



Bioremediation: Current Research Trends and Applications

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Abstract

Environmental pollution due to heavy metals, nuclear wastes, pesticides, nuclear gases, hydrocarbons, etc. seems to be of significant concern. The only possible solution to this is remediation using microbial processes (bioremediation). Bioremediation employs microbial metabolism under optimum environmental conditions and sufficient nutrients to degrade contaminants. This has proven to be effective and reliable due to its eco-friendly nature. Both in situ and ex situ techniques of bioremediation can be employed to reduce pollutant concentration. A diverse range of methods and strategies like bioaugmentation, biostimulation, bioventing, bioattenuation, etc. with their own merits and demerits are in use for the bioremediation process.

Keywords

Bioremediation · Bioaugmentation · Biostimulation · Bioventing · Bioattenuation

5.1 Introduction

The past two decades have experienced advances in bioremediation, aiming to restore polluted environments using eco-friendly techniques in a cost-effective manner. Several bioremediation techniques are developed by researchers. The nature and type of pollutants make use of numerous combinations of techniques to restore

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polluted environments. The natural microbes present in the polluted environment solely solve the problems associated with biodegradation and bioremediation of pollutant (Verma and Jaiswal 2016) only when the environmental conditions are conducive for their growth and metabolism. The physical and chemical methods of remediation are harmful as compared to the eco-friendly and cost-effective bioremediation techniques. Biodegradation is involved in a process called bioremediation. In bioremediation, microbes are able to convert and modify toxic pollutants to obtain energy and biomass. These toxic pollutants are converted to less toxic or non-toxic forms. Thus microbes like fungi, bacteria and archaea are also termed as bioremediators (Strong and Burgess 2008).

The nature of the pollutant, i.e. agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear wastes, plastics and sewage decides the process that is to be involved in the removal of pollutant. Bioremediation techniques are categorized to ex situ or in situ. This categorization is chosen to take into account the nature of pollutant, degree of pollution, type of environment, location, cost and environmental policies (Smith et al. 2015). Besides this, oxygen and nutrient concentrations, temperature, pH, and other abiotic factors are also given importance. The bioremediation techniques have a diverse application focusing on hydrocarbons polluting soil and groundwater (Frutos et al. 2010; Firmino et al. 2015). These techniques may be economical and efficient. This review focuses on the brief knowledge of remediation techniques highlighting their principle, merits, demerits, applications and prospects.

5.2 Principle

The principle of bioremediation is the biological degradation of organic wastes to an innocuous state or to levels below concentration limits as established by regulatory authorities (Mueller et al. 1996). This biological degradation of contaminants is carried by naturally occurring bacteria, fungi or plants to substances nonhazardous to human health and the environment. These microbes are either indigenous to a contaminated site or are isolated, cultured separated and then inoculated at the contaminated site for the remediation process. Practical bioremediation can be achieved by microbes through an enzymatic attack on the contaminants, thereby converting them to harmless products. The environmental conditions play an important role in microbial growth and activity, along with the degradation process (Kumar et al. 2011). Bioremediation can be carried under both aerobic and anaerobic conditions (Colberg and Young 1995).

However, some contaminants such as chlorinated organic or high aromatic compounds are resistant to microbial enzymatic degradation. They are either slowly degraded or not at all degraded, making it difficult to predict the degradation rate.

5.3 Factors Affecting Bioremediation

Bioremediation depends on factors like the chemical nature and concentration of the pollutants, the physicochemical characteristics of the environment and their availability to the microbes (Strong and Burgess 2008). The rate of degradation seems to be affected as microbes, and the pollutants do not contact each other and are non-uniformly spread in the environment. Efficient bioremediation is achieved by the following factors:

5.3.1 Biological Factors

Here, microbes play a key role (Fig. 5.1). The microbes of interest can be isolated from any environmental condition, cultured and inoculated at the contaminated site.

