Review Article

Bioremediation: Sustainable Approach for Pollution Control

Abstract

Bioremediation is one of the most imminent technological approaches to meet hazardous waste problems, which transforms toxic chemicals into less toxic or nontoxic substances using bacteria or fungi. Instead of direct chemical or physical treatment, such biological transformation is more striking where microorganisms are used as efficient, cost-effective and non-disruptive tools for eliminating hazardous materials. Major destructive impacts of pollutants are perinatal, respiratory, cardiovascular and mental disorders, mortality, allergy, cancer etc. Remarkable results leading to destruction of contaminants can be obtained using genetically concocted microbes that produce many modified enzymes through ecological new technology. Microorganisms utilize metabolic degradation pathways in situ to degrade the foreign undesirable matter directly instead of simply relocating them to another medium to minimize interference of the restoration place. It is certain that for remediation of polluted sites, bioremediation will play a key role in paving the way to greener pastures. The aim of this review article is to provide appropriate information regarding research findings on bioremediation, its types and techniques of eliminating pollutants by microbial consortia. This will facilitate to understand the strategic role of bioremediation at different levels and to apply latest research and knowledge to remove toxicity and to develop strategies to manage the waste generatedin industries, which is a global alarm. Until now, not many microbial enzymes have been exploited and huge microbial diversity is yet to be explored.

***Key words-* Bioremediation, Persistent chemicals, Environment toxicity, hazardous pollutants, microorganisms,**

1. Introduction

Many chemicals produced by contemporary technologies offer a severe threat to the ecological balance and degrading processes. Several of these pollutants have intricate structures, making them difficult to breakdown them. These contaminants are building up in the environment at a startling rate. The use of biotechnology in environmental control of such dangerous chemicals through bioremediation has been beneficial. This method is additionally known as bio-restoration or bio-treatment. Using naturally occurring microbes to hasten the breakdown of biological components and the decomposition of different chemicals is known as bioremediation. This method significantly accelerates the cleanup procedure. The fundamental idea behind bioremediation is that organic pollutants are broken down into innocuous substances like carbon dioxide, water, salts and other simple organic molecules.

Bioremediates have been divided into five categories-

(i) Halogenated aromatic hydrocarbons

(ii) Polyaromatic hydrocarbons

(iii) Pesticides

(iv) Organic solvents

(v) Munitions wastes

Polyaromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, xylene

Dichlorodiphenyltrichloroethane, toluene, trinitrotoluene as well as ethylbenzene are persistent impurities in the atmosphere known to be lethal and have been categorized as the pollutants of main concern by EPA. Decontamination is necessary to avoid healthcare associated illnesses and to reduce the number of people who get other people's infections (HAI). Decontamination techniques can range from straightforward cleaning with soap and water to autoclave or ethylene oxide treatment. Decontamination techniques include sterilization, disinfection and antisepsis. Since the major goal of decontamination is to remove the toxic substances in order to stop anyone from being badly impacted by it, it is crucial that it should be done in an appropriate manner.

For instance, if the dangerous substance is a virus or hazardous chemical, anyone who comes into contact with it could experience severe health issues. During the coronavirus epidemic, it was important to stress the need of stopping the spread of pathogens. Using conventional waste disposal techniques like land filling and cremation, the cost to disinfect toxic waste sites in the USA is approximately one trillion USD [1].

2. Environmental Biotechnology

Biotechnology can be used to evaluate the ecosystems' sustainability. It can develop ecologically safe production and disposal processes, produce recyclable materials from renewable sources and transform pollutants into harmless compounds, all of which contribute to sustainable industrial development. To reduce costs and the negative environmental consequences that enterprises that produce textiles, paper, pulp, and chemicals have on the environment, many tools are being made available to firms. Also, the environmental performance has improved in comparison to what are frequently accomplished using conventional chemical methods. Biotechnology has a lot of potential in terms of reducing reliance on economic growth, environmental destruction, and human well-being. It exploits duly competent fauna and implements genetic engineering to ameliorate the efficacy and expenditure, which are crucial elements in the subsequent extensive utilization of microbes to reduce the burden of virulent substances on the environment.

Taking into consideration the intense need of a methodical environmental biotechnological process, an approach, termed as bioremediation, has been devised by the researchers, which is an emerging tool for the rehabilitation of sectors adulterated by contaminants or otherwise vandalized by way of biosphere abuse. Bioremediation is looked upon as an effective and cost-effective process for the purification of the environment.

3. Bioremediation

The term "bioremediation" consists of two parts: "bios" denotes survival and "to remediate" denotes the removal of environmental problems such contamination in soil or sediment. The process of bioremediation uses bacteria, fungus and other microorganisms to break down pollutants in the environment and return the area to its original natural state [2]. It is a method for sanitizing contaminated aquatic, terrestrial or both types of habitats. Using a wide range of metabolic processes that microbes are capable of, pollutants can be transformed into safe byproducts by mineralization, transformed into microbial biomass or there is production of carbon (IV) oxide and water [3,4].

