Microbial Exploration and Their Metabolic Capacity for Detoxification and Restoration of Natural Ecosystems

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1 Introduction

The microbial communities are distributed and allocated widely on the earth, due to their inbuilt metabolic capacity and ability, which is remarkable and they can comfortably grow in a wide range of environmental situations. The beneficial and nutritional versatility of microbial population can be used for remediation and biodegradation of contaminants and different contaminated ecosystems. Such type of phenomenon is known as bioremediation. Microbes utilize energy from the toxic contaminants and convert them to other less toxic substances and are therefore called bioremediators. The bioremediation efficient steps and processes not only collect the contaminant and utilize it, but the process of bioremediation is a microbiological well-organized procedural action that is applied to break down or transform contaminants and polluted ecosystems into less toxic or non-toxic elemental and compound forms with the help of microbial population including fungi and bacteria. These bioremediators are efficient biological agents used for bioremediation to clean up polluted sites. Microbial population like fungi, archaea, and bacteria are typical primary bioremediators. The efficient bioremediation used as a biotechnological process includes microbial population for solving, detoxifying, and removing toxicity of many contaminants through effective biodegradation from the contaminated systems. The effective bioremediation and biodegradation terms are more interchangeable processes and words. These microbial communities act as significant contaminant removal tools in water, sediments, and soil. These microbial

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R. A. Bhat et al. (eds.), *Microbial Bioremediation*,
https://doi.org/10.1007/978-3-031-18017-0_9

communities are restoring and detoxifying the original natural ecosystems and restricting further degradation and pollution.

The efficient and effective process of bioremediation converts organic pollutants mainly to carbon dioxide, water, and biomass. The pollutants can be immobilized by attaching to the humic substance fraction. The degradation process may occur under aerobic and anaerobic conditions. The primarily aerobic process is used for bioremediation, and classified as ex situ and in situ. An appropriate selection of technology among the variety of bioremediation technologies is developed to treat pollutants based on basic three principles: an amenability of the contaminant to biological transformation, the accessibility of the contaminant to microorganisms, and the opportunity for optimum biological actions. With the appropriate selection of technologies and conditioning, the process of degradation is enhanced and the efficacy of degradation is improved which primarily reduces the cost of treatment (Mohapatra, 2008). The ex-situ bioremediation methods are best used to clean pumped-out contaminated groundwater and excavated polluted soils. The bioremediation through in situ methods are defined as those which are used to soil and groundwater at the contamination site with minimal disturbance. These techniques of different types are among the most efficient and desirable options due to least disturbances and lower cost since microbial communities ensure action in place avoiding pollutant transportation and excavation. However, the depth of the soil that can be effectively treated limits in situ treatment. Almost in soils, effective oxygen diffusion for desirable rates of bioremediation extends to a range of only some centimeters around 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in many cases (Vidali, 2001). The process of bioremediation has been observed as an alternative to traditional physico-chemical methods to restore contaminated sites. Being a cost-effective, less labor-intensive, safe, and environment-friendly technique rapid development and advances are happening in this field for the past decades. Bioremediation observed effective for a wide range of soil pollutants including PAH, PCB, CAH, pesticides, explosives, even heavy metals, and radionuclides. However, in many cases, the bioremediation method combines several treatment techniques and can last for a long time (years and decades) and are often applied in combination with other techniques; therefore, it is difficult to estimate the efficacy of the same. In this context, more interdisciplinary research should be carried out with process optimization, validation, its impact on the eco-system and the effectiveness and predictability should be demonstrated to make it generalized.

The term bioremediation comprises of two parts: "bios" means life and refers to living organisms and "to remediate" means to solve a problem. The term "bioremediate" means to use microorganisms to solve an environmental issue such as contaminated soil or groundwater. The term bioremediation is the use of microorganisms to degrade environmental contamination or to prevent pollution. Especially, it is a method for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution (Table 1).