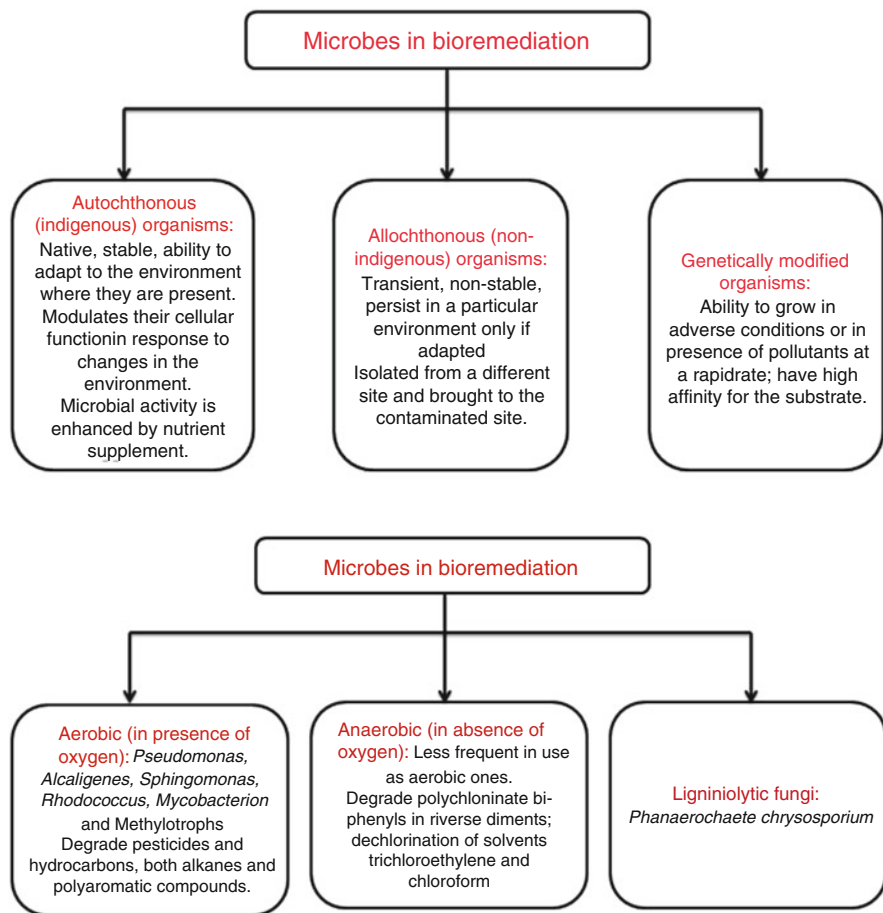


Fig. 5.1 Microbes used in the process of bioremediation

They have the adaptability to grow ranging from subzero temperatures to extreme heat from water to a condition in presence or absence of oxygen. They need a source of energy and carbon to sustain. Their adaptability to various conditions helps them degrade or remediate environmental hazards (Cheung 2013).

The interaction between biological, chemical and physical processes forms the basis of microbial remediation (Cheung 2013), the mechanism of which is depicted in Fig. 5.2.

5.3.2 Environmental Factors

The interaction between the microbes and the targeted contaminant depends upon the environmental parameters of the site of interaction. Microbial growth and activity are determined by pH, temperature, moisture, soil structure, solubility in water, nutrients, site characteristics, redox potential and oxygen and physio-chemic bioavailability of pollutants (Table 5.1) (Madhavi and Mohini 2012; Adams et al. 2015).

5.3.2.1 Nutrients

Addition of nutrients, especially nitrogen (N) and phosphorus (P) balances microbial growth and reproduction affecting rate and effectiveness of biodegradation. This optimizes the bacterial carbon (C): N:P ratio. The nutrient addition in appropriate quantity enhances the metabolic activity and biodegradation rate in a cold environment (Couto et al. 2014; Phulia et al. 2013).

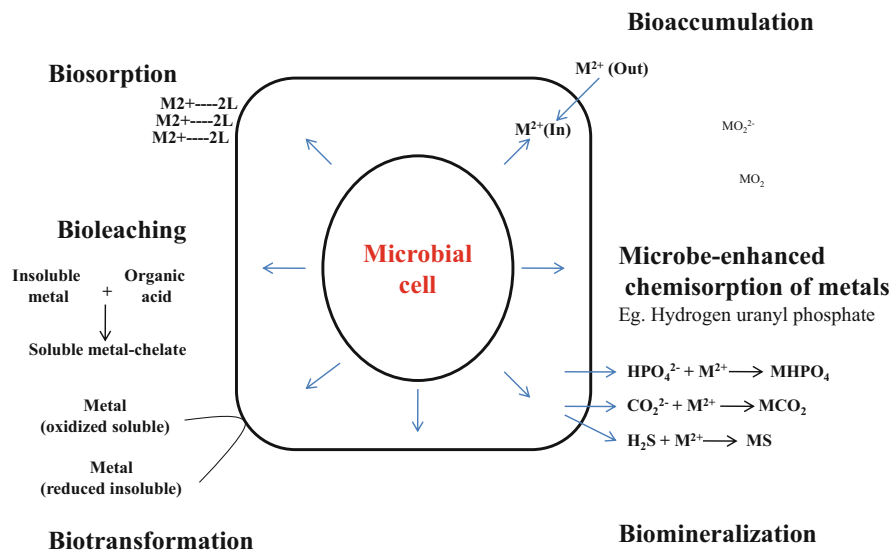


Fig. 5.2 Mechanism of microbial remediation (Cheung 2013)

Table 5.1 Environmental conditions affecting bioremediation (Sutar and Das 2012)

Parameters	Conditions for microbial activity
Soil moisture	25–28% of water holding capacity
Soil pH	5.5–8.8
Oxygen content	Aerobic, minimum air-filled pore space of 10%
Nutrient content	N and P for microbial growth
Temperature (°C)	15–45
Contaminants	Not too toxic
Heavy metals	Total content 2000 ppm
Soil type	Low clay or silt content

5.3.2.2 Temperature

Temperature is crucial to determine the survival of microbes and composition of hydrocarbons. A specific temperature is essential for the degradation of a specific compound. By affecting the microbial physiological properties, the temperature can accelerate and decelerate the bioremediation process. With an increase in temperature, the rate of microbial activities increases and reaches a maximum at an optimum temperature. However, it declines further with an increase or decrease in temperature and eventually stops after reaching a specific temperature (Macaulay 2014; Das and Chandran 2011).

5.3.2.3 Oxygen Concentration

Biological degradation can be carried out both in aerobic and anaerobic conditions. Aerobic conditions degrade hydrocarbons while anaerobic conditions degrade chlorate compounds (Macaulay 2014).

