**BIOREMEDIATION**

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**Introduction:**

Worldwide there is a tendency to develop simple methods, fast, cheap and efficient, ensuring their application in-situ blocking the migration of pollutants in underground or other neighbourhoods, destroying pollutants and restoring the natural. Decontamination technologies biodegradation (bioremediation) can help the biodegradation process to be accelerated. The biodegradation of petroleum hydrocarbons existing in different environments, particularly in the ground, is based on one hand on the use of microorganisms indigenous existing in nature and adapted to the pollutant in question and, on the other hand the introduction of micro-organisms specific species .

The term "bioremediation" refers to the employment of biological processes to essentially eliminate pollutants or other impairments from soil and water. The concept of bioremediation is accepted as a summation of the processes of decomposition of constituents of natural or synthetic, by activating certain strains of microorganisms specialized resulting in end products useful or acceptable in terms of its environmental impact. Detoxification, reduction, degradation, or transformation of hazardous chemicals into less toxic ones is the primary mechanism underlying this procedure. The nature of the contaminants, such as the type of pesticides, agricultural chemicals, xenobiotic compounds, heavy metals, plastics, organic halogens, greenhouse gases, etc., is the only factor that influences the bioremediation process. Nuclear waste processing also employs this technique.

**1. Advantages and disadvantages of bioremediation:**

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| **Advantages** | **Disadvantages** |
| Bioremediation is an eco-friendly and sustainable approach | Bioremediation technology is restricted to biodegradable compounds. |
| Bioremediation does not use any toxic chemicals. Sometimes it utilizes nutrients such as fertilizers to activate the microbial population. | Sometimes the new product developed after biodegradation may be more toxic to the environment than the initial compound. |
| Bioremediation is less labor-intensive and is economical. | The process of bioremediation is time-consuming. |
| It does not generate waste and self sustaining. | Bio remediation is difficult to extrapolate from the laboratory scale *i.e.* at field level. |
| Microorganisms have the ability to degrade a large number of contaminants and are also harmless to the environment. | Lack of oxygen and other nutrients in the soil may limit the performance of the soil microorganisms. |
| Bioremediation can be made on the site directly without any ecosystem disruption. | Changing in the hydrological and geochemical conditions over the time could cause a remobilization of contaminants previously stabilized. |
| The transport and oprating cost for waste management can be eliminated with bioremediation. | Ex situ bioremediation required excavation and pumping. |
| Bioremediation can be combined with other treatment technologies. | Public awareness and education for wide acceptance of bioremediation. |

**2. Principles of bioremediation:**

The utilisation of living creatures, particularly microorganisms, in the reduction of toxic contaminants to less toxic chemicals is the fundamental principle behind bioremediation. This technology detoxifies or degrades dangerous compounds from the environment using bacteria, fungi, and/or plants. Living organisms change pollution through metabolic processes including enzyme synthesis. Several microorganisms are used in this approach to break down contaminants at the contaminated site.

**3. Techniques of Bioremediation**

**Ex-situ and in-situ site of application are two options for bioremediation treatment (Figure 1). The selection criteria that are taken into account while choosing any bioremediation technique include the type of pollutant, the depth and volume of contamination, the type of environment, the location, the cost, and environmental policies. The efficiency of bioremediation processes is determined by oxygen and nutrient concentrations, temperature, pH, and other abiotic variables. (**Frutos et al. 2012; Smith et al. 2015).

**3.1 Ex situ bioremediation techniques**

**Ex-situ bioremediation strategies entail removing pollutants from contaminated areas and transferring them one at a time to another location for restoration. The depth of pollution, kind of pollutant, degree of pollution, cost of treatment, and location of the contaminated site are frequently taken into consideration when ex-situ bioremediation strategies are being evaluated. Ex-situ bioremediation technology selection is governed by performance standards as well.**

**3.1.1 Solid-phase treatment**

**In ex-situ  solid-phase bioremediation, the contaminated soil is removed from its natural environment and piled up. It also comprises home, industrial, and municipal trash, as well as organic wastes like leaves, animal dung, and agricultural wastes. Pipes installed all over the piles are used to transport bacterial growth. For ventilation and microbial respiration, air must be pulled via the pipes. In comparison to slurry-phase procedures, solid-phase systems needs more area, and cleanups takes longer time to carry out. Processes for solid-phase treatment include composting, land farming, windrows, biopiles, and etc.(**Kulasrestha et al. 2014**).**

