and xylene isomers (BTEX) are the major components of hazardous fuel that can be accidentally spilled into the environment with the release of gasoline products from the leakage of pipelines and fuel storage tanks. This is one of the primary sources of groundwater impurity. Intrinsic bioremediation process is considered as a potential method for the cleanup and removal for the sites of petroleum-hydrocarbon-contaminated areas (Chen et al. 2006). In the process of biosparging, indigenous microorganisms are used to degrade the organic substances in the saturated zone. Oxygen and nutrient are introduced into the saturated zone for the enhancement of microbial activity. Biosparging technique is utilized for the reduction of petroleum components which are dissolved in groundwater and within the capillary fringe. Biosparging differs from air sparging techniques in the sense that in the air sparging process contaminants are removed through volatilization method, while biosparging stimulates biodegradation of ingredients. If any volatile constituent is present in the form of contaminants, biosparging is frequently combined with soil vapour extraction and other remedial technologies. Whenever combinational process of biosparging and extraction of vapour occurs, the vapour extraction system can produce negative pressure in the unsaturated zone of groundwater through the extraction wells, which could also control the vapour plume migration.

2. *Bioventing*

   This is one of the important in situ remediation processes in which microorganisms can be utilized for the degradation of organic matter which is usually adsorbed with soil in the unsaturated zone. Bioventing system essentially has similar components as present in the soil venting system. It consists of a series of air induction (influent), blower and venting (effluent) wells. Bioventing usually occurs at specific site where air is injected into the soil or extracted from the soil. It can also be accomplished with excavated soils. In the sparging method, air is utilized to provide oxygen for the biodegradation and it can transfer the volatile contaminants from groundwater to the vadose zone in the soil. In the bioventing process, withdrawn air (off-gas) needs treatment (Hoeppel et al. 1991). The off-gas is usually treated by activated carbon, adsorption against thermal pressure and catalysis in a biofilter. During carbon adsorption the air treatment contains a very high cost of venting project (Hoeppel et al. 1991); therefore, as an alternate option, the vented air is used as a biofilter that can pass through unsaturated and uncontaminated soil (Miller and Bartha 1989). With the usage of bioventing process, the cost remediation process can be minimized and the need for off-gas treatment is also eliminated (Lee and Swindoll 1993).

3. *Bioaugmentation*

   Bioaugmentation is an in situ method for the removal of undesired hazardous compounds through the genetically modified microorganisms (hydrocarbon-degrading microbes) from soil and groundwater (Mrozik and Piotrowska-Seget 2010). The foundation of this approach is the augmentation of pollutant-degrading microorganisms for degradation of complex pollutants (Omokhagbor Adams et al. 2020). In this process microorganisms containing the specific metabolic activity are augmented to enhance the rate of waste degradation at the contaminated site. Contamination of chlorinated ethenes, such as tetrachloroethylene and trichloroethylene in the groundwater or soil, can be completely eradicated using in situ microbes. These contaminants are converted to non-toxic substances such as chloride and ethylene (Singh 2014).

4. *Biostimulation*

   Biostimulation is a cost-effective, eco-friendly and extremely efficient bioremediation process. It refers to the addition of micronutrients such as phosphorus, nitrogen, oxygen and electron donors in harshly polluted area for stimulation and to improve the efficient degradation ability of inhabitant microorganisms. Biostimulation technique is applied to accelerate the rate of decontamination and degradation of the toxic contaminants and hazardous compounds. The rate of biostimulation is influenced by various environmental conditions such as moisture content, pH and temperature which predominate the ecological physiology (Abdulsalam and Omale 2009). Biostimulation technique plays an important role in the degradation of petroleum products, hydrocarbons and their derivatives (Kaouther Zaafouri 2014). In the petroleum-contaminated areas, the microbial population and their activity are very low. Poor metabolic activity of microbial population can be remediated through the process of biostimulation (Tyagi et al. 2011).

Ex Situ Remediation

In ex situ remediation, the process is carried out in any other place from the contaminated site (Singh 2014). By using this method, it gives favourable result in decreasing the total load of toxic xenobiotic compounds from the waste (Kumari et al. 2014). Ex situ bioremediation technologies can be facilitated by different methods such as biopiling and landfarming and bioreactors.