The earth has many bioremediants available that can be used against a broad range of pollutants. The process of bioremediation is a useful technique for the

Table 1 Microorganisms that remediate heavy metal pollution

S. No	Elements	Symbol	Microorganisms
01	Copper	Cu	*Bacillus* sp., *Pseudomonas aeruginosa, Chlorella vulgaris, Pleurotus ostreatus, Phormidium valderium, Volvariella volvacea, Daedalea quercina*
02	Nickel	Ni	*Pseudomonas aeruginosa, Zooglea* sp., *Chlorella vulgaris, Phormidium valderium*
03	Zinc	Zn	*Bacillus* sp., *Chlorella vulgaris, Aspergillus niger, Pleurotus ostreatus, Daedalea quercina*
04	Uranium	U	*Pseudomonas aeruginosa, Citrobacter* sp., *Chlorella vulgaris, Aspergillus niger*
05	Cobalt	Co	*Zooglea* sp., *Phormidium valderium*
06	Cadmium	Cd	*Ganoderma applantus, Zooglea* sp., *Citrobacter* sp., *Aspergillus niger, Pleurotus ostreatus, Stereum hirsutum, Phormidium valderium*
07	Lead	Pb	*Stereum hirsutum, Citrobacter* sp., *Chlorella vulgaris, Ganoderma applantus, Volvariella volvacea, Daedalea quercina*
08	Mercury	Hg	*Chlorella vulgaris, Rhizopus arrhizus, Volvariella volvacea, G. metallireducens*
09	Gold	Au	*Chlorella vulgaris, G. metallireducens*
10	Silver	Ag	*Aspergillus niger, Rhizopus arrhizus, G. metallireducens*
11	Chromium	Cr	*D. vulgaris, D. acetoxidans, D. fructosovorans, D. norvegicium*

degradation and detoxification of a wide range of contaminants present in different ecosystems. There are many compounds available within ecosystems that are detrimental and harmful, can be biotransformed or converted to less toxic or harmless products with the help of these microorganisms. Bioremediation restricts the bioaccumulation of highly toxic and detrimental pollutants from lower to higher trophic levels and movement from one ecosystem to another. Bioremediation eliminates or reduces the need to transport quantities of waste from the contaminated and can often be completed on-site, often without causing a major disruption of normal activities. Bioremediation has proven to be effective and cost-effective than other technologies for the cleanup of contaminated sites. Microorganisms used for such processes should be healthy and active, i.e., must be present in their active or exponential phase so that they can perform their work efficiently. Oxygen quantity will decide the efficiency of microbes; if sufficient amount of oxygen is present then contaminants and toxins can easily be converted into water and carbon. Xenobiotics such as nitroglycerine which is an explosive can also be cleaned up through bioremediation. Bioremediation is a natural attenuation with no or little human efforts. Furthermore, adding natural or engineered microorganisms can enhance the ideal catalytic abilities.

In bioremediation, microorganisms (bacteria, fungi, and algae) or plants are used to degrade and detoxify the hazardous pollutants present in different ecosystems and convert them into CO_2, H_2O, microbial biomass, and other less toxic metabolites. Bacteria is considered as one of the most efficient agents of biological degradation. In 1974, Williams and Murray made the first report of bacterial degradation of benzene derivatives by *Pseudomonas putida* which has a special enzymatic route to degrade these compounds and use them as a carbon source. A large number of bacterial species have been identified as efficient degraders of various xenobiotic compounds. The maximum members were from the genera of *Mycobacterium, Alcanivorax, Burkholderia, Cellulomonas, Sphingomonas, Micrococcus, Streptomyces, Bacillus, Haemophilus, Enterobacter Pseudomonas*, etc. These microorganisms can degrade polycyclic aromatic hydrocarbons (PAHs), pesticides, and azo dyes and remove or change the redox state of certain heavy metals. The degradation by xenobiotics via oxidases has been better studied in fungi as compared to bacteria although ligninolytic–like enzymes have been also found in bacteria. Enzymes identified as yellow laccases have low redox potential than fungal laccases. The normal peroxidase activity of bacteria is limited although another kind of peroxidase activity known as dye-decolorizing peroxidases has been extensively studied. Throughout the large umbrella of these enzymes, several oxidases can activate many xenobiotic compounds through the production of free radicals for their further mineralization and polymerization, rendering them non-bioavailable. Enzymes such as catalases and superoxide dismutase have been involved in PAH degradation. Pesticide compounds such as organophosphate group and carbamates can also be oxidized using bacteria with heavy metal being an exception. The bioremediation consists primarily of adsorption of these compounds into the cell wall, compartmentalization on vacuole or other organelles in eukaryotes, or changing their redox state into a less soluble form and thus making them less bioavailable.