 Bioremediation Techniques

 Ex Situ

 In situ

Biopiling

Windrow

Bio

reactor

Land farming

Natural attenuation

Enhanced

Bioslurping

Bioventing

Biosparging

Phyto

remediation

Permeable reactive barrier

Solid phase

Slurry phase

**Fig.1.** Bioremediation techniques

**3.1.1.1 Types of ex-situ bioremediation**

**3.1.1.1.1 Biopile**

In order to improve bioremediation by effectively boosting microbial activity, biopile-mediated bioremediation requires piling excavated contaminated soil above ground, followed by nutrient supplementation and occasionally aeration. Aeration, irrigation, nutrient and leachate collecting systems and a treatment bed are the elements of this technique. Due to its beneficial qualities, such as cost effectiveness, which enables effective biodegradation under the condition that nutrient, temperature, and aeration are sufficiently managed, the application of this specific ex situ technique is being studied more frequently (Whelan et al. 2015). The use of biopile to remediate contaminated environments, such as very cold regions, can be useful in reducing the volatilization of low molecular weight (LMW) contaminants (Dias et al. 2015; Gomez and Sartaj 2014; Whelan et al. 2015).

**3.1.1.1.2 Windrows:**

Windrows rely on the frequent rotation of the piled polluted soil to increase the degradation activities of the native and/or transitory hydrocarbonoclastic bacteria present in the polluted soil. The regular turning of contaminated soil and addition of water promote aeration, uniformly spreading of pollutants, nutrients, and microbial degradative activities and it increases the rate of bioremediation by assimilation, biotransformation, and mineralization (Barr, 2002). When compared to biopile treatments, windrow treatments showed a higher rate of hydrocarbon removal; nevertheless, the better effectiveness of the windrow towards hydrocarbon removal was due to the soil type, which was said to be more friable (Coulon et al. 2010).The establishment of an anaerobic zone inside heaped polluted soil is associated with CH4 (greenhouse gas) emission. (Hobson et al. 2005).

**3.1.1.1.3 Land farming:**

It is typically viewed as ex situ bioremediation, while it is occasionally seen as an in situ bioremediation technology. The treatment site is at the centre of this controversy. Whether land farming can be done in situ or ex situ depends significantly on the depth of the pollution. The common procedure in land farming is to excavate and/or till contaminated soils. Excavated polluted soil might be considered in situ when it is treated there; otherwise, it is ex situ because it shares more characteristics with other ex situ bioremediation approaches.According to reports, when a pollutant is less than one metre below ground level, bioremediation may proceed without excavation, but when it is more than one and a half metres below ground level, transporting the pollution to the surface is necessary for effective bioremediation (Nikolopoulou et al. 2013). Typically, excavated polluted soils are carefully put on a fixed layer support above the ground surface to enable aerobic biodegradation of pollution by native microorganisms (Philp and Atlas 2005; Paudyn et al. 2008; Volpe et al. 2012; Silva-Castro et al. 2015).It was revealed that tillage and irrigation without nutrient addition in a soil with adequate biological activity boosted heterotrophic and diesel-degrading bacterial counts, accelerating the pace of bioremediation. Dehydrogenase activity was also found to be a good indicator of biostimulation treatment and may be employed as a biological parameter in land farming technique.(Silva-Castro et al. 2015).

**Advantages of land farming**

* It is the simplest bioremediation technique.
* Low cost and less equipment requirement for operation

**Limitations of land farming**

* Large operating space requirement.
* Reduction in microbial activities due to unfavourable environmental conditions.
* Additional cost due to excavation
* Reduced efficacy in inorganic pollutant removal
* Not suitable for treating soil polluted with toxic volatiles due to its design and mechanism of pollutant removal (volatilization), especially in hot (tropical) climate regions.
	+ 1. **Slurry-phase bioremediation**

Slurry-phase bioremediation is a somewhat quicker process than the other types of treatment. In the bioreactor, contaminated soil is blended with water, nutrients, and oxygen to provide the microorganisms with the best possible environment in which to break down the pollutants that are present in the soil. Stones and rubble are separated from the contaminated soil during this operation. The concentration of additional water is influenced by soil physicochemical characteristics, the rate of biodegradation, and the concentration of contaminants. After this procedure is finished, the soil is extracted and dried using centrifuges, vacuum filters, and pressure filters. The next step is to dispose of the soil and treat the resulting fluids in advance.