5.3.2.4 Moisture

Pollutant degradation is affected by moisture content. The moisture content has an adverse effect on biodegradable agents.

5.3.2.5 pH

It impacts microbial activity and influences the biodegradation. A measure in soil pH justifies microbial growth (Asira 2013). A slight change in pH affects the microbial process (Wang et al. 2011).

5.3.2.6 Metal Ions

Small quantity of metal ions in microbes directly or indirectly impact the degradation rate.

5.3.3 Types of Bioremediation

Bioremediation of pollutants is carried either in situ or in ex situ conditions (Fig. 5.3). Both these remediation strategies depend on community dynamics of organisms, their development and existence, structure and function.

5.4 Ex situ Bioremediation Techniques

It involves excavating pollutants from polluted sites for subsequent treatment in another site.

Factors considered:

1. The cost of treatment.
2. Type of pollutant.
3. Depth and degree of pollution.
4. Geographical location and geology of polluted site.

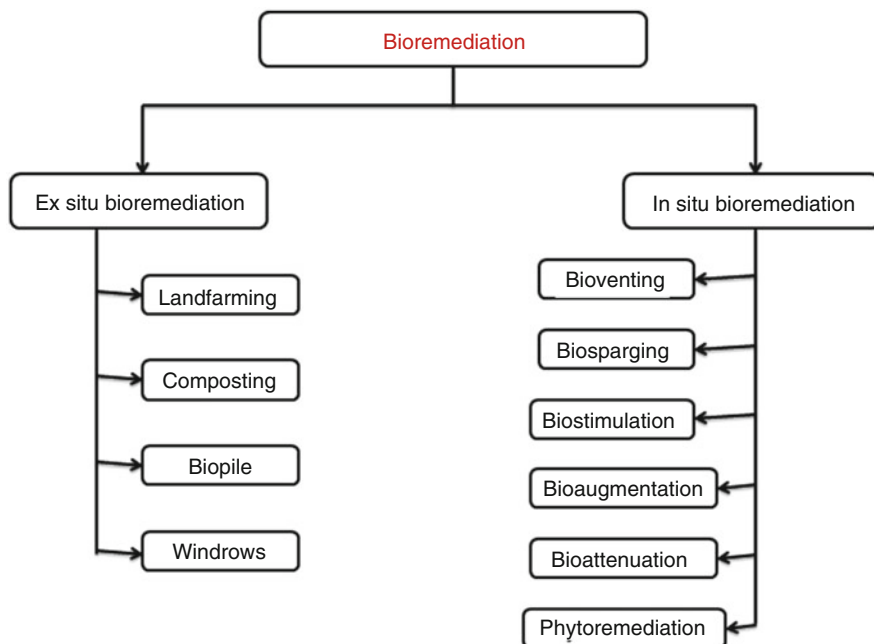


Fig. 5.3 Schematic representation of bioremediation techniques

5.4.1 Landfarming

It involves the excavation of contaminated soil to a prepared bed where it is periodically tilled to degrade pollutants. Here aerobic degradation of contaminants through indigenous microbes is achieved (Silva-Castro et al. 2015). Limitation involves the treatment of superficial (10–35 cm) soil. However, it is widely used as a disposal alternative as it needs less monitoring, maintenance costs and less operational equipment with low cleanup liabilities. This is used to treat voluminous polluted soil with meagre environmental impact and energy requirement (Maila and Colete 2004). This technique is employed for remediation of hydrocarbon polluted sites, including polyaromatic hydrocarbons (Cerqueira et al. 2014). This technique complies with government regulations and can be used in any climatic condition and location (Besaltatpour et al. 2011).

5.4.2 Composting

The contaminated soil is enriched with nonhazardous organic materials such as manure or agricultural wastes so as to develop a rich microbial population and enhance temperature suitable for composting.

5.4.3 Biopile

This technique involves above-ground piling of excavated polluted soil, followed by nutrient amendment and aeration to enhance bioremediation. This technique relies on enhancement in microbial activities through microbial respiration resulting in higher degradation of adsorbed petroleum pollutant (Emami et al. 2012). A system of pumps either forces air under positive pressure or negative pressure (Delille et al. 2008).

Components:

1. Aeration.
2. Irrigation.
3. Nutrient and leachate collection systems.
4. Treatment beds.

This technique (von Fahnestock et al. 1998) is cost-effective with an effective biodegradation provided nutrient, temperature and aeration are adequately controlled (Whelan et al. 2015). This technique is a hybrid of landfarming and composting. These treat surface contamination with hydrocarbons of petroleum. Biopiles conserve space compared to other ex situ techniques like landfarming. However, robust engineering, cost of maintenance and operation, lack of power supply at remote areas are significant drawbacks of this technique. Overheating of air can lead to drying of soil undergoing bioremediation, which will result in inhibition of microbial

activities and promote volatilization (Sanscartier et al. 2009). Indigenous aerobic and anaerobic microbes are favoured in this method.