Microorganism like fungi is among the top priorities and most promising for bioremediation since they produce a surfeit of oxidative and hydrolytic enzymes which are very effective in the process of bioremediation. One of the most important advantages of using fungi in situ is the presence of hyphae which covers a wide surface area in a single instant. Fungi can decompose lignin, cellulose, and hemicellulose, which have recalcitrant structures. The most widely studied fungal species for bioremediation are *Basidiomycota, Trametes, Phanerochaete, Pleurotus, Bjerkandera, Coriollopsis, Aspergilli, Trichoderma*, and *Fusarium*. The most important fungal-derived oxidases are laccases, peroxidases, lytic polysaccharides, and monooxygenases, which can degrade different compounds. Fungal enzymes involved in the mineralization of xenobiotic compounds are glucose oxidase, aryl alcohol oxidase, quinone oxidoreductase, and cellobiose dehydrogenase. These organisms can be indigenous to the site or can be isolated and transferred to a contaminated site. The bioremediation of pollutant compounds requires the action of several microbes, therefore sometimes potential microbes are used from other contaminated sites for the effective degradation process, and this process is called bioaugmentation (Table 2).

Biodegradation depends on favorable environmental conditions, the pollutant type, solubility of the pollutant compounds, and the bioavailability of the pollutant to the microbes. Environmental conditions are controlled to allow sufficient microbial growth for fast and effective biodegradation. Microbes with degradation potential have been isolated from contaminated environments, such as heavy metal-polluted sites, landfills, petroleum-contaminated sites, pesticide-contaminated sites, and

Table 2 Microorganisms having potential to degrade different organic pollutants

S. No	Pollutant	Microorganisms
01	Benzene, anthracene, hydrocarbons, PCBs	*Pseudomonas* spp.
02	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	*Alcaligenes* spp.
03	Benzene, hydrocarbons, pentachlorophe-Nol, phenoxyacetate, polycyclic aromatic	*Arthrobacter* spp.
04	Aromatics, long-chain alkanes, phenol, cresol	*Bacillus* spp.
05	Halogenated hydrocarbons, phenoxyacetates	*Corynebacterium* spp.
06	Aromatics	*Flavobacterium* spp.
07	Naphthalene, biphenyl	*Rhodococcus* spp.
08	Aromatics, branched hydrocarbons benzene, cycloparaffins	*Mycobacterium* spp.
09	Hydrocarbons	*Nocardia* spp.
10	Hydrocarbons, polycyclic hydrocarbons	*Xanthomonas* spp.
11	Phenoxyacetate, halogenated hydrocarbon diazinon	*Streptomyces* spp.
12	PCBs, polycyclic aromatics, biphenyls	*Cunniughamela elegans*

wastewater treatment plants. These microbes use hazardous contaminants as their source of energy and carbon source in aerobic and anaerobic conditions in other words metabolic activity can reduce or convert the pollutant to less or nontoxic metabolites. These soil microbes and pollutants should be in close contact for an effective degradation of the pollutant and it can be done by the application of surfactants. Aerobic bacterial species such as *Mycobacterium, Alcaligens, Sphingomonas,* and *Pseudomonas* are known for their aerobic degradation of hydrocarbons (alkanes and polycyclic aromatic hydrocarbons) and pesticides. A few of the aerobic methylotrophs are also recognized for the degradation of dichloroethane and trichloroethylene. There are many anaerobic bacterial species that are known for the degradation of PCBs, chloroform, and trichloroethylene (chlorinated solvent). Along the bacterial species, a few of the fungal species, such as *Phanerochaete chrysosporium*, are also reported to be efficient in the remediation of a variety of toxic and persistent toxic contaminants.