**3.1.2.1** **Bioreactor:**

A bioreactor is a container where raw materials are transformed into certain products as a result of a chain of biological reactions. The bioreactor can be operated in a variety of ways, such as batch, fed-batch, sequencing batch, continuous, and multistage. The market economy and capital investment play a major role in determining the operating mode. An ideal growth environment is created in a bioreactor by imitating and maintaining the natural environment of the cells being studied. In either instance, using a bioreactor to clean polluted soil has various benefits over alternative ex situ bioremediation methods. Polluted samples can be put into a bioreactor as dry matter or slurry. One of the main benefits of bioreactor-based bioremediation is its excellent control of the bioprocess parameters (temperature, pH, agitation and aeration rates, substrate and inoculum concentrations). The ability to regulate and alter a bioreactor's process parameters suggests that the biological reactions taking place inside can be improved to significantly speed up bioremediation.

**3.1.3 Advantages of ex-situ bioremediation**

* Effective against a variety of pollutants
* Can be evaluated very easily from site investigation data.
* Due to the more manageable, predictable, and controlled polluted environment found in bioreactor systems, biodegradation occurs more quickly than in solid-phase systems.

**3.1.4 Disadvantages**

* Non-permeable soil requires further processing;
* Not applicable to heavy metal pollution or chlorinated hydrocarbons, such as trichloroethylene.
* Before adding soil to a bioreactor, the pollutant might be removed physically or by washing the soil.

**3.2 In situ bioremediation techniques**

In situ bioremediation techniques involve treating polluted substances at the site of pollution. It does not require any excavation; therefore, it is accompanied by little or no disturbance to soil structure. Ideally, these techniques ought to be less expensive compared to ex situ bioremediation techniques, due to no extra cost required for excavation processes. Some in situ bioremediation techniques might be enhanced (bioventing, biosparging and phytoremediation), while others might proceed without any form of enhancement (intrinsic bioremediation or natural attenuation). In situ bioremediation techniques have been successfully used to treat chlorinated solvents, dyes, heavy metals, and hydrocarbons polluted sites (Folch et al. 2013; Kim et al. 2014; Frascari et al. 2015; Roy et al. 2015).Notably, the availability of nutrients, moisture content, pH, and temperature are among the crucial environmental factors that must be adequate for an in situ bioremediation to be completed (Philp and Atlas 2005).

**3.2.1 Types of in-situ bioremediation**

In-situ bioremediation is two types; these are natural (intrinsic) and enhanced (engineered) bioremediation.

**3.2.1.1 Natural(intrinsic) bioremediation**

Natural or intrinsic bioremediation involves passive repair of polluted environments without the use of any outside force (human intervention). Polluting compounds, especially those that are refractory, are biodegraded via both aerobic and anaerobic microbial processes. The lack of an outside force suggests that the method is less expensive than other in situ methods. To prove that bioremediation is continuing and sustained, however, the process must be observed; hence, the phrase "monitored natural attenuation" (MNA). Additionally, MNA is frequently used to denote a more comprehensive strategy for intrinsic bioremediation.Three requirements must be met for intrinsic bioremediation, according to the United States National Research Council (US NRC): proof of contaminants loss from contaminated sites, evidence based on laboratory tests that microorganisms isolated from contaminated sites have the innate potentials to biodegrade or transform contaminants present at the contaminated site from which they were isolated, and proof of realisation of biodegradation (Philp and Atlas 2005). Due to the cold temperature conditions that are likely to have a negative impact on the biodegradation process, MNA is increasingly becoming accepted in the majority of European countries, with the exception of a very small number.(Declercq et al. 2012). Furthermore, the primary mechanism for pollutant elimination during intrinsic bioremediation is biodegradation. Given that no outside force is used to speed up the cleanup process, intrinsic bioremediation has a number of significant drawbacks, one of which is that it may take longer to reach the goal level of pollutant concentration. Therefore, risk assessment must be done before intrinsic bioremediation  to guarantee that the remediation duration is shorter than the time allotted for the pollutant to reach the exposure point relative to the closest human and animal populations. Additionally, it was shown that intrinsic bioremediation does not sufficiently remove polyaromatic hydrocarbons (PAHs), which would reduce the ecotoxicity of polluted soil (Garcia-Delgado et al. 2015).