Bioremediation and biodegradation should not be considered as same but are entirely two different processes. Biodegradation is an exclusive method applied in the course of bioremediation as only few microorganisms are able to degrade a part of biodegradable contaminants. Consequently, to evaluate the biodegrading capacity of microorganisms would be of much significance. Bioremediation is as an eco-friendly science used for at least a century for cleansing the environment. It's typical use is the regular bacteriological decontamination of municipal waste water, where various methods including activated sludge and fixed films are used in waste water treatment facilities in accordance with the metabolic activities of bacteria [5].

Bioremediation, according to the USEPA, is a spontaneous process that uses microbiological activities to reduce toxins to non-toxic levels in order to remediate or completely remove environmental contamination [6,7]. Solvents, explosives and polychlorinated biphenyls (PCBs) connected to polycyclic aromatic hydrocarbons have all witnessed an increase in the previous 15 years, where bioremediation technology is used [8].

The effectiveness of bioremediation approaches depends on having the right microorganisms such as physiologically and metabolically active bacteria and fungus, at the right location under ecologically sound conditions [9]. Already, industrial areas are being cleaned up using microorganisms that have the natural ability to digest some pollutants [10]. Biotechnology has accelerated the generation of microorganisms through genetic engineering. Using organisms to remove contaminants is predicated on the idea that all living things can take things out of their surroundings to use for their growth and metabolism [11–16]. According to studies, *Saccharomyces cerevisiae* is an efficient and affordable biosorbent for eliminating heavy metals from food. It has effective sorption properties for a number of heavy metals. Reducing the amount of nutrients in the media has little impact on *S. cerevisiae's* growth [17,18].

There are largely two ways of bioremediation depending upon the site of elimination of refuse -

3.1 In Situ Bioremediation

It is used to degrade pollutants in saturated soils and ground water. Due to its cheap shipping costs and employment of harmless microbial organisms to decompose the pollutants, it is a popular method for cleaning places that have been contaminated. Microbial populations that already exist are stimulated or fed with nutrients and oxygen to increase their metabolic activity. The potential for simultaneous treatment of soil and groundwater is another benefit of in situ bioremediation. However, compared to other remedial techniques, in situ bioremediation takes more time. Microbial activity varies seasonally due to constant exposure to the complex and uncontrollable environmental conditions. Further, issues could arise from the usage of additives. The type of waste materials i.e. organic or inorganic determines the bioremediation yield, if the wastes give the necessary nutrients and energy, the microorganisms can bioremediate; if not, the loss of bioactivity may be compensated through stimulation of native microbes.

3.1.1 Two types of in situ bioremediation are distinguished based on the origin of the microorganisms applied as bioremediants:

I Intrinsic bioremediation: This method involves enriching naturally occurring populations as well as existing microfauna by enhancing their nutritional and aeration status, without the use of basic microbial alteration, in the contaminated areas.

(ii) (ii) Engineered bioremediation: This process involves introducing a small amount of micro fauna to a contaminated site. Since unfavorable conditions prevail for the growth and bioactivity of the extrinsically remediated microorganisms, hence at this juncture, the environment is altered in a way so as to provide better physico-chemical conditions. Nutrients like nitrogen, phosphorus, oxygen and electron acceptors are required to boost microbial growth.

Experiments using *Streptomyces tritici D5*, cyanide-resistant bacteria to treat the effluents from the sago industry have indicated that the best organic pollutant reduction occurred between 35 and 45 °C. Moreover, *S. tritici D5* dried biomass had 69.56% of the total protein in the form of single cell proteins (SCP) after 30 days of culture [19]. The degradation of high and medium molecular mass chemicals was discovered by studying the molecular size distribution of control and bacterially treated samples from pulp and paper mill effluents. The outcomes also proved that Bacillus cereus has a great deal of potential as a key candidate for color removal [20].

3.2 Ex Situ Bioremediation

Here, groundwater or contaminated soil must be delivered to the site and bioremediation must occur away from the source of pollution. Since it takes a lot of time and money, this method is less effective.

Ex situ bioremediation is separated into two phases depending on the type of contamination used-

(i) Solid phase system- Solid-phase soil bioremediation uses three different processes: land farming, soil biopiling, composting and biofilter. It is applied for the bioremediation of organic, difficult-to-recycle home and industrial wastes, municipal solid wastes and sewage sludge.

(ii) Slurry phase systems- Compared to other treatment methods, slurry phase bioremediation is a relatively short procedure. Here, polluted soil is mixed with water and other additives before being combined in a huge vessel called a bioreactor, where the mixture is then assorted in a solid-liquid suspension to bring the native microorganisms close to the soil contaminants. Nutrient levels, oxygen concentrations and other factors are changed to create the ideal conditions for microbial bioremediation in the bioreactor. After the procedure is finished, the water is drained away and the solid wastes are either thrown away or treated to remove any pollutants that may have been left out.