5.4.4 Windrows

Here, bioremediation is enhanced by periodic turning of piled polluted soil to increase degradation activities of indigenous and transient bacteria residing in polluted soil. Furthermore, when water is added increases aeration, uniform distribution of pollutants, nutrients and microbial degradative activities speeding up the rate of biodegradation (Barr 2002). Higher rate of hydrocarbon removal was observed in windrows as compared to biopile treatment (Coulon et al. 2010). Windrows treatment has been used in greenhouse gas release (Hobson et al. 2005).

Ex situ bioremediation does not require an extensive preliminary assessment of polluted site before remediation making the preliminary stage short less labourious and less expensive.

5.5 In situ Bioremediation Techniques

These techniques are applied to soil and groundwater at site avoiding excavation and transport of contaminants with minimal disturbance to soil structure. It involves low cost making it the most desirable option. However, the cost of design and on-site installation of some sophisticated types of equipment to enhance microbial activities are of significant concern. It is limited by the depth of the soil that can be effectively treated.

Some in situ bioremediation techniques are enhanced using bioventing, biosparging and phytoremediation methods, while other techniques like intrinsic bioremediation or natural attenuation involve no enhancement.

These techniques treated chlorinated solvents, dyes, heavy metals, and hydrocarbon polluted sites (Folch et al. 2013; Roy et al. 2015). Some of the major factors taken into account for achieving a successful in situ bioremediation are electron acceptor, soil porosity, moisture content, nutrient availability, pH and temperature.

5.5.1 Bioventing

This process stimulates the growth of natural or introduced microbes through a vent that supplies oxygen by means of direct air injection to the soil. It is more functional in aerobically degradable compounds. The microbial activities are sustained through modulating airflow rates to provide the necessary oxygen for biodegradation minimizing volatilization and release of contaminants to the atmosphere. It also works for contamination deep under the surface. It is used to treat soil contaminated with fuels, non-halogenated volatile organic compounds, semi-volatile organic

compounds, pesticides and herbicides. This technique is used for biodegradation of petroleum-contaminated soil (Lee et al. 2006; Agarry and Latinwo 2015).

5.5.2 Biosparging

This process involves the injection of air under pressure below the water table to increase groundwater oxygen concentration which increases the rate of biological degradation of contaminants by indigenous bacteria. It treats groundwater contamination by fuels, non-halogenated volatile organic compounds, semi-volatile organic compounds, pesticides, organics and herbicides. It requires indigenous microbes, nutrients for their growth and specific containment availability.

5.5.3 Biostimulation

It deals with the injection of water-based solution carrying nutrients, electron acceptor or other amendments at the contaminated site (soil/groundwater) to stimulate the activity of indigenous microbes. Microbial activity is stimulated by supplying fertilizers, growth supplements, trace elements, environmental requirements like pH, temperature and oxygen to enhance their metabolism and pathway (Adams et al. 2015; Kumar et al. 2011). This is used to treat soil and groundwater contaminated by fuels, non-halogenated volatile organic compounds, semi-volatile organic compounds, pesticides and herbicides. Bioremediation process is enhanced when biostimulation is coupled with advanced tools and techniques.

5.5.4 Bioaugmentation

It involves augmenting the biodegradative capacity of indigenous microbial populations on contaminated site through the addition of natural/exotic/engineered pollutant degrading microbes. These microbes may include bacteria, protozoa, nematodes, rotifers and fungi. These microbes are collected from the remediation site, cultured, genetically modified and returned to the contaminated site for degradation. These in situ microbes can degrade sites contaminated with chlorinated ethenes to ethylene and chloride that are non-toxic (Niu et al. 2009; Malik and Ahmed 2012; Alwan et al. 2013; Gomez and Sartaj 2014). The genetically engineered microbes are more competent than that of natural species in degrading contaminants. These genetically engineered microbes can remediate soil, groundwater and activated sludge, exhibiting a higher degradative ability of chemical and physical pollutants (Sayler and Ripp 2000; Thapa et al. 2012).

5.5.5 Bioattenuation/Natural Attenuation

It is a proactive approach that monitors natural remediation processes (Khan et al. 2004). It is a method of eradication of pollutant concentrations from the surrounding environment. It uses natural methods to limit the spread of contamination, reducing the concentration and amount of pollutants at the contaminated sites (Boparai et al. 2008; Khan et al. 2004). It involves biological processes like aerobic and anaerobic biodegradation; physical phenomena like advection, dispersion, dilution, diffusion, volatilization, sorption/desorption; and chemical reactions like ion exchange, complexation and abiotic transformation. This process often involves terms like passive remediation, in situ bioremediation, intrinsic remediation or biotransformation (Mulligana and Yong 2004).

This process is categorized either as destructive or non-destructive (Gelman and Binstock 2008). This process is applied to fuels, halogenated organics, non-halogenated volatile organic compounds, semi-volatile organic compounds, pesticides, herbicides and hydrophobic contaminants.

For chemical pollutants in the environment, nature can clean up in the following ways (Li et al. 2010):

1. The chemicals are consumed by microbes dwelling in the soil and groundwater, converting them into the water and harmless gases.
2. The chemicals stick to the soil, holding them in the soil, preventing them from contaminating the groundwater.
3. The chemical pollutant on movement through the soil and groundwater dilutes it before mixing with the clean water.
4. Chemical pollutants in oil and solvents can evaporate and mix with the air where sunlight destroys them.
5. If the process of bioattenuation is not achieved, enhanced bioremediation techniques like biostimulation or bioaugmentation may be used.