**3.2.1.2 Enhanced(engineered) in situ bioremediation**

Enhanced (engineered) in situ bioremediationincludes introducing certain microorganisms to the contaminated site. By improving the physicochemical conditions to promote the growth of microorganisms, genetically engineered microorganisms employed in in-situ bioremediation to speed up the degradation process.

**3.2.1.2.1 Bioventing**

This method uses a regulated airflow stimulation to promote bioremediation by boosting the activity of local bacteria by supplying oxygen to the unsaturated (vadose) zone. The eventual goal of bioventing is to promote microbial transformation of pollutants into a harmless condition. Amendments are made by adding nutrients and moisture to enhance bioremediation (Philp and Atlas 2005). This method has become more popular than other in situ bioremediation methods, particularly for cleaning up areas where light petroleum compounds have been spilled (Hӧhener and Ponsin 2014). Although the purpose of bioventing is to promote aeration in unsaturated zones, it can also be utilised for anaerobic bioremediation, particularly for the treatment of vadose zones contaminated with chlorinated chemicals that are resistant to aerobic treatment. In this latter method, hydrogen serves as an electron donor and a mixture of nitrogen, low amounts of carbon dioxide, and hydrogen can also be added in place of air or pure oxygen to reduce chlorinated vapour (Mihopoulos et al. 2000; Shah et al. 2001).

**3.2.1.2.2 Bioslurping**

This method combines soil vapour extraction, bioventing, and vacuum-enhanced pumping to remediate soil and groundwater by indirectly supplying oxygen and encouraging pollutant biodegradation (Gidarakos and Aivalioti 2007). The method is made to recover free products such light non-aqueous phase liquids (LNAPLs), which can be used to remediate saturated, unsaturated, and capillary zones. Additionally, it can be used to clean up soils that have been contaminated with organic volatile and semi-volatile substances. A "slurp" that extends into the free product layer is used in the system to suck up liquids (free products and soil gas) from this layer in a way akin to how a straw draws liquid from any vessel.LNAPLs are propelled upward to the surface by the pumping mechanism, where they are cut off from water and air. After all free products have been removed, the system can easily be configured to function as a standard bioventing system to finish the remediation process (Kim et al. 2014). In this method, too much soil moisture restricts air permeability and slows the pace at which oxygen is transferred, which in turn lowers microbial activity. The technique saves money because it produces less groundwater as a result of the operation, which reduces the expenses associated with storage, treatment, and disposal even though it is not ideal for remediating soil with low permeability (Philp and Atlas 2005).One of the main issues with this particular in situ technique is creating a vacuum on a deep high permeable site. Another is the fluctuating water table, which could lead to saturated soil lenses that are challenging to aerate.

**3.2.1.2.3 Biosparging**

Biosparging is quite similar to bioventing in that it involves injecting air into the subsurface of the soil to encourage microbial activity to aid in pollution removal from polluted regions. Contrary to bioventing, air is pumped at the saturated zone, which might transfer volatile organic molecules upward to the unsaturated zone to encourage biodegradation. The soil permeability, which affects the pollutant's bioavailability to microorganisms, and the pollutant's biodegradability are the two main parameters that influence how effective biosparging is (Philp and Atlas 2005).Similar to soil vapour extraction (SVE) and bioventing, in situ air sparging (IAS) is a closely related approach that uses high airflow rates to produce pollutant volatilization while biosparging encourages biodegradation. Similar to this, neither of the pollutant removal mechanisms for each technique is mutually exclusive. Diesel and kerosene contamination of aquifers has been successfully treated by biosparging in many cases.According to Kao et al. (2008), biosparging of a benzene, toluene, ethylbenzene, and xylene (BTEX)-contaminated aquifer plume led to a change from anaerobic to aerobic conditions, which was shown by an increase in dissolved oxygen, redox potentials, nitrate, sulphate, and total culturable heterotrophs and a decrease in dissolved ferrous iron, sulphide, methane and total anaerobes and methanogens.