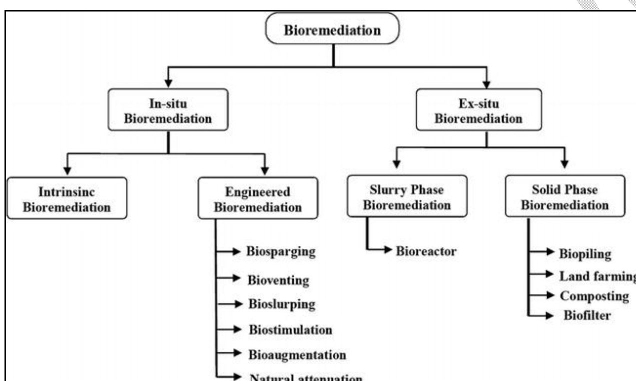

Figure-1 - Bioremediation types and techniques

4. Bioremediation Techniques

Numerous techniques/procedures are applied for bioremediation [21]. A few of them are mentioned as follows -

4.1 Biosparging

In this technique, the polluted sites are activated by injecting air into the soil subsurface. The microbial activities on certain sites are enhanced, which encourage the elimination of contaminants [22]. The two main variables that affect the efficacy of biosparging are soil permeability and pollutant biodegradability. Biosparging, in general, has been used in treating water bodies and aquifers polluted with kerosene and diesel.

4.2 Bioventing

By introducing oxygen into the infected medium, bioventing encourages microbial activity and growth. It is a typical in situ treatment technique in which contaminated soil is supplied with the nutrients and air through the wells to encourage the growth of local microorganisms. Low air flow rates and enough oxygen for biodegradation exists to reduce volatilization and pollutant escape into the atmosphere. This method works for simple hydrocarbons and in situations where the pollution is deep underground. The effective depth of oxygen diffusion for bioremediation in many soils ranges from a few centimetres to roughly 30 cm or even more [23]. Among other in-situ bioremediation approaches, this one has become more well-known [15].

Both biosparging and bioventing systems are integrated with different treatment strategies such permeable reactive barrier and soil vapour extraction technologies [24].

4.3 Bioslurping

To accomplish soil and ground water remediation, bioslurping involves a blend of bioventing, vacuum-enhanced pumping and soil vapor extraction [16]. This technique intends to recover products from light non-aqueous phase liquids, capillary, saturated, and unsaturated zones. Moreover, it is used to clean up soils that have been contaminated with organic volatile and semi-volatile substances. With this technique, liquids are held up by a "slurp" that spreads into the free product layer. Light non-aqueous phase liquids are brought to the surface by a pumping device and separated from air and water. Moisture in the soil limits air permeability and lowers the rate at which oxygen is transferred, which in turn decreases microbial activity. Although this method is ineffective for remediating less permeable soils, it is nevertheless regarded as a cost-effective operating procedure because of its low groundwater penetration, storage and treatment disposal expenses.

4.4 Biostimulation

By changing the environment and adding a range of rate-limiting nutrients and electron acceptors such as nitrogen, phosphorus, oxygen or molasses, it entails promoting the presence of bacteria that are capable of bioremediation. Biostimulants encourage the microorganisms already present to break down the poisonous and dangerous chemicals found in highly contaminated sites [25]. For the remediation of soil contaminated with a high concentration of atrazine, a combined bioaugmentation and biostimulation strategy has been proposed [26]. An insignificant improvement in the atrazine mineralization was seen after bioaugmentation using *Pseudomonas sp.* ADP alone. Contrarily, bioaugmentation improved the bacterial degrader's cell viability and as a result, herbicide mineralization coupled with citrate and succinate which were used as biostimulants. Biostimulation holds a promising future in constructing workable technology for the removal of herbicide pollutants from the environment, mostly thanks to advancements in biotechnology combined with the use of the omics data on various soil degrading bacteria.

4.5 Bioaugmentation

In insitu processes, the most frequently used method is introduction of bacteriological cultures into a contaminated medium. Limitations in the use of bacterial cultures in the treatment of a land unit are-

(a) Foreign cultures rarely compete appropriately with the inhabitants to strengthen desirable population levels.

(b) Properly managed land units develop indigenous microorganisms due to prolonged exposure to eco-friendly waste, that are effective degraders [23]. Studies have shown that using white-rot fungi to remediate old, industrially-polluted soils enriched with heavy hydrocarbons has a number of drawbacks that have been extensively described. Instead, using bioaugmentation of native fungal species may improve the bioremediation of aged hydrocarbon pollution in soils. Without raising the risk of ecotoxicology, cytochrome P450 and fungal laccases may be highly important in the microbial bioremediation of oil hydrocarbons. The oxidative enzymatic machinery necessary for the manufacture of melanin has also been linked to melanized fungus, such as the bioaugmented *Ulocladium sp.* [27].