5.5.6 Phytoremediation

The term “phytoremediation” was coined in 1991. It is an emerging technology where plants are used for treatment and mineralization of pollutants from contaminated sites (Raskin and Ensley 2000).

The overview below shows the applications of phytoremediation (Fig. 5.4).

This technique is used at the very large field site where other methods of remediation are not cost-effective or practicable; at sites with a low concentration of contaminants where only polish treatment is required for a more extended period and besides with other techniques where vegetation is used as a final cap and closure of the site.

Limitation:

1. Longer duration of time for remediation.

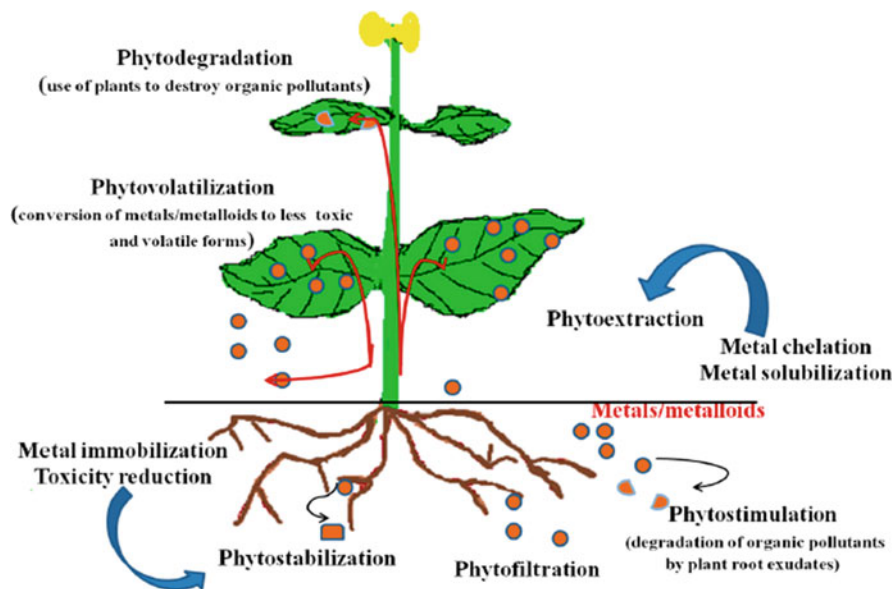


Fig. 5.4 Processes involved in phytoremediation of contaminants (heavy metals) (Ojuederie and Babalola 2017)

2. Potential contamination of vegetation and food chain.
3. Difficulty in establishing and maintaining vegetation at some sites that have high toxic levels.

5.6 Bioreactor

The bioreactor is an engineered device or vessel specifically designed and developed for abundant growth and metabolic activity of microbes through a biocatalyst, enzyme or microbial or animal or plant cells. The basic raw material is an organic or inorganic chemical compound. The internal condition of a bioreactor should support the growth of the cell, which is further enhanced by their biocatalytic activity (Singh et al. 2014). Bioreactors are much different from conventional chemical reactors supporting biological growth. Bioreactors should have a robust environment that must control the process upset and contaminants. It should enhance biological activities and minimize undesirable activities. Additionally, coenzymes are added that influence the kinetics of bioreactors (Singh et al. 2014). Most of the bioreactors operate by providing culture control, optimization, standardization, scale-up feasibility and automatic operation for cultivation of cells (Paopo 2014). Microbial bioreactors are classified to batch, fed-batch and continuous bioreactors (Figs. 5.5, 5.6 and 5.7).