The metabolic characteristics of the selected microorganisms and physicochemical properties of the targeted contaminants determine to a large extent possible interaction during the process of bioremediation. The actual successful interaction between the microbes and pollutant however depends on the environmental conditions of the site of the interaction. Growth and activity of microbes are largely affected by pH, temperature, moisture, soil structure, solubility in water, nutrients, site features, oxygen content, redox potential, and physico-chemical bioavailability of pollutants. These factors determine the kinetics of the degradation process. The process of biodegradation can occur under a wide range of pH. A pH of 6.5–8.5 is mainly optimum for biodegradation in most aquatic and terrestrial ecosystems. Moisture influences the rate of contaminant metabolism because it influences the kind and amount of soluble content that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems. The natural process of bioremediation is a slow and time-consuming process. Microbes degrade the contaminant and increase their population when the contaminant is still present. When the pollutant is degraded, the microbial population declines. Many of the hazardous compounds can be transformed into less toxic products, and this feature eliminates the chance of future liability associated with treatment and disposal of contaminated media. Bioremediation process does not use any synthetic and toxic chemicals. The nutrients especially biofertilizers added to make active and fast microbial growth are easily biodegraded. The natural technique of remediation is eco-friendly and sustainable. The contaminants are destroyed, or sometimes simply transferred to less toxic forms.

Bioremediation process is either in situ or ex situ. The basic bioremediation methods are biostimulation, attenuation, augmentation, venting, and piles.

2 Biostimulation

This type of strategy is operated through the injection of specific nutrients at the site of contamination to stimulate the activity of indigenous microbes. Naturally existing microbial communities are stimulated primarily by supplying fertilizers, growth supplements, and trace minerals for growth and active metabolism. Other environmental requirements like pH, temperature, and oxygen also need to be kept at optimum to speed up their metabolism rate and pathway.

3 Bioattenuation

Bioattenuation or natural attenuation is the eradication of pollutant concentrations from the contaminated site. It is carried out biologically (aerobic and anaerobic biodegradation), physically (dispersion, diffusion, advection, volatilization, dilution, sorption/desorption), and chemically (complexation, abiotic transformation, ion exchange).

The contaminants moves through soil and groundwater, they often can mix with water which reduces or dilutes the pollution. Many chemicals, like oil and solvents, can evaporate, they change from liquids to gases within the soil. Meanwhile, if these gases escape to the air at the ground surface, sunlight may destroy them. If the natural attenuation is not quick enough or complete enough, bioremediation will be enhanced by either biostimulation or bioaugmentation.

4 Bioaugmentation

The addition of additional contaminant degrading microorganisms (natural/exotic/engineered) to augment the biodegradative capacity of indigenous microbial populations on the contaminated area is known as bioaugmentation. The microorganisms are first collected from the remediation site, then separately cultured, sometimes genetically modified and returned to the contaminated site. Bioaugmentation is also referred to as the process of adding engineered microbes to a system, which act as bioremediation to quickly eliminate complex pollutants. The natural species must be genetically modified through DNA manipulation to facilitate genetically engineered microbes; as naturally they are not efficient enough to break down certain compounds. Genetically modified microbes act much faster than the naturally existing species and are highly competitive with the indigenous species, predators, and also various ecological factors. The genetically engineered microbes have shown potential for bioremediation of soil, groundwater, activated sludge, and oil spills in oceans.