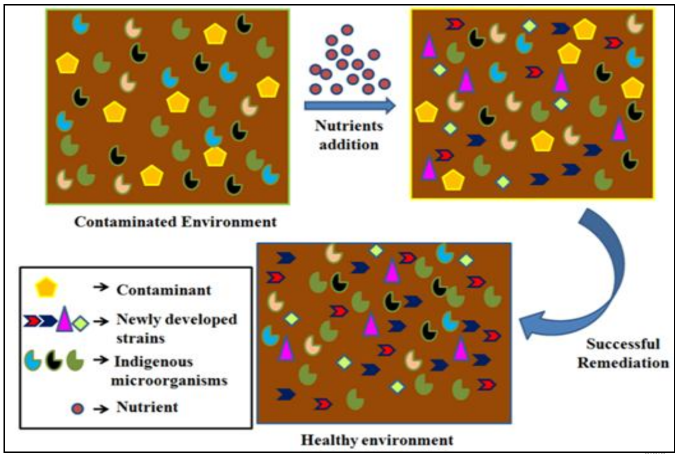

Figure-2 Bioaugmentation

4.6 Natural attenuation

In order to transform or immobilize contaminants into a less hazardous or bioavailable state, engineering biotechnology principles are applied to soil and groundwater systems in the form of natural bioreactors. In order to reduce or "attenuate" pollutant concentrations in soil and groundwater, natural attenuation relies on natural processes. To ensure that natural attenuation is occurring, scientists keep an eye on these parameters. Collecting samples of soil and groundwater for analysis to look for pollutants and other site features is a common part of monitoring. '*Monitored natural attenuation'* is the name given to the entire process. The majority of polluted sites experience natural attenuation; nonetheless, for a site to be adequately and swiftly cleaned, the necessary circumstances must exist underground. To ensure monitored natural attenuation remains functional, regular monitoring must be done.

How does it function?

When the environment is contaminated with dangerous chemicals, nature may act in five different methods to clean it up:

i) When microbes consume pollutants and break them down into minute quantities of water and gases by digestion, biodegradation takes place. In the soil and groundwater, microorganisms can be found. Some of these microbes use pollutants as food and energy.

ii) Contaminants adhere to soil particles as a result of sorption. Although sorption prevents toxins from migrating further underground or from leaving the site with groundwater flow, it does not actually eliminate them.

iii) As contaminants pass through and mix with pure groundwater, their amounts are diluted.

iv) Pollutants such as petrol and industrial solvents vaporize during evaporation, turning them from liquids to gases in the soil. These gases could be destroyed by sunlight if they are allowed to escape into the air near the ground's surface.

v) Contaminants underground may undergo chemical processes that change them into less hazardous forms.

For instance, the very toxic "Chromium 6" can be changed into the considerably less lethal and mobile form "Chromium 3" in low-oxygen settings underground when it combines with naturally existing iron and water. Where the source of contamination has been eliminated, MNA performs best. For instance, any waste that is buried underground must be dug up and properly disposed of, or removed utilising other cleanup techniques that are available. Natural mechanisms may be able to get rid of the last, smaller amount of toxins in the soil or groundwater, once the source is gone. In order to accomplish cleanup goals and ensure that toxins are not spreading, the site is routinely assessed for contaminants.

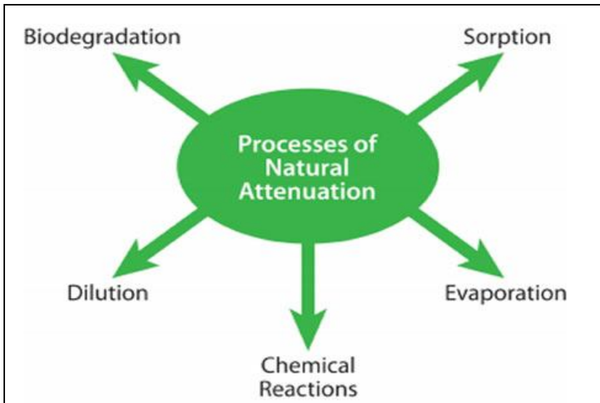

Figure-3 Natural attenuation

4.7 Bioreactors

It uses biological processes in a confined space to treat a comparatively small amount of trash. Slurries or liquids are treated in bioreactors. Water is pumped up from a polluted plume using aqueous reactors for ex situ remediation of polluted soils. The target contaminants can be broken down by slurry, but it is also used to produce a three-phase mixing environment that has the effect of accelerating the bioremediation of pollutants that are water-soluble and those linked to soil. In general, biodegradation is more efficient in a bioreactor system because the contained environment is simple to monitor and control.

But before it is put in a bioreactor, pretreatments like mining/stripping of the contaminated soil are required. This can be done by washing the soil or using a vacuum extractor [23]. To treat soils and other materials contaminated with petroleum wastes, bioreactors have been used [28, 29].