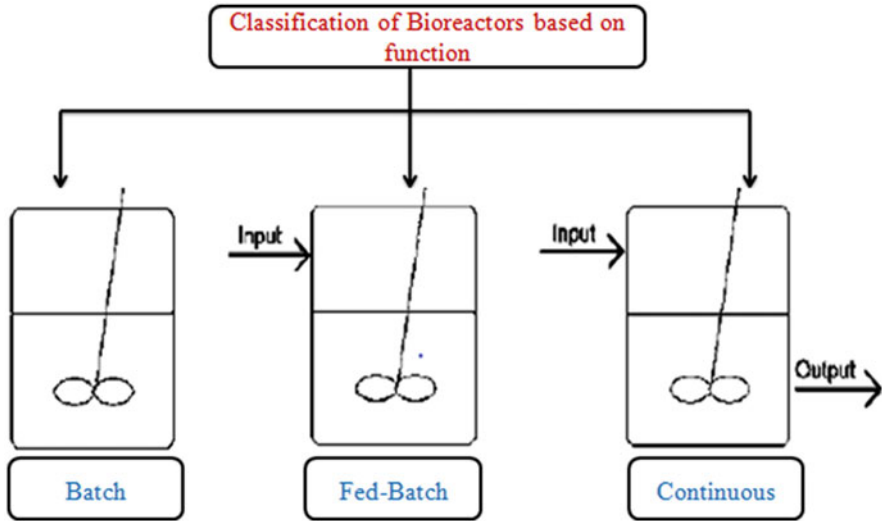


Fig. 5.5 Classification of bioreactors based on functions

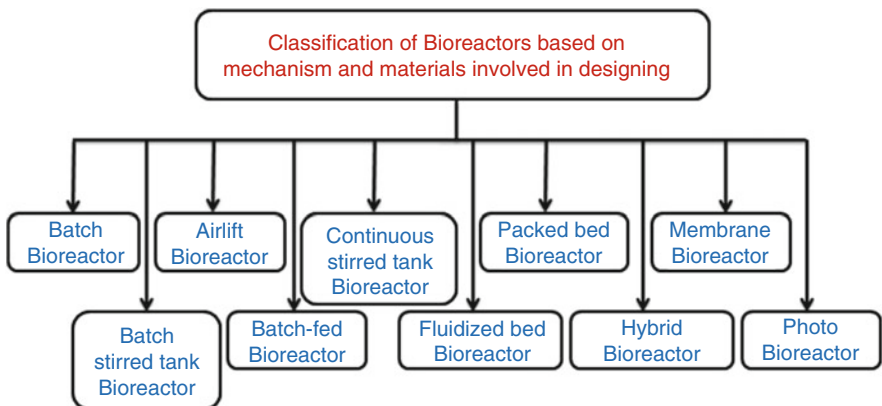


Fig. 5.6 Classification of bioreactors (Praharaj 2012; Sarkar and Mazumder 2014; Mandenius 2016)

5.7 Merits of Bioremediation

1. Effective and economical than that of other conventional technologies.
2. Less labour-intensive.
3. Complete destruction of pollutants.
4. Since there is no use of harmful chemicals, it is eco-friendly (as it releases harmless/less toxic by-product) and sustainable.

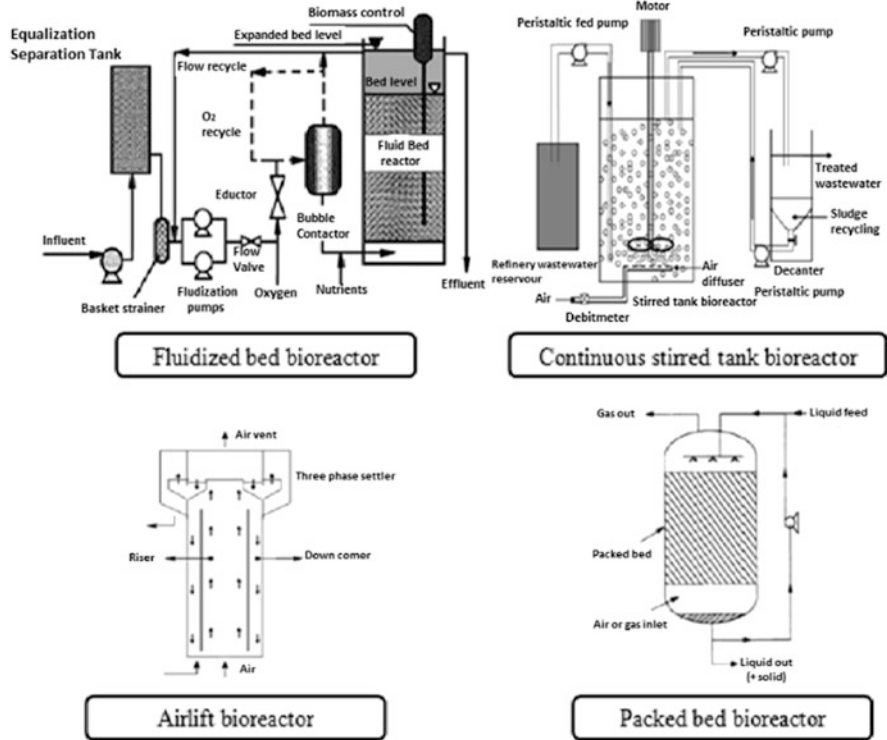


Fig. 5.7 Types of bioreactors

5. Relatively easy in implementing the technique.
6. On-site treatment possible without causing a major disruption of normal activities.
7. Do not affect natural flora, making it an effective way of remediating the natural ecosystem.

5.8 Demerits of Bioremediation

1. Biodegradable compounds can undergo biodegradation.
2. The specific environmental condition required.
3. Specific microflora required.
4. Appropriate levels of nutrients and contaminants required.
5. Long time required to remove or transform contaminant.
6. Some end products may be persistent or toxic than their parent compound.

5.9 Applications

5.9.1 Microbial Remediation of Contaminants (Heavy Metals)

Microbes can tolerate metal toxicity in numerous ways. Microbes have been used to sequester, precipitate or alter the oxidation state of numerous heavy metals (Gupta et al. 2016; Kang et al. 2016). A consortium of bacterial strain is more effective than a single strain culture for successful bioremediation. Wang and Chen (2009) studied that the bacterial mixture of *Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46 and *E. cloacae* KJ-47 had more excellent resistance and efficiency than the single strain culture in bioremediation of a mixture of Pb (lead), Cd (cadmium) and Cu (copper) from contaminated soils. The microbial mechanism for bioremediation is given in the following flowchart (Jan et al. 2014) (Fig. 5.8).

Figuroa et al. showed that several microbes exhibited high resistance to 19 metalloids. These strains exhibited metal or metalloid, reducing capacity and have been used successfully to synthesize nanostructures. Tables 5.2 and 5.3 show different kinds of in situ and ex situ techniques that used to bioremediate of different contaminants.