**3.2.1.2.4 Phytoremediation**

In order to reduce the hazardous effects of pollutants, this strategy uses plant interactions (physical, biochemical, biological, chemical, and microbiological) in polluted areas. There are a number of mechanisms (accumulation or extraction, degradation, filtration, stabilisation, and volatilization) involved in phytoremediation, depending on the pollutant type (elemental or organic). Extraction, transformation, and sequestering are the main methods used to eliminate elemental contaminants (toxic heavy metals and radionuclides). In contrast, organic pollutants (such as hydrocarbons and chlorinated chemicals) are primarily eliminated through degradation, rhizoremediation, stability, and volatilization with the possibility of mineralization when certain plants, such willow and alfalfa, are utilised (Meagher 2000; Kuiper et al. 2004). Some important factors to consider while choosing a plant as a phytoremediator are plant’s root system, which may be fibrous or tap-like depending on the depth of the pollutant, above-ground biomass, which shouldn't be suitable for animal consumption, the toxicity of the pollutant to plants, the plant's ability to survive and adapt to the environment, the plant's growth rate, site monitoring, and most importantly the amount of time needed to reach the desired level of cleanliness are some of the key considerations. More over, the plant should also be immune to pests and illnesses (Lee 2013). According to Miguel et al. (2013), in some contaminated situations, contaminant removal by plants includes three steps: uptake, which is primarily a passive process; translocation from roots to shoots; and accumulation in shoot. Additionally, transpiration and the division of xylem sap between adjacent tissues are required for translocation and accumulation, respectively. Nevertheless, the procedure may vary depending on other elements including the type of contamination and the plant. The majority of plants living on any polluted site are likely effective phytoremediators. In order to maximise the remediation capacity of native plants growing in contaminated areas, either through bioaugmentation with endogenous or exogenous plant rhizobacteria or through biostimulation, is essential for the success of any phytoremediation strategy. In light of the fact that plant growth-promoting rhizobacteria (PGPR) tend to increase biomass output and plant tolerance to heavy metals and other unfavourable soil (edaphic) conditions, it has been suggested that PGPR utilisation could be crucial in phytoremediation (Yancheshmeh et al. 2011; de-Bashan et al. 2012). The fact that some precious metals can bioaccumulate in specific plants and be retrieved after remediation, a process known as phytomining, is one of the main benefits of employing plants to clean up polluted sites. The following are some benefits of phytoremediation:

* Cheap cost,
* Environmental friendliness,
* Widespread use,
* Affordable installation and maintenance,
* Preservation of soil structure,
* Reduction of erosion, and
* Prevention of metal leaching (Van Aken 2009; Ali et al. 2013).
* Better soil fertility may result from phytoremediation due to the addition of organic materials (Mench et al. 2009).

Limitations of phytoremediation are:

* Longer remediation times,
* Pollutant concentrations, toxicity, and
* Bioavailability to plants,
* Depth of plant roots, and
* Sluggish plant growth rates (Kuiper et al. 2004; Vangronsveld et al. 2009; Ali et al. 2013).
* In some instances, collecting plants for biomass management after remediation may result in extra costs (Wang et al. 2012a, b).
* It is also possible that accumulated hazardous pollutants will go up the food chain because plants lack the catabolic enzymes necessary to completely mineralize organic contaminants to carbon dioxide and water because they are autotrophic (unable to utilise organic substances as sources of carbon and energy), (Lee 2013).

There are several different types of phytoremediation mechanisms.

1. **Rhizosphere biodegradation.** In this process, the plant releases organic compounds through its roots, feeding soil microbes with nutrition. These organisms in turn accelerate the biological decay.
2. **Phyto-stabilization:** Instead of degrading impurities throughout in this process, the plant's chemical byproducts immobilise the toxic compounds.
3. **Phyto-accumulation** (also called phytoextraction): The pollutants are absorbed by plant roots during this process, along with other nutrients and water. The contaminated mass is not removed; instead, it becomes part of the plant's leaves and branches. This technique is mostly applied to metal-containing trash.
4. **Hydroponic Systems for Treating Water Streams (Rhizofiltration)**: In contrast to phytoaccumulation, rhizofiltration uses plants that are grown in greenhouses with their roots submerged in water. Ex-situ groundwater remediation can be done using this growth technique. In order to irrigate these plants, groundwater is pumped to the surface. An artificial soil medium, like as sand blended with perlite or vermiculite, is typically used in hydroponic systems. The roots are removed and discarded as soon as they are completely saturated with pollutants.
5. **Phyto-volatilization:** In this procedure, plants absorb contaminated water that contains organic substances and then expel those substances into the atmosphere through their leaves.
6. **Phyto-degradation:**In this process, plants actually metabolize and destroy contaminants within plant tissues.
7. **Hydraulic Control:** By restricting the circulation of groundwater during this process, trees indirectly remediate. When a tree's roots descend to the water table and form a massive root mass that absorbs a lot of water, they operate as natural pumps. Example a cottonwood tree may absorb up to 350 gallons of water per day, whereas a poplar tree can extract 30 gallons of water from the ground each day.