1. *Biopiling*

   Biopiling-mediated remediation process is employed for the piling of excavated polluted soil above the ground and nutrient modification, followed by aeration. It enhances the remediation process by increasing the microbial activities. Ex situ technique has been always considered for their cost efficiency. Biopiling can control the nutrient condition, aeration rate and temperature during the process (Whelan et al. 2015). Biopile can also be useful for the limited volatilization of low molecular weight pollutants. It can be effectively utilized for the remediation of pollutants of psychrophilic environments, i.e. cold regions (Dias et al. 2015). In the biopile heating system, augmentation of microbial activity is combined with contaminant availability which enhances the rate of biodegradation. Moreover, heated air is inserted into biopile design to deliver the air and heat in tandem, to facilitate enhanced bioremediation (Aislabie et al. 2006). In the processing of contaminated soil, sieving and aeration are provided by biopile (Delille et al. 2008). However, biopile systems have disadvantages over ex situ bioremediation process that include robust engineering, lack of power supply, maintenance cost, and land farming, especially at remote areas. Further, excessive heating of air can lead to drying of soil undergoing bioremediation, which results in the inhibition of microbial activities, reduction of biodegradation process and promotion of volatilization (Sanscartier et al. 2009).
2. *Landfarming*

   Landfarming is useful mainly for the control of pollutions from the soil by pesticides. In the landfarming process, a bilayer of fresh soil, clay or concrete are prepared from the fresh soil and combination of clay and concrete. The fresh soil is placed at the bottom part and the concrete layer is always placed in the superior layer. Then, natural microbial deprivation is permitted. In this process air, nutrient and humidity are necessary to exhibit the protocol by maintaining the 7.0 pH through liming (Singh 2014).

3. *Bioreactors*

   In the ex situ treatment, slurry bioreactors are used for the removal of stained soil and water pumped up which contaminated the column. Bioremediation by bioreactors involves the removal of contaminated solid materials such as soil, residues and sludge. In this reactor system the rate of biodegradation is higher than in situ remediation, because the contained environment is more convenient, predictable and manageable. The disadvantages of bioreactor is pretreatment of contaminated soil by excavation or vacuum extraction method (Mary 2011).

16.6.4.1.2 Culture-Independent Approach

In this approach, sequencing technique is being utilized for the analysis of genomes of all nucleotides such as DNA, RNA and protein. Various processes are involved in this culture-independent method like processing of whole DNA sequence from specific environmental condition known as metagenomics, whole-genome sequencing, whole-transcriptome sequencing (isolation of RNA from a single and pure culture), metatranscriptomics (RNA isolated from a sample of ecological unit) and so on (Rathour et al. 2017). In nature microorganisms are omnipresent; therefore, to explore their microbial diversity, "next-generation sequencing" would be in assistance to overcome the concealed potential of microbiome functionally, taxonomically and morphologically (Gupta et al. 2019).

16.6.4.1.3 Bioremediation of Rubber Waste

The use of rubber is increasing day by day in vehicles (Holst et al. 1998). Tire is composed of high-grade black carbon and synthetic polymers for maintaining the strength of the rubber tire (Larsen et al. 2006). About 12% of rubber is considered as solid waste. With regard to its physical composition, rubber can neither be degraded easily nor recycled (Conesa et al. 2004). A major environmental problem arises due to the burning of rubber; it produces a large number of toxic chemical components such as zinc oxides and carbon monoxide (Zabaniotou and Stavropoulos 2003). The toxic component from rubber has been preliminarily eliminated by using *Recinicium* fungi. In the second step, rubber can be devulcanized by sulphur-oxidizing or sulphur-reducing bacteria like *Thiobacillus ferrooxidans* and *Pyrococcus furiosus*. Devulcanized rubber can be recycled (Stevenson et al. 2008). The best way of waste management is to control the combustion of rubber and the liberated heat is also utilized for the generation of energy (Conesa et al. 2004).

16.7 Roles of Microorganisms in Wastewater Treatment

Application of microbial technology is now being also focused on the removal of heavy metals, toxic substance, dissolved inorganic dyes and nutrients. Microorganisms in sewage treatment are manifested through their metabolic activities, degradation potential as well as detoxification capacity. Microorganisms regulate a series of chemical reactions, and metabolize the pollutants by utilizing organic matter as a nutrient source present in wastewater. Microbial technology also utilizes degradation of organic matter into inorganic substance and detoxification. On the basis of metabolic capacity of microbes under anaerobic or aerobic conditions, microbial process is divided into aerobic and anaerobic biological treatment.