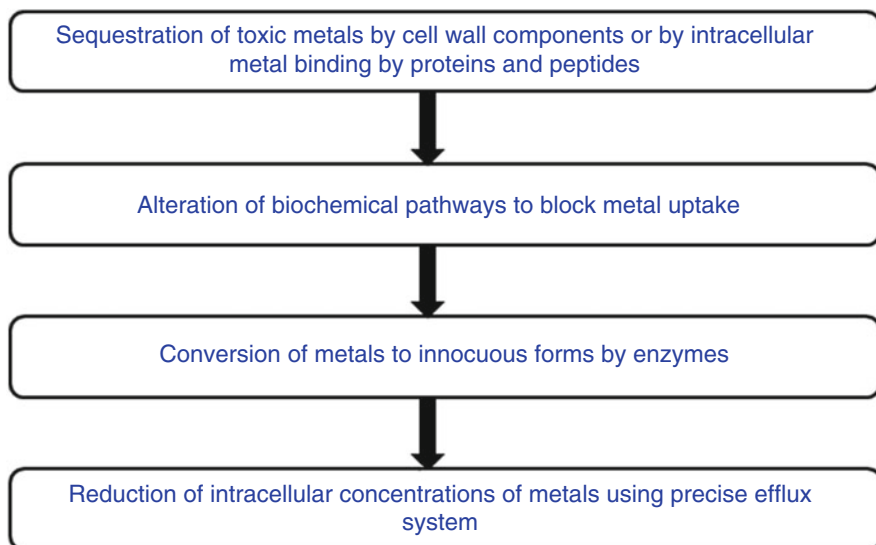


Fig. 5.8 Microbial mechanism for bioremediation

Table 5.2 Application of in situ bioremediation techniques

In situ technique	Contaminant	References
Bioventing	Diesel oil in the soil	Agarry and Latinwo (2015)
	Petroleum hydrocarbons	Mosco and Zytner (2017) and Xiao and Zytner (2019)
	Benzene and toluene	Amin et al. (2014)
Biostimulation	Chlorinated solvents	Henry (2010)
	TCE (Trichloroethane)	
	n-Alkane in diesel	Kahraman et al. (2017)
	Kerosene	
Biosparging	Sulphur and metallic ions	Rodrigues et al. (2020)
	Petroleum hydrocarbons	Shahsavari et al. (2017)
Bioaugmentation	Crude oil	Wang et al. (2019)
	Chlorpyrifos	Zhang et al. (2012)
	Chlorinated solvents	Mao et al. (2012)
Phytoremediation	Arsenic	Upadhyay et al. (2018)
	Mercury and lead	Kumar et al. (2017)
	Heavy metal	Yan et al. (2020)

Table 5.3 Application of ex situ bioremediation techniques

Ex situ techniques	Contaminant	References
Landfarming	Heavy metal	Kapahi and Sachdeva (2019)
	Hydrocarbon	Bergsveinson et al. (2019)
	Crude oil	Ali et al. (2020)
Composting	Heavy metal	Rahman and Singh (2020)
	Phenolic compounds	Tripathi and Dixit (2016)
	Hydrocarbon	Paladino et al. (2016)

5.9.2 Bioremediation of Pesticides

Pesticide usage has afflicted both the soil and water affecting many lives. Thus decontamination of these harmful pesticides is highly needed (Gavrilescu 2005) (Fig. 5.9).

5.10 Trends in Bioremediation

The bioremediation techniques effectively restore sites polluted with numerous pollutants. Since microbes are crucial in this process, their diversity, abundance and community structure in polluted sites are important only when the environmental parameters for their metabolic activities are maintained. Bioremediation can be

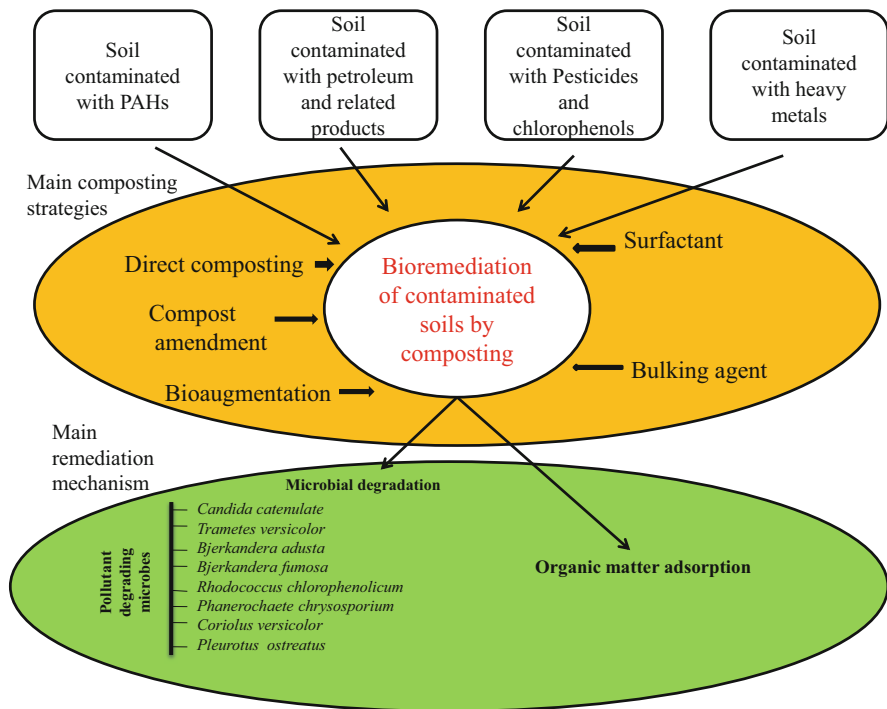


Fig. 5.9 Biodegradation of pesticide phenanthrene in compost amended soil (Puglisi et al. 2007)

enhanced through the application of several tools and techniques, as mentioned below (Fig. 5.10).