**3.2.2 Permeable reactive barrier (PRB)**

This technique is mostly perceived as a physical method for remediating contaminated groundwater, due to its design and mechanism of pollutant removal. In general, PRB is an in-situ method for cleaning up groundwater that has been contaminated with a variety of contaminants, such as heavy metals and chlorinated substances, such as natural pyrite (FeS2), zero-valent iron (ZVI) powder, sodium citrate, etc. In this method, the path of contaminated groundwater is covered with a permanent or semi-permanent reactive barrier (medium), which is primarily composed of zero-valent iron (Garcia et al. 2014; Zhou et al. 2014). Pollutants become trapped and undergo a series of reactions as contaminated water passes through the barrier under its natural gradient, producing clean water in the flow through (Thiruvenkatachari et al. 2008; Obiri-Nyarko). Ideally, the barriers are usually reactive enough to trap pollutants, permeable to allow the flow of water but not pollutants, passive with little energy input, inexpensive, readily available and accessible (De Pourcq et al. 2015). The type of media utilised, which is impacted by the type of pollutant, biogeochemical and hydrogeological conditions, environmental and health effects, mechanical stability, and cost, is largely responsible for this technique's efficacy (Obiri-Nyarko et al. 2014; Liu et al. 2015). Researchers have recently concentrated on combining PRB and other techniques, such electrokinetics, for treating various classes of pollutants (Garcia et al. 2014; Mena et al. 2015; Ramirez et al. 2015). Although sustaining barrier reactivity is important for PRB technique performance, retaining barrier permeability is equally important for PRB success and may be accomplished by preserving the proper particle size distribution (Mumford et al. 2014). One of the main operational issues with the PRB technique is the decrease in long-term performance caused by the barrier's decreased reactivity, zero-valent iron (ZVI), loss of porosity, and inability to apply the technique to sites contaminated with some chlorinated hydrocarbons and recalcitrant compounds.

**3.2.3 Microorganisms used in bioremediation**

In nutritional chains, which are a crucial component of the biological balance in life, microorganisms play a significant role. With the aid of bacteria, fungi, algae, and yeast, polluted materials are removed during bioremediation. Under the presence of hazardous substances or any waste stream, microbes are capable of growing in temperatures below zero as well as in severe heat. The biological system and adaptability of microorganisms make them suited for the cleanup process. The fundamental ingredient needed for microbial action is carbon. Microbial consortiums worked in many situations to do bioremediation. *Achromobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Flavobacterium*, *Mycobacterium*, *Nitrosomonas*, *Xanthobacter*, etc. are some of these microbes. The following categories of microorganisms are employed in bioremediation: *Acinetobacter*, *Sphingomonas*, *Nocardia*, *Flavobacterium*, *Rhodococcus*, and *Mycobacterium* are few examples of complex chemicals that can be broken down by aerobic bacteria. According to reports, these bacteria can break down polyaromatic chemicals, hydrocarbons, alkanes, and pesticides. A large number of these bacteria utilise the pollutants as a source of carbon and energy. Anaerobic: Compared to aerobic bacteria, anaerobic bacteria are not used as frequently. Aerobic bacteria are utilised in bioremediation to break down and transform contaminants into less harmful forms, including polychlorinated biphenyls, chlorinated aromatic compounds, and the solvents trichloroethylene and chloroform.