4.8 Biopiling

To improve bioremediation of the soil by microbial metabolic activities, it is piled above the ground, aerated, irrigated, supplied with nutrients etc. This technique, besides being cost effective, is progressively being considered due to effectively controlled operative biodegradation conditions like temperature, pH, nutrient concentration and aeration. Biopiling acts upon volatile low molecular weight pollutants and successfully remediates extremely cold polluted environments. Reduced remediation time is made possible by the biopile's adaptability [30–32]. Heating systems can be used into biopile designs to accelerate biodegradation by increasing contaminant availability and microbial activity [33]. Moreover, hot air can be introduced into the biopile design to deliver both air and heat together, improving bioremediation. Instead of biodegradation, air heating may cause soil undergoing bioremediation to become dry [34]. This will prevent microbial activity and increase volatilization.

4.9 Land farming

In a soil treatment cell, land farming can be used as an ex situ or an in situ procedure. Until the contaminants are broken down, the contaminated soil is dug up, spread out across a bed, and occasionally turned over. The goal is to activate locally present biologically active bacteria that can aerobically break down pollutants. Only the top 10 to 35 cm of the soil can be treated using this procedure. Land farming is an excellent waste disposal alternative due to its ability to reduce surveillance and repair costs, clean-up liabilities etc [23]. Bioremediation of overflowed oil and wood-preserving wastes has been carried out using this technique [35,36].

4.10 Composting

Composting involves adding nontoxic primal matter such as organic or agricultural wastes to the contaminated soil. A key characteristic of composting is the establishment of a large microbial population at a high temperature, which is supported by this stock [23]. Composting temperatures typically vary from 55 to 65 °C. The rise in temperature is due to heat generated during the compost degradation.

4.11 Windrow composting

In the initial stage, digging and screening of the contaminated soils is carried out to take away debris and large rocks [37, 38]. The soil is then transferred to a composting pad and kept in isolated and controlled weather conditions. Materials including manure, lucerne, wood chips, straw and agricultural wastes are placed into long piles known as windrows, which are then completely mixed with a windrow turning machine. These materials also serve as supplements for carbon sources and to give bulk to the soil. The windrows are demounted, and the compost is transferred to the final disposal area after continual monitoring of moisture, pH, temperature, and explosives after the completion of the composting time.

4.12 Biofilter

In a process known as biofiltration, organic pollutants in the vapour phase, such as fuel hydrocarbons, are forced through a soil bed where they bind to the soil's surface and decompose by soil microorganisms. To preferentially breakdown a particular substance, certain bacteria strains may be added to the filter [39]. Comparing the biofilter to traditional activated carbon adsorbers, there are various benefits. Since biofilters regenerate themselves, their adsorption capacity is always at its highest. Coconut fibre is a superior choice for biofiltration of inlet air with low relative humidity, and commercial activated carbon has been reported to be 60% more suited to pack a biofilter with intermittent loads than the rest of the packing media. Furthermore, investigations show that wetting these packing materials significantly reduced their ability to bind hydrophobic substances like toluene, which has significant ramifications for the development of buffering systems for load equalization [40].

In comparison to bacterial systems, it is thought that either a fungal biofiltration system or a synthetic microbial biofiltration system may help to achieve higher removal rates. In order to efficiently treat volatile organic compounds, researchers have recently been interested in the fungi *Paecilomyces variotii* and *Scedosporium apiospermum*. Recently, a fungal biofiltration system using these two fungi strains was reported to have a maximum elimination capacity of 245 g m-3 h-1 for the treatment of toluene. A new microbial strain that may be utilized exclusively for degrading a specific pollutant or a mixture of pollutants in a pure culture biofiltration system may be generated with the advancement of recombinant DNA technology. In the near future, this will unquestionably improve a biofilter's EC and removal efficiency.

4.13 Phytoremediation

As plants interact with pollutants in polluted soils in physical, chemical, biological, microbiological, and biochemical ways, the toxicity of the pollutants is reduced. This process is known as phytoremediation. Phytoremediation involves a number of mechanisms including uprooting, degradation, clarity, accumulation, fixation, and dehumidification, depending on the kind and quantity of the pollutant. A combination of extraction, transformation, and sequestration is used to remove radionucleides and heavy metals. By degrading, rhizoremediating, volatilizing and stabilising some organic and chlorinated substances as well as hydrocarbons, they are typically removed from the environment. For plants like willow and lucerne, mineralization is also possible [41, 42].

Fibrous or tap root system are the foremost characteristics of plants to act as phytoremediators based upon pollutant depth, terrestrial biomass, toxicity of pollutants to the plants, plant life and their capacity to acclimatize. Growth rate of plants, regular site inspection and time are the key factors responsible to attain the optimum level of purity. Besides these, plants must be disease and pest resistant [43]. By using a process called phytoremediation, pollutants are removed by being absorbed by plants and moving from the roots to the shoots, which in turn depends on transpiration and segregation [44]. Ionic chemicals can be absorbed by plants through their root systems at even low soil concentrations. By spreading their root systems into the soil matrix, plants produce the rhizosphere ecosystem, reclaiming the polluted soil and preserving its fertility. This process involves aggregating heavy metals and changing their bioavailability [45-47].