5 Bioslurping

In this process, combination of vacuum-enriched pumping, soil vapor extraction along with bioventing is used for remediation of soil and groundwater providing indirect oxygen supply and stimulating the biodegradation of contaminants. This technique can also be used for remediation of semi-volatile and volatile organic compounds from contaminated soils. This method is not appropriate for remediation of soil having little permeability.

6 Genetically Engineered Microorganisms (GEMS)

Genetically engineered microorganism is a microorganism whose genetic material has been changed by applying genetic engineering techniques inspired by the natural otherwise artificial genetic exchange between microorganisms. These kinds of artistic work and scientific procedures are mainly termed recombinant DNA technology. The recent genetic engineering has improved the utilization and elimination of hazardous unwanted wastes under laboratory conditions by creating genetically modified microorganisms. Recombinant living organisms can be obtained by recombinant DNA techniques or by the natural genetic material exchange between microorganisms. Currently, development is required in gene production having the potential to degrade complex toxic substances into eco-friendly substances. The genetically engineered microorganisms (GEMs) are efficient for bioremediation applications in soil, groundwater, and activated sludge environments, exhibiting enhanced degradative capabilities encompassing a wide range of synthetic contaminants. Recently, many opportunities forward for improving degradative performance using genetic engineering actions for rate-limiting steps in known metabolic pathways, which can be genetically manipulated to yield increased degradation rates. In GEMs, four activities/strategies to be done are modification of enzyme specificity and affinity, pathway construction and regulation, bioprocess development, monitoring, and control, bioaffinity bioreporter sensor applications for chemical sensing, toxicity reduction, and endpoint analysis. The primary genes of bacteria are carried on a single chromosome but genes specifying enzymes essential for the catabolism of some of these unusual substrates may be carried on plasmids. Plasmids have been implicated in catabolism. Therefore, GEMs can be used effectively for biodegradation purposes and leads to represent/indicate a research frontier with broad implications in the future time. The major function is to speed up the recovery of waste-polluted sites, increase substrate degradation, displays a high catalytic or utilization capacity with a small amount of cell mass, crate safe and purified environmental conditions by decontamination or neutralizing any harmful contaminant.

7 Bioventing

Bioventing helps with in situ bioremediation of pollutants present in soil by providing enough supply of oxygen to microorganisms involved in converting pollutants into a harmless product. Bioventing requires low airflow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil using wells. Although rate of airflow and air interval are the most important factors of bioventing, still accomplishment depends on the number of air injection points for uniform distribution of air. The adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil. Effective bioremediation of petroleum-contaminated soil using venting has been proved by many researchers.

8 Biopiles

This is a complete treatment technology in which excavated soils are mixed with nutrients to enhance microbial activities and placed on a treatment bed with main components such as irrigation, aeration, leachate, and nutrient collection systems. Various environmental and physico-chemical parameters viz. heat, moisture, nutrients, pH, and oxygen can be controlled for further enhancement of biodegradation. Filtering and ventilation of polluted soil, addition of bulking agents like saw dust, straw, wood chips, or any other organic materials can further help in enhancement of efficiency. Biopiling can be effectively used to control volatilization of low molecular weight pollutants and can work even under extreme cold environments. Biopile can be used for treatment of huge quantity of contaminated soil in less space in comparison to different ex situ bioremediation methods comprising land farming. Biopiles are also known as biocells, bioheaps, biomounds, and compost piles. In this process, the air is supplied to the biopile system during a system of piping and pumps that forces air into the pile under positive pressure or draws air through the pile under negative pressure. The microbial activity is enhanced through microbial respiration then the result in the degradation of adsorbed petroleum pollutants became high.

The bioremediation process is broadly categorized into in-situ remediation and ex-situ bioremediation, based on the origin, transportation, and removal of pollutants from contaminated sites, as shown in Fig. 1.