**3.2.3.1 Factors affecting microbial bioremediation**

The bioremediation technique involves using bacteria, fungi, algae, and plants to break down, remove, change, immobilise, or detoxify various chemicals and physical pollutants from the environment. Microorganisms' enzymatic metabolic pathways speed up the biochemical processes that aid in pollution breakdown. Only when microorganisms come into touch with substances that aid in their ability to produce energy and nutrients for cell division do they begin to react to contaminants. The chemical composition and quantity of contaminants, the physicochemical properties of the environment, and their accessibility to already-existing microorganisms are only a few of the variables that affect how effective bioremediation is. The key contributing components include the microbial population's capacity to degrade pollutants, the ease with which contaminants may be accessed by the microbial population, and environmental conditions such soil type, pH, temperature, and oxygen level and nutrients.

**3.2.3.1.1 Biotic or biological factors**

**Biotic factors are beneficial for the breakdown of organic compounds by microorganisms with limited carbon sources, antagonistic interactions between microorganisms, or the protozoa and bacteriophages. The concentration of the contaminant and the amount of catalyst present in the biochemical process are typically correlated with the rate of contaminant breakdown. Enzyme activity, interactions (competition, succession, and predation), mutation, horizontal gene transfer, its growth for biomass production, population size, and composition are among the main biological factors ( Madhavi et al. 2012; Boopathy et al. 2000).**

**3.2.3.1.2 Abiotic or environmental factors**

Metabolic activity and physicochemical characteristics of the microorganisms targeted during the process in relation to environmental pollutants. The environmental conditions affect how successfully bacteria and pollutants interact. Temperature, pH, moisture, soil structure, water solubility, nutrients, site conditions, oxygen content and redox potential, resource depletion and physico-chemical bioavailability of pollutants, concentration, chemical structure, type, solubility, and toxicity are all factors that affect microbial growth and activity. These variables regulate the kinetics of deterioration.

In most aquatic and terrestrial environments, the pH range of (6.5-8.5) is generally ideal for contaminant biodegradation. Moisture influences contaminant metabolism because it is influenced by the kind and availability of soluble components also the pH and osmotic pressure of terrestrial and aquatic systems (Cases et al. 2005).

**4. Prospects of bioremediation**

There are several different bioremediation strategies, and many have proven successful in repairing polluted environments. The variety, abundance, and community structure of microorganisms in polluted settings provide insight into the likelihood that any bioremediation strategy will provide other environmental elements that can restrict microbial activity. Microorganisms play a crucial role in bioremediation. Advanced molecular approaches, such as "Omics," which comprises genomics, proteomics, metabolomics, and transcriptomics, have aided in the identification of microorganisms as well as their functions and metabolic and catabolic processes. The availability of nutrients, a lack of or low population of microorganisms with the ability to degrade materials, and the bioavailability of pollutants can all cause a delay in the completion of bioremediation.

Biostimulation and bioaugmentation techniques speed up microbial activities in polluted sites since bioremediation rely on microbial processes. A contaminated sample is biostimulated by adding nutrients to boost microbial activity. It is notable that pollutant-degrading microbes are naturally present in polluted contaminated sites; their growth and metabolic activities may depend on the type and concentration of pollutants; later, we can use agro-industrial wastes, which contain nitrogen, phosphorus, and potassium as a nutrient source for most polluted sites. Pollutants are reportedly degraded more effectively by microbial consortiums than by isolated isolates (Silva-Castro et al. 2012). When these isolates are combined, this activity could result in the complete and quick degradation of pollutants due to the metabolic diversities of individual isolates, which potency is created by their isolation source, adaptation process, pollutant composition, and synergistic effects (Bhattacharya et al.2015). Additionally, compared to a non-adjusted setup (control), both bioaugmentation and biostimula-tion were successful in eliminating pollutants such polyaromatic hydrocarbons (PAHs) from a substantially polluted sample (Sun et al. 2012). Bioaugmentation has been proven to be an efficient strategy, but it has also been demonstrated to speed up the breakdown of several chemicals. Specific microorganisms can be added as "introduced organ-isms" to improve the current populations if proper biodegrading microorganisms are not present in the soil or if microbial populations decreased due to contaminant toxicity. However, this method is very uncertain because there is a chance that the inoculated microorganisms may not survive in the new environment. The practise is referred to as bioaugmentation. Using the bioremediation process, sewage or contaminated water or soil is treated using naturally occurring or genetically modified microorganisms with specific metabolic profiles. Some of the issues with bioaugmentation can be resolved by using carrier materials including alginate, agar, agarose, gelatin, gellan gum, and polyurethane (Tyagi et al., 2011).