Wastewater treatment process is associated with the following group of microorganisms:

1. ***Bacteria:*** Bacteria play a pulsating role in the treatment of wastewater. They hold the principal responsibility for the removal of organic compounds. Organic matters are utilized by them to get energy and they use this energy for their growth. Most common bacteria present in wastewater are *Achromobacter* sp., *Arthrobacter* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Bacillus* sp., *Chromobacterium* sp., *Cronobacter* sp., *Citramonas* sp., *Enterobacter* sp., *Escherichia coli*, *Flavobacterium* sp., *Klebsiella* sp., *Kosakonia oryzae*, *Leclercia* sp., *Pseudomonas* sp. and *Serratia* sp. (Silva-Bedoya et al. 2016).
2. ***Filamentous bacteria:*** They are normally associated with biomass present in activated sludge. Their presence is noteworthy for the formation of floc. Their population size varies with nutrient conditions such as the amount of nutrients, DO, sludge age, pH and temperature of wastewater system. Common filamentous bacteria found in activated sludge are *Alcanivorax* sp., *Beggiatoa* sp., *Microthrix* sp., *Sphaerotilus natans* and *Thiothrix* sp. (Paillard et al. 2005).
3. ***Algae:*** They are photosynthetic organisms that have a substantial role in nutrient (nitrogen and phosphorous) removal and accumulation of some xenobiotic compounds, toxic substances (both organic and inorganic) and heavy metals. Some common algae present in wastewater are *Chlamydomonas* sp., *Euglena* sp., *Limnothrix*, *Lyngbya*, *Microcystis*, *Oscillatoria*, *Phormidium autumnale* and *Synechocystis* (Martins et al. 2010).
4. ***Protozoa:*** Protozoans are unicellular eukaryotic organisms that digest and eliminate free-swimming bacteria and other suspended particles from wastewater. Requirement of oxygen varies among different species of protozoan for their survival. Common ciliated protozoans found in wastewater are *Aspidisca* sp., *Carchesium* sp., *Chilodonella* sp., *Opercularia coarctata*, *Trachelophyllum* sp. and *Vorticella* sp. (Amaral et al. 2004).
5. **Fungi:** They are multicellular eukaryotes having ability to hydrolyze organic matter even at low pH. They secrete hydrolytic enzymes to degrade substrate and adsorb suspended solids through mycelia to accomplish their nutrient requirements during wastewater treatment. Common fungi in waste removal process are

Aspergillus, *Absidia*, *Fusarium*, *Sphaerotilus*, *Penicillium* and so on (Akpor et al. 2013).

16.8 Microbial Technology for Wastewater Treatment

The strategy for treatment of wastewater begins in the early twentieth century. Technology application for wastewater treatment has been focused on the quality of waste present in sewage. The major pollutants present in wastewater are biodegradable and volatile organic compounds, nutrients (nitrogen and phosphorus), suspended solids, toxic metals, recalcitrant xenobiotics as well as microbial pathogens. Microbial technologies are environment friendly and sensible choice for hazardous waste from wastewater. Waste present in water is usually treated primarily by physical treatment methods to remove the physical pollutants. Microbes were usually applied as a secondary treatment to remove organic matter present in waste. The choice of technique is based on the quality of waste, aerobic or anaerobic method of degradation and several other parameters. The British Royal Commission declares their goal for waste water treatment to decrease BOD of wastewater up to 20 mg/L and the final yield of effluent and suspended solids should also decrease to 30 mg/L. BOD (biochemical oxygen demand) is defined as "the amount of oxygen required by microbes present in waste water for the oxidation of organic nutrients". It measures the strength of the organic waste present in wastewater; the more organic matter present, the higher the BOD value. We included some common and advanced technology used for wastewater treatment.