5.10.1 Biosurfactants

These are surface-active substances prepared by microbial cells. Their diversity, eco-friendly nature, large scale production, selectivity, performance under extreme conditions and usage in environmental protection have enhanced their interest (Rahman et al. 2002; Bodour et al. 2004). The usage will decrease the hydrophobic nature of the contaminant, making it readily available to the biological system for its remediation.

5.10.2 Oxygen Releasing Compounds

Aerobic condition ensures faster degradation of petroleum hydrocarbons than the anaerobic conditions. So the addition of oxygen or oxygen releasing compounds enhances bioremediation rate. These compounds release oxygen slowly when it

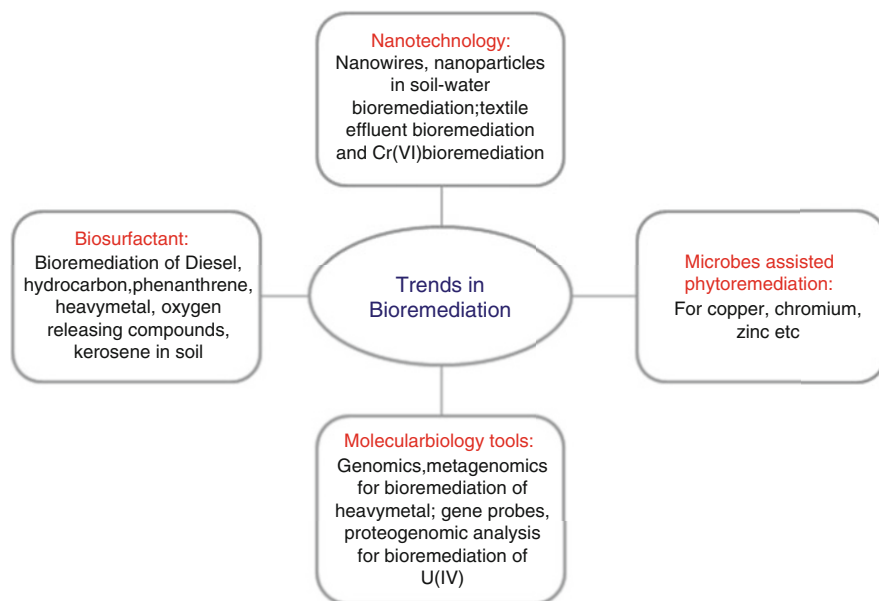


Fig. 5.10 Schematics of trends in bioremediation

comes in contact with water. Oxygen releasing compound product similar to Milk of Magnesia is used to address dissolved phase contamination, such as total petroleum hydrocarbons and BTEX (Benzene, Toluene, Ethylbenzene and Xylene).

5.10.3 Molecular Biological Tools and Techniques

Though many microbes are uncultivable in the laboratory, yet their importance cannot be neglected in the bioremediation process. Certain molecular techniques like genomics, metagenomics, gene probes, etc. are used to trace these microbes, their genes and enzymes involved in the bioremediation process. These techniques track the metabolic pathway employed by the microbe to degrade the contaminant.

5.10.4 Bioinformatics

The tools of bioinformatics helps identify, analyse various components of the cells, viz. gene and protein functions, interactions, metabolic and regulatory pathways. These tools facilitate the analysis of cellular processes to understand their cellular mechanism. Bioinformatics is helpful in structure-function determination and pathways of biodegradation of xenobiotics (Fulekar and Sharma 2008).

5.10.5 Nanotechnology

The use of nanotechnology seems like a fascinating and highly promising approach to clean the environment contaminated with pollutants.

5.11 Conclusion

Bioremediation is a green approach that cleans the environment. The microbial activity is employed in remediating, cleaning, managing and recovering techniques for degrading environmental contaminants. The rate of degradation depends on the biological agents, nutrient supply, external abiotic conditions and availability of the pollutant. Understanding microbial communities and their response to the natural environment and pollutant, studying the genetics of microbes to increase the capabilities to degrade pollutant and field trials of existing and new remediation techniques are the significant insights to be focused. It offers the advantage of being efficient, eco-friendly and cost-effective. Successful bioremediation depends upon site characterization, which establishes suitable bioremediation technique (ex situ or in situ). The efficient method to treat polluted site depends upon the geological feature of the polluted site (viz. soil type, pollutant depth and type, site location, etc.). The increasing popularity of these techniques shows that merits outnumber demerits. The advanced tools in bioremediation need field trials to be further implemented. Mostly, environmental factors and toxic nature of the contaminant hamper the usage of available technology urging the need for the development of hybrid technologies that can adjust to environmental parameters and toxicity of the contaminant.

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