As a solar-powered, autotrophic system, phytoremediation is useful since it is practical from an economic standpoint. As a result, it requires little care and is easy to manage. It is safe for the environment, eco-friendly and has a wide range of applications. Also, because it stabilizes heavy metals, it reduces the danger of contamination by preventing erosion and metal leaching. Also, by allowing different organic materials to enter the soil, it can increase soil fertility. [46,48,49].

Any phytoremediation process' effectiveness mostly depends on bioaugmentation with either inland or foreign plants, which increases the ability of local plants growing in contaminated areas to remediate the environment. Studies found that even after some precious metals bioaccumulate in plants, they can still be extracted through the process of phytomining. According to research, phytoremediation of heavy metals has emerged as one of the most promising areas for future growth [50]. Current research on phytoremediation of settings contaminated with heavy metals has advanced as a result of the finding of hyper accumulator plants for internal growth and developmental mechanisms of plants [51, 52]. Researchers have examined the properties of heavy metal stress-resistant plant seed germination and seedling growth and demonstrated the role of metallothioneins and phytochelatins in the defense mechanisms against the stress generated by heavy metals. Moreover, *Phaseolus calaratus* L. had dramatically increased shoot and root length, fresh and dry weight, chlorophyll and carotenoid levels, and 2,2-diphenyl-1-picryl-hydrazyl-hydrate activity with the addition of citric acid and chromium [55].

4.14 Mycoremediation

Mycoremediation is using fungi to clean up the environment after contamination [56]. It has been demonstrated that the use of fungi to remove a variety of toxins from polluted settings or waste water is an economical, efficient and environmentally responsible method. Heavy metals, organic pollutants, textile dyes, chemicals and wastewater from leather tanning, petroleum fuels, polycyclic aromatic hydrocarbons, pharmaceuticals and personal care items, pesticides and herbicides in land, fresh water and marine environments are just a few of these contaminants [57].

The remediation process can be made even more profitable by the byproducts, which can be valuable resources in and of themselves, such as laccase enzymes and edible or therapeutic mushrooms [56,58]. In extremely cold or radioactive situations where conventional cleanup approaches prove too expensive or are not practical due to the harsh conditions, some fungi are helpful in the biodegradation of pollutants. Enzymes are also a biobased and environmentally acceptable method of bioremediation. For the isolation of active enzymes against certain contaminants, microorganisms exposed to contaminated locations and those pollutants are desirable sources. A significant metabolite of the insecticide chlorpyrifos is 3,5,6-trichloro-2-pyridinol (TCP), which is harmful to both human and animal health. This is demonstrated by the presence of TCP- and chlorpyrifos-degrading enzymes in the cow rumen micro biome [59]. With the encapsulation approach, which uses pellets of fungus spores covered with agarose, mycoremediation can also be utilized for fire management. In the burned forest, this pellet is added to a substrate, breaking down environmental pollutants and promoting development [60].

5. Biotechnology in Bioremediation

5.1 Sustainable Remediation of SOCs via Genetically Modified Biological Agents

Researchers are improving organic compounds created during paper production through the use of sophisticated techniques for identification and degradation. Given serious consideration is biotechnological intervention using synthetic and systems biology to create genetically modified organisms specifically for potential breakdown of synthetic organic chemicals (SOCs). These chemicals are degraded by enzymes secreted by microorganisms, primarily bacteria and fungi that take part in several metabolic pathways. For the breakdown and removal of newly emerging chemicals, standard physicochemical bioremediation techniques (in situ and ex situ) are inefficient [61,62]. Many genetically modified microbes have been created utilising a variety of methods for the remediation of synthetic organic chemicals as a result of advancements in the fields of genetic engineering and recombinant DNA technology [63]. *Escherichia coli* strains created specifically for phenol breakdown were found to be successful [64]. It was possible to develop *Pseudomonas putida* to degrade organophosphates, carbamates and pyrethroids [65].

Oleophilic microbes are employed for the breakdown and cleanup of petroleum-based contaminants from terrestrial and aquatic systems because they are low-cost, environmentally benign, and the best natural mechanism for preserving ecosystem and environmental sustainability. [66, 67]. The standard and widely used procedure for the aerobic degradation of petroleum hydrocarbons by microorganisms is shown in Fig. 4. In this method, certain anaerobic and aerobic enzymes, as well as emulsifiers and biosurfactants, are utilised under controlled circumstances to fix microbial cells to the substrates.