16.8.1 Fixed-Film Processes

Fixed-film microbiological processes are based on attachment of high concentration of microorganisms on a solid support material such as plastic, gravels, sand or stone particles. Microbial growth is influenced on the substratum by factors such as geometric configuration of particles and the flow rate of wastewater. There are several advantages of fixed-film processes such as low specific growth rate of microorganisms, suitability for small size reactor and lower operation cost. The disadvantage associated with this process is overgrowth of microorganisms on the solid substratum and biofouling which affects heat exchange process unpleasantly. Two major categories of fixed-film reactors, trickling filters and rotating biological contactors, are described here.

1. *Trickling Filters*

   The treatment of wastewater by trickling filters is conducted in a rectangular or circular reservoir filled with filter medium with a depth of approximately 100–250 cm. Large surface area available in filter medium is suitable for growth of microorganisms and also provides adequate void space for diffusion of air.

Usually crushed stones, granite, hard coal, ceramic material, plastic and treated wood are used in filter media. The size of support matrix is based on microbial attachment and void space. Smaller sized matrix has higher surface area for microbial attachment, but void space is less. Wastewater is sprayed upon the surface of the bed with sprinklers that allow uniform hydraulic load. Wastewater containing organic matter is dripped above the filter media that provides nutrients for the growth of microorganisms on filter surface. During the flow, the wastewater undergoes aerobic decomposition by microorganisms. An underdrain system is also connected that collects the treated wastewater as well as sloughed off microbial biomass. A final clarifier separates the microbial biomass from the treated wastewater. Depending on the amount of treated water, the trickling filters may be subdivided into flushing and percolating filters.

Microbes in trickling filter: Biofilm formation occurring at filter surface is called zoogleal film which consists of algae, bacteria, protozoa and fungi. Biofilm formation on the surface of filter media is similar to naturally occurring aquatic environments. Common bacteria found in zoogleal film are *Achromobacter*, *Alcaligenes*, *Flavobacterium*, filamentous bacteria, nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*), *Pseudomonas* and *Zooglea*. Bacteria adsorb and anchor on the substratum using polymer-containing matrix, glycocalyx. After acclimatizing to the substratum, the filter surface colonized by bacteria is also further occupied by successional life forms (Rani et al. 2019). Common fungi found in zoogleal film are *Aspergillus*, *Geotrichum*, *Fusarium*, yeasts, *Mucor* and *Penicillium*. The growth of fungal hyphae supports the transfer of oxygen at the depths of the biofilm. Several types of algae also flourish in biofilm such as *Anacystis*, *Chlorella*, *Euglena*, *Phormidium* and *Ulothrix*. Algae produce oxygen and some of them also fix nitrogen. The protozoa occurring in biofilms are amoeba, arcella, ciliates (*Colpidium*, *Vorticella*) and flagellates (Bodo, Monas). Microbial biofilm degrades the organic matter present in wastewater and continues to grow (Arezoo et al. 2017). The increase in thickness of biofilm acts as a limiting condition for diffusion of oxygen in the deeper layers, hence creating an anaerobic environment. It also reduces supply of organic substrates into the deeper layer. These biofilms are later sloughed off and further a new biofilm forms.

Advantages and Disadvantages: Trickling filters are reliable, are easy to operate and have little maintenance costs. Both domestic and toxic industrial effluents can be treated. Its disadvantage is associated with the clogging of filter under higher organic load. It results into excessive growth of slime bacteria that cause restriction in air circulation, low oxygen availability and hence foul odour.

2. *Rotating Biological Contactors (RBCs)*

This is a fixed-film bioreactor in which the disk surface is used for adsorption of microbes. A thin biofilm of 1–4 mm thickness is accountable to decrease BOD level. RBC comprises a horizontal shaft with a series of microbial immobilized disks straddling on it. Disks are around 40% submerged and rotate slowly in the wastewater. The rotation enhances oxygen transfer required for microbial activity. It also improves contact between immobilized biomass and wastewater that reduces BOD at a faster rate. There is successional growth of microorganism on the rotating disk. Biofilms on RBCs encompass diverse microbial community such as filamentous and eubacteria, metazoan, protozoa and filamentous algae such as *Oscillatoria* (Aguilera et al. 2007). RBCs mostly remove organic materials and also oxidized ammonia. Several advantages associated with RBCs are low operation cost, short residence time, low maintenance costs and release of dewatered sludge.