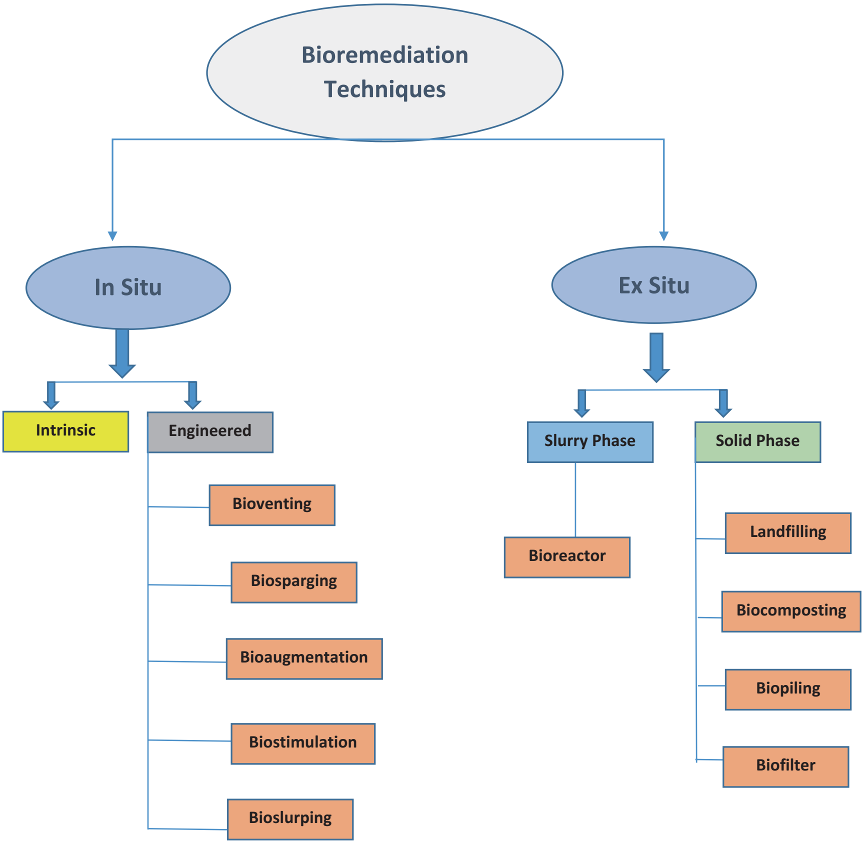

Fig. 1 Types of bioremediation

The process of biodegradation occurs under aerobic and anaerobic conditions; majority of bioremediation systems are designed to operate and degrade contaminants aerobically. Organic compounds are degraded aerobically, undergo oxidation to form less toxic compounds such as CO_2 and water. Throughout the anaerobic degradation, persistent intermediate compounds may be formed. Meanwhile, the anaerobic biodegradation of chlorinated aliphatic solvents can produce lower substituted chlorinated hydrocarbons, such as chloroethane or vinyl chloride. There are some compounds that are not readily degraded under anaerobic conditions and maybe more toxic than the original contaminant. The biodegradation of contaminants occurs as direct or co-metabolic processes. Direct bioremediation processes include the microbes that use the contaminants as a source of food or energy. When contaminants cannot be used as a food source, biodegradation may occur through co-metabolism in which the pollutant is degraded by an enzyme or cofactor produced during microbial metabolism of another compound.

Bioremediation is an emerging technology that can be simultaneously used with other physical and chemical treatment methods for the complete management of a diverse group of environmental pollutants. It seems like a sustainable approach for environmental pollution management, and hence, there is a need for more research in this area. Efforts need to be made to generate a synergistic interaction between the environmental impact on the fate and behavior of environmental contaminants and the assortment and performance of the most suitable bioremediation technique that can sustain the effective and successful operation and monitoring of a bioremediation action required. The current efforts of research and development will direct future regulations, dealing with bioremediation targets, contaminant availability, and their potential threat to the human and natural ecosystems.

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