Chemically speaking, biosurfactants are comparable substances with green and biodegradable characteristics. However, using bio-surfactants to a polluted site would be economically unviable due to their high building costs and limited scalability. Combining agricultural and industrial wastes provides nutrients for the growth of biosurfactant producers throughout the fermentation process. Utilizing a variety of bioremediation strategies will help improve remediation effectiveness (Cassidy et al., 2015). It is a good idea to use genetically engineered microorganisms (GEM) strategically to improve bioremediation capacity. This is because it is possible to create a designer biocatalyst that can break down pollutants including resistant substances by merging new, effective metabolic pathways, expanding the range of substrates for existing pathways, and enhancing the stability of catabolic activity (Paul et al.2005). Parallel gene transfer and GEM expansion in an environmental application, however, are encouraging strategies. Systems for containing bacteria that allow any GEM to escape and recreate a dirty environment. Additionally, a biological strategy for genetically engineering microorganisms with a particular pollutant component could improve the effectiveness of bioremediation. Because of their increased surface area and lower activation energy, nanomaterials decrease the toxicity of pollutants to microorganisms, which speeds up and lowers the cost of bioremediation (Rizwan et al.2014).

**5. Bioremediation applications**

To be effective, bioremediation must be applied to all environmental states of matter, including solids (soils, silt, and sludge), liquids (ground water, surface water, and industrial waste water), gases (industrial air emissions), and subsurface environments (saturated and vadose zones).

The three main methods of bioremediation are: intrinsic (natural) bioremediation, biosimulation (environmental changes made through fertiliser application and aeration), and bioaugmentation (addition of microbes).

The natural soil microflora typically makes up the biological community used in bioremediation. However, it is also possible to manage higher plants to improve toxicant elimination (phytoremediation), particularly for cleaning up soils that have been poisoned with metal.

**6. Limitations of bioremediation**

The use of bioremediation is restricted to biodegradable substances. This process is prone to quick and total deterioration. Products of biodegradation may be more harmful or persistent in the environment than the parent chemical.

1. Specificity: The biological processes that take place are quite particular. The availability of metabolically competent microbial populations, optimal environmental growth conditions, and appropriate quantities of nutrients and pollutants are crucial site elements that are essential for success.

2. The bioremediation technique cannot easily be scaled up from batch and pilot scale investigations to large-scale field operations.

3. Development of new engineering bioremediation solutions that are appropriate for sites with composite combinations of pollutants that are not evenly dispersed in the environment is still needed. It might exist in the form of solids, liquids, or gases.

4. The bioremediation procedure is time-consuming compared to alternative treatment options including excavation and soil removal from contaminated sites.

5. Lack of regulatory consensus: Since there is no universally agreed-upon definition of clean, we cannot state with certainty that remediation is 100% accomplished. There is no acceptable endpoint for bioremediation treatments as a result, making it impossible to evaluate their effectiveness.

**Conclusion**

When it comes to remediating, cleaning, maintaining, and recovering methods for resolving a polluted environment through microbial activity, biodegradation is a very profitable and alluring alternative. The rivalry between biological agents like fungi, bacteria, and algae as well as unfavourable external abiotic factors (aeration, moisture, pH, and temperature) and limited bioavailability dictate how quickly undesired waste materials degrade. The effectiveness of bioremediation depends on a number of variables, including but not limited to cost, site features, and the kind and quantity of pollutants. Site description is the first step in a successful bioremediation since it aids in the creation of the most effective and promising bioremediation technique (ex-situ or in-situ). Due to the excavation and removal from the archaeological site, ex-situ bioremediation methods are typically more expensive. They can, however, be utilised to cure a variety of contaminants. Contrarily, in-situ techniques do not incur additional costs for excavation; yet, some inefficient in-situ bioremediation approaches can be reduced by the on-site installation cost of equipment, attached with successfully, and controlling the subsurface of a polluted site. When choosing the most effective bioremediation method to successfully treat polluted sites, geological properties of the polluted site, including soil, pollutant kind and depth, human habitation site, and performance of each bioremediation approach, should be taken into consideration.

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