16.8.2 Activated Sludge

This is the most extensively used suspended-growth wastewater treatment process. It utilizes microbial culture to degrade organic matter under aerobic condition. The treatment consists of oxidation of organic matter to NH_4, CO_2 and H_2O with the formation of cellular biomass. Activated sludge forms floc for separation of solid in settling tank during the aeration of wastewater. Activated sludge process is constructed with two major objectives:

1. By using aerobic oxidation process, soluble organic matter is converted into new biomass of biodegradable organic matter
2. Activated sludge technique is applied for the separation of biomass from treated effluent

Activated sludge system consists of aeration and sedimentation tank. Aerobic oxidation of organic matter using microorganism is carried out in aeration tank. Wastewater treated after preliminary method is pass in the tank and assorted with sludge or return activated sludge (RAS) to form mixed suspended solids. Aeration is provided by mechanical method. Air is utilized by microorganisms to develop a biological floc. During return activated sludge, a huge amount of the microbial biomass is recycled, maintaining an enormous number of intial microbes for effective oxidation of organic matters in a reasonably short time. The total content activated sludge present in aeration tank is called mixed liquor, whereas the total amount of microorganisms, mineral and organic suspended solids in the assorted with liquor is called mixed liquor suspended solids (MLSS). MLSS possess both organic and inorganic portions. The organic portion comprises live or dead microorganisms, lacking microbes in the organic matter, and cellular debris is denoted by MLVSS.

The MLSS is shifted from the aeration tank to the sedimentation tank for the separation of sludge from the treated effluent. Sedimentation tank is utilized for the sedimentation of microbial sludge formed in aeration tank during oxidation of organic substance. The amounts of regimented solids can be varied by waste activated sludge (WAS) and returned activated sludge (RAS). A rational settled activated sludge is returned back to the aeration tank for the treatment of incoming raw wastewater (RAS). The remnants or excess sludge is removed to balance the ratio of food for the microorganisms (F/M). The food to microorganism ratio is significant to maintain the balance between organic loads to biomass generation in activated sludge system conveyed in terms of kilogram BOD per kilogram of MLSS per day. Mean cell resident time (MCRT) or solid retention time (SRT) is an important component of activated sludge that measures the contact time of micro-organism with substrate. Activated sludge process is controlled by several parameters including food to microorganism ratio, sludge volume index, mean cell residence time, sludge age, dissolved oxygen and biochemical oxidation demand (Johnston et al. 2019).

The solid part of flocs absorbs impurities present in wastewater, while microorganisms oxidize the absorbed substances. Activated sludge has a loose and porous structure. These microbial cells occur as flocs or agglomerates whose density is abundant for sedimentation in the sedimentation tank. Sedimentation is monitored by using “secondary clarification” for the separation of microorganism and solids from treated wastewater.

Microbes in Activated Sludge: The activated sludge flocs contain numerous microorganisms but mostly bacteria, organic substance and inorganic particles. The size of floc varies between <1 mm and >1000 mm. Microelectrodes are used to measure microbial activity and estimate the concentration of oxygen, ammonia, nitrate, redox potential, pH or sulphide profiles within flocs. The major bacterial genera present in flocs are *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Comamonas*, *Flavobacterium*, *Pseudomonas* and *Zooglea*. Fungal growth is usually not favoured; however, some genera such as *Alternaria*, *Cladosporium*, *Cephalosporium*, *Geotrichum* and *Penicillium* are present in activated sludge (Yang et al. 2020). Several conditions such as oxygen deficiency and overloading of aeration tanks do not favour proper floc formation; this phase is called as active-sludge swelling. There are two discrete types of sludge swelling: fibrous and non-fibrous swelling. The growth of *Sphaerotilus*, *Beggiatoa* or *Thiothrix* is observed during fibrous swelling and the amount of mucous secretion is increased during non-fibrous swelling.

16.8.3 Biosorption Technology

Microorganism-based sorbent materials have been recently implicated for the retrieval of heavy metals from wastewater. Biosorption is a specific property of some microbes that concentrate the organic or inorganic substances from liquid solutions. Though biosorption techniques are principally applied to sequester heavy metals, metalloids, radionuclides and rare earth elements, recently it has been implicated for the removal of organic dyes (Kaushik and Malik 2009). Microbial EPS (extracellular polymeric substance) and cell wall play an important role in absorption and act as an alternative of synthetic adsorptive substances.