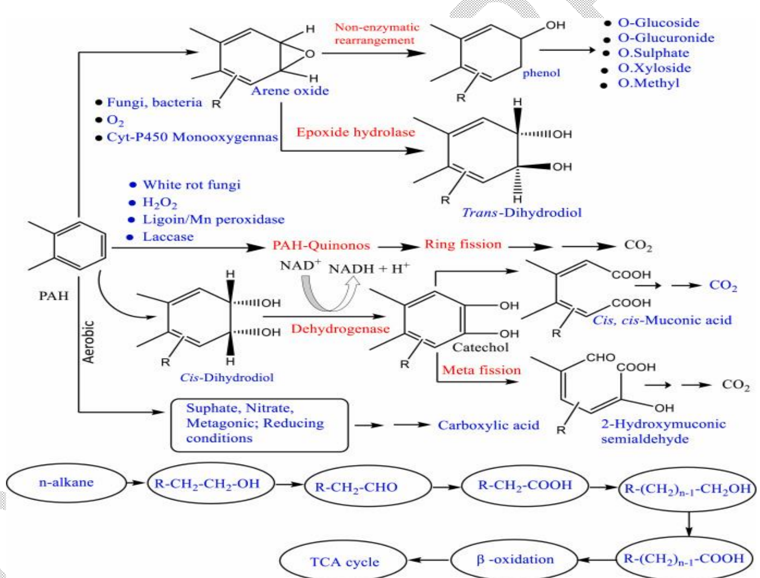

Figure-4. Graphical flowchart for the decomposition of PHs and alkanes via microbial processes (Koshlaf and Ball, 2017)

An oxygenase enzyme attack on the petroleum hydrocarbons is followed by ring cleavage during the aerobic transformation process. The cations of peroxides can also use fungi to oxidise polycyclic aromatic hydrocarbons into phenolic and quinone derivatives [68]. The enzymes utilise the molecular oxygen generated during the aerobic transformation process to oxidise polycyclic aromatic hydrocarbons in the substrate to make hydroxylation rings and other types of intermediate oxidised products [69]. A bio-transformation process involving anaerobic digestion and sulfate-reducing bacteria was used to degrade fluorine and phenanthrene. Molybdate synthesized as a result of the experiments, suggests that phenanthrene and fluorene undergo biotransformation through the processes of hydration and hydrolysis, followed by decarboxylation with the production of p-cresol and phenol [70]. In light of this, the production of these metabolites can be viewed as a novel biotransformation pathway for phenanthrene and fluorene. Owing to the complexity of the field scale, research studies suggest that most petroleum hydrocarbons' degrading mechanism modes are not fully understood and additional study and investigations are required in the future as illustrated in Fig. 5

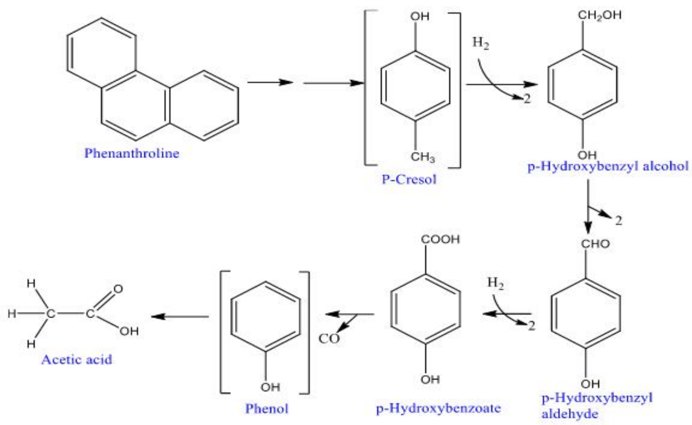

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Figure-5. Anaerobic and sulfate-reducing bacteria used in the biotransformation of phenanthrene (Tsai et al, 2009)

Table1 lists a few latent bacteria that can break down petroleum hydrocarbons.

Table 1. A list of some probable microorganisms that could break down petroleum hydrocarbons.

Microorganism	Compound	Amount removed	Reference
Aspergillus species	Crude Oil	Recovery of 59-87.7% of crude oil	[71]
Aspergillus ocharaceus CBAI 849	Benzo[a]pyrene	Bioremediation from salty settings, 76.6–99.7%	[72]
Penicillium sp. RMA1 and RMA2	Crude oil	Achieved degradation with RMA1- 57% and RMA2- 55%	[73]
Aspergillus species RFC-1	Different Petroleum Hydrocarbons	Removal of PHs from aquatic environments - 60.3% to 97.4%	[74]
Ochobactrum species	Crude oil	Crude oil degradation was 83.49%, or 3% volume-to-volume.	[75]
Candida tropicalis	Diesel	Diesel degradation- 83%	[76]
Pseudomonas species	Petrol, diesel and waste engine oil	Degradation of fuel (76%), diesel (83%) and used motor oil (69%).	[77]
Bacillus sorensis D11,*Pseudomonas stutzeri* D13 and*Bacillus cereus* D12	Petroleum-diesel TPH-DRO	Strains consortium removed upto 80% petroleum-diesel, extract at 31.1 mg g-1 TPH-DRO	[78]

5.2 Nanotechnology and Enzyme Technology

Enzymes and nanotechnology work very well together to make nanomaterials less harmful to the environment. In the presence of nanomaterials, hydrocarbons and enzyme molecules reduce the amount of cell contact by stearic hindrance and lower the surface energy [79]. Nanomaterials are more adaptive and effective in bioremediation and production of green energy because enzymes are environmentally friendly and have a unique catalytic function that is supplementary to that of nanoparticles. Because of their recalcitrant character and the durability of created nanoparticles, there is worry about their application for the large-scale cleanup of petroleum hydrocarbons from damaged soil [80]. Before they are applied widely, it is crucial to have a better understanding of the environmental fate [81].