EPS is a high molecular weight natural polymer secreted by several microorganisms, and their chemical composition also varies with microbial genera. EPS is utilized by microbe primarily to protect themselves from metal toxicity. Diverse structure, chemical stability, metal binding property, selectivity and high reactivity of microbial EPS advocate them as great competitor. Factors for biosorption include the type of metal and its ionic form, metal binding site and external environmental of microbes. Biomass of algae, bacteria, cyanobacteria, filamentous fungi and yeast was commonly used for biosorption.

These microbes could be isolated from their natural habitat or obtained from waste by-product of fermentation industries to make the process economic. The surface area of bacteria and yeast has been increased for enhanced absorption using genetic engineering methods. Water inhabitants as algae and cyanobacteria were reported to absorb a variety of toxic metals in natural water and wastewater. They show various degrees of binding and specificity with Ni, Pb, Cd, Zn, Co, Cu, As, Mn, Mg and Zn. *Anabaena doliolum* Ind1, having surface group's carboxyl, hydroxyl, carbonyl, sulphate and amide groups, has great ability to bind with Cd (II) (Goswami et al. 2015). Similarly, *Providencia vermicola* strain SJ2 and *Paenibacillus peoriae* strain TS7 have shown specificity to bind with Pb (II) (Arumugam et al. 2017). *Streptomyces rimosus* and *Rhodococcus opacus* also show their ability to bind with Al (III) (Cayllahua and Torem 2010). *Rhizobium radiobacter* strain VBCK1062 which is commonly found in contaminated soil is a highly specific strain that binds with (V).

Metal ions were extraordinarily fixed by bacterial cell wall. Two mechanisms are conveyed in metal binding capacity of microbial EPS and cell wall:

1. Ion exchange performed by high quantity of negatively charged functional group in microbial EPS.
2. Complex formation with the charged group present in EPS or cell wall.

A thick layer of peptidoglycan found in gram-positive bacterial cell wall and lipopolysaccharide in gram-negative bacteria plays an important role in binding with metal ions (Flemming and Wingender 2001). Gram-positive bacteria and actinobacteria are comparatively more capable of adsorbing metal on their cell wall when exposed to selective metals. Copper was efficiently removed by desulphurization bacteria from wastewater. White-rot fungi and yeasts were also used to absorb toxic substances such as chromium, lead and other constituents of wastewater in China. Microbial technology for adsorption was also integrated with activated sludge treatment.

Advantage: Biosorption process offers several advantages such as low operating cost, high efficiency for even low metal concentrations, minimization of chemical uses, free from nutritional requirements, free from disposal of organic or inorganic sludge and avoidance from metal toxicity issues.

16.8.4 Microbial Electrochemical Technology

Microbial electrochemical technology (MET) is an emerging technology that amalgamates microbiology with electrochemistry (Schröder et al. 2015). In MET method, electroactive bacteria are capable of using a solid electrode as electron acceptor or electron donor (Rabaey et al. 2006). This electrode acts as an alternative to traditionally used nitrate/oxygen as electron acceptor or hydrogen/organic matter as electron donor (Karanasios et al. 2010). Depending on the quality of groundwater or pollutant present in them, MET system can be operated as microbial electrolysis cell (MEC) or as microbial fuel cell (MFC) (Schröder et al. 2015). MFC device differs from MEC in the sense that in MFC, energy can be extracted, while in MEC energy is supplied to allow or enhance bio-electrochemical process. MFC differs substantially from the conformist fuel cells. For conversion of fuel cell microorganisms act as biocatalysts for the cathodic and anodic substrate to catalyze the electrochemical reactions. Direct electron transfer in MFC was initially demonstrated in *Saccharomyces cerevisiae*, where it was grown in enriched medium, for the separation the platinum cathode and anode electrodes; porous cylinder is utilized in the field.

Microbial fuel cells emerged as a new bioremediation technology that is primarily used to recover toxic metal or mobilize pollutants present in wastewater. Several groups of bacteria, yeast, algae and fungi were found to remediate the heavy metal ions (Pous et al. 2017). Microbial electrochemical technologies are effectively applied for in situ or ex situ treatment of groundwater contamination. However, ex situ MET is widely applicable. During ex situ treatment, wastewater or groundwater has to be pumped to the other location, where intensive treatment is applied for reckless removal of pollutants. Different MET patterns have been utilized for the treatment of different groundwater pollutants such as aromatic hydrocarbons, chlorinated hydrocarbons (Aulenta et al. 2007) and metals (Huang et al. 2013).

16.8.5 Wastewater Treatment Using Oleaginous Microorganisms

Oleaginous microorganisms are well-known sources to produce microbial biofuels of comparable fatty acid configuration present in higher plants and animal oils. However, a major limitation of synthesis of biofuel from oleaginous microorganisms is the high cost of raw material (Azócar et al. 2010). Therefore, nutrient-rich wastewater was employed as cheaper substrates for oleaginous microorganisms. It was not only feasible economically but also important for environment-friendly biodiesel production (Huang et al. 2013). Different oleaginous microorganisms have been studied for simultaneous biofuel production along with simultaneous wastewater treatment. Certain groups of microbes such as bacteria, yeast microalgae and fungi are acting as single cell oils (SCOs) or oleaginous microorganisms (Arous et al. 2019).

Yeast: Oleaginous yeast *Debaryomyces etchellsii* is able to accumulate a substantial quantity of lipids from agroindustrial wastewaters such as wastewater obtained from olive mill, expired soft drinks and confectionary industries. Wastewater obtained from milk candy was effectively used by *Rhodosporidium toruloides* to produce sufficient quantity of reserve lipids (Zhou et al. 2013). Strain *Rhodotorula glutinis* TISTR 5159 was found to convert corn starch wastewater into lipids under semicontinuous fermentation conditions with higher efficiency of 65% reduction of COD value. Oleaginous yeasts belonging to genus *Trichosporon* also showed their high ability to remove pollutants, removing more than 55% COD from industrial wastewaters. Wastewater obtained from bioethanol industry was efficiently utilized by oleaginous yeast *Rhodosporidium toruloides* Y2 to decrease 72% BOD level. Other group of oleaginous yeast such as *Cryptococcus* sp. (Fernandes et al. 2014), *Lipomyces starkeyi*, *Rhodosporidium toruloides* and *Yarrowia lipolytica* (Louhasakul et al. 2016) was found to be highly efficient for utilization of wastewater.

Algae: Oleaginous microalgae show significant advantages over yeasts such as natural habitat to grow in wastewater and the requirement of low nutrition owing to their autotrophic characteristics (Cai et al. 2013). Some oleaginous microalgae such as *Neochloris*, *Arthrospira*, *Botryococcus*, *Chlorella* and *Scenedesmus* are able to remove pollutants from wastewater along with the production of microbial lipids (Perez-Garcia et al. 2011).

Fungi: An oleaginous fungus *Aspergillus oryzae* is able to produce biodiesel using starch-rich industrial wastewater (Muniraj et al. 2013).

Bacteria: An oleaginous bacterial strain of *Alcanivorax* was able to solve environmental pollution by removal of spilled petroleum. Their population significantly increased in oil spilled water by utilizing them as nutrients. Researchers also observed that soil-borne bacteria *Geobacter* are able to electroplate uranium, rendering it in insoluble form; therefore, it cannot dissolve and contaminate groundwater. There will be a wide application of *Geobacter* at the uranium contamination sites such as nuclear plants and mines in order to limit the catastrophic spillages.

16.8.6 Removal of Synthetic Dye Using Microbial Technology

Synthetic dyes with various structural diversities are frequently used in textile, paper, leather tanning, agricultural research and photochemical research. They cause extensive environmental pollution, deteriorate the quality of wastewater, increase toxicity and are also hazardous for health. Several wastewater treatment methods have been adopted to handle artificial dyes. The solicitation of microbial technology for degradation of synthetic dye is highly effective, economic and environment friendly. Different wastewater treatment methods using microbes have been adopted for the removal of synthetic dye. Rhodamine B and methyl violet have been removed during activated sludge process using cattle dung derived from microorganisms (Kanekar and Sarnaik 1991). Certain microbial culture was used for degradation of Acid Orange 7 dye in biofilm. For the assimilation of immobilization and decomposition of azo dye, multistage rotating biological reactor is used (Ogawa and Yatome 1990).