Under ideal conditions of pH, temperature, concentration, contact duration etc., the pollutant substrate can identify various microbial enzymes for efficient transformation into harmless products. Using oxidation, reduction, elimination and ring-opening mechanisms, the best representative enzymes such as cytochrome P450, laccases, dehydrogenases, hydrolases, proteases, lipases and dehalogenases have the potential to degrade polymers, dyes, detergents, aromatic hydrocarbons, halogenated & agrochemical compounds, among other substances.

Table-2 Function of microbial enzymes used in bioremediation -

S.No.	Enzymes	Function	Reference
1	Cytochrome P450	Cells can synthesise and metabolise a variety of compounds and chemicals, able to oxidise substances like steroids, xenobiotics and fatty acids.	[82]
2	Laccases	Create free radicals by causing the ring cleavage of aromatic molecules and lowering oxygen levels in water.	[83]
3	Dehalogenases	Eliminate halogens by breaking the carbon-halogen link.	[84,85]
4	Dehydrogenases	Produce energy by oxidising organic molecules.	[86,87]
5	Hydrolases	Transform proteins and fats	[88]
6	Proteases	Used to clean wastewater and degrade proteins like keratin and casein, which results in the dehairing of leather.	[89]
7	Lipases	In order to produce fatty acids and glycerol, lipases hydrolyze mono, di, and triglycerides,also play a role in esterification and trans esterification reactions	[90]

6. Positive aspects of bioremediation

1. It is a simple, natural and less time consuming waste treatment process for contaminated soils. There is reduction in biodegradative populations after degradation of the contaminant. The treatment products like carbon dioxide, water and cell biomass are generally non toxic.
2. It can be easily carried out on the site without affecting normal microbial activities. Hence it involves less effort as well as eradicates the potential hazards to human wellbeing and the environment.
3. It is economical as compared to other traditional methods used to treat oil contaminated areas. It transforms toxic & hazardous compounds into less harmful products, ensuring their complete degradation and disposal.

7. Limitations and Concerns

Bioremediation is only possible for substances that degrade naturally. The presence of metabolically active microbial populations, adequate environmental growth circumstances, nutrient levels and contaminants are crucial components needed for its success. In some situations, the biodegradation's byproducts may be more hazardous or more persistent than the parent chemical. To enhance the development of the most advanced designed bioremediation systems suitable for the sites with complex pollutants, new investigations, research and discoveries are required. As there is no accepted definition of what constitutes "clean," it is ambiguous to declare that remediation has been completely accomplished. Since there is no acceptable limit for bioremediation treatments, performance evaluation of bioremediation is therefore difficult. Degradation may be slowed or stopped at low temperatures. To maintain high removal efficiency, moisture levels, pH, temperature and other filter parameters like fungal development should be watched carefully. Biofilters weren't made to handle chlorinated chemicals until recently. New experiments, nevertheless, seem to indicate that they can also be employed to eliminate these chemicals. Filters may become clogged as a result of the buildup of surplus microorganisms, necessitating routine mechanical cleaning. There is a chance that fugitive fungi will be released.

8. Conclusion and Perspectives

Recent improvements in biochar modification research (physical, chemical, and biological) create more opportunities to create tailored biochar with better surface properties than their native forms for better removal efficiencies of significant and potentially harmful organic pollutants like antibiotics and pesticides. By further enhancing the bioremediation level and removing contaminants from the environment, microbial interactions with biochar also improve the ecological and socio-economic conditions. Ecological remediation of possible organic contaminants using green, affordable and sustainable biochar offers reasonable treatment options as well as a secure environment [91]. By preventing the enzyme acetylcholine esterase (AChE), pesticides become hazardous to humans. For a sustainable environment and resilient agriculture, bacterial mediated degradation is an eco-friendly and sustainable method in pesticide-contaminated soils. We can recognise and distinguish the constituents and traits of core micro biomes at the contaminated location using high throughput next-generation sequencing and ‘in silico’ analysis [92].

It can be concluded that with the growing population on the planet, use of science and technology for bioremediation would eventually become a prerequisite of present day’s modern life. The variety of bioremediants, the extensiveness of methodologies accessible and the range of substrates utilized by bioremediants in both terrestrial and watery ecosystems, portend positive outcomes in the days to come. Fungi are a class of unicellular, filamentous micro fauna with a high level of biodiversity that have been isolated from many settings and are currently thought to be an efficient class of bioremediants. With specified recent developments in genetic and metabolic engineering, it seems that fungi will play a larger role in the bioremediation of toxins and wastes.

In order to quickly address the difficulties of the present and future world, it is projected that bioremediation will eventually expand to more precise scientific areas based on studies, investigations, and available data.

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