Removal of synthetic dye tartrazine and azo dye from wastewater was done by some bacterial species under anaerobic conditions. Both individual strains as well as mixed cultures were used under aerobic and anaerobic conditions for removal of synthetic dye. The combined aerobic and anaerobic sequential method has been effectively engaging on the disintegration of anthraquinone and monochlotriazine dyes (Panswad and Luangdilok 2000). More than 100 fungal and some bacterial laccase producers such as *Bacillus subtilis*, *Coprinus cinereus*, *Melanocarpus albomyces*, *Pycnoporus cinnabarinus*, *Thielavia arenaria* and so on have been reported that efficiently decolourize dye from wastewater (Mishra et al. 2019). Some dye decolourizer and degrader group of microorganisms have been mentioned below.

Fungi: White-rot fungi have attracted researchers in recent years due to their potent biodegradation capacity of highly stable natural molecule such as cellulose, lignin and hemicellulose. They are able to synthesize the wide range of extracellular enzymes such as laccase, phenol oxidase, lignin peroxidase, manganese-dependent peroxidase and manganese-independent peroxidase. They have been extensively investigated for decolourization of wastewater (Young and Yu 1997). A white-rot fungus, *Phanerochaete chrysosporium*, has been employed for the decolouration of Azure B, Congo red, Orange II and Tropaeolin O dye under aerobic conditions. This fungus is able to remove these dyes from wastewater between 6 and 9 days (Cripps et al. 1990). *Trametes versicolor* were able to decompose azo, anthraquinone and indigo-based dyes. Highly sensitive azo dyes have been efficiently removed from the wastewater by *Aspergillus foetidus*. Another dye Remazol Brilliant Blue R has been successfully decolourized by fungus *Pycnoporus cinnabarinus* using packed-bed bioreactor (Schliephake and Lonergan 1996). Anthraquinone dyes *and* triarylmethane and indigoid dye present in environment were efficiently degraded by *Trametes hirsute* (Abadulla et al. 2000). One of the efficient fungi *Kurthia* sp. is capable to decolourize the synthetic dyes such as brilliant green, pararosaniline, crystal violet, magenta and malachite green (Sani and Banerjee 1999).

Bacteria: *Bacillus subtilis* is able to degrade *p*-aminoazobenzene to produce *p*-phenylenediamine and aniline compound under anoxic condition (Zissi and Lyberatos 1996). Under fixed-film reactor conditions, *Pseudomonas mendocina* are used for the decolourization of methyl violet in the textile industrial effluent wastewater. *Pseudomonas luteola* and *Klebsiella pneumoniae* bacteria were used to decolourize reactive azo dyes from wastewater.

Algae: Reactive azo dyes have been also degraded by algae such as *Chlorella vulgaris*, *Chlorella pyrenoidosa* and *Oscillatoria tenuis*. An enzyme "azo reductase" found in some algae is responsible for the breakdown of azo linkage, hence converting azo dyes into aromatic amines.

16.9 Conclusions and Future Prospects

Hazardous waste is a major threat for environmental safety that needs to be eradicated in various ways. Microbial technology is the amalgamation of microbial process with technology in which selective microbes are used by scientific methods. Microbial technology has been extensively used for the removal of waste from terrestrial area and wastewater. Removal of waste using microbes is not only cost-effective, efficient but also environment friendly. A major section of the waste is organic matter which can be properly handled by suitable microorganisms using the phenomena of reduction, reutilization and recycling of waste. Besides the organic matter, several microorganisms have been explored that are able to remove oil, synthetic dyes, toxic heavy metals and xenobiotic compounds. Modern techniques such as biosorption and microbial electrochemical technologies were implemented for toxic metal recovery. Exponential increase of hazardous waste still creates pressure to further explore newer microbial technology or extend the existing techniques with underexplored microbes to solve the global problem. Therefore, genetically engineered microbes and high-throughput screening of specific microorganism have been promoted for microbial technology as more convenient and effective for waste removal. Careful monitoring of multiple strain-based inoculums could also reduce waste in rapid rate. Generation of energy using microbes during waste management is an economic way that needs to be implemented in the future. The potentials of hidden domains of microbes have great prospect that can be further explored for the treatment of waste.

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