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. The significant degradation of pollutants can be upgraded utilizing genetically engineered microorganisms that produce many recombinant enzymes through eco-friendly new technology. Microorganisms utilize metabolic degradation pathways in situ to degrade the foreign undesirable matter directly rather than merely transferring them from one medium to another to reduce disturbance of the cleanup site. 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 and research findings on bioremediation, its types and techniques of eliminating pollutants by microbial consortia. This willfacilitate 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

Various chemicals generated by the modem technologies are posing a great threat to the natural degradation processes and the natural mechanisms of maintaining ecological balance.Many of these pollutants are complex in nature and are hence difficult to break down. Such pollutants are accumulating in the natural environment at an alarming rate. The application of biotechnology has helped in the environmental management of such hazardous contaminants by bioremediation. This process is also referred to as bio-restoration or bio-treatment. Bioremediation involves the use of naturally existing microorganisms to speed up the breaking down of biological substances and the degradation of various materials.This process adds substantial momentum to the process of cleaning up. The basic principle of bioremediation is the breaking down of organic contaminants into simple organic compounds like carbon dioxide, water, salts and other harmless products.

Bioremediates have been divided into five groups[1] -

(i) Halogenated aromatic hydrocarbons

(ii) Polyaromatic hydrocarbons

(iii) Pesticides

(iv) Organic solvents

(v) Munitions wastes

Polyaromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, DDT, toluene, ethylbenzene, xylene and trinitrotoluene are tenacious pollutants in the environment known to cause carcinogenic and or

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mutagenic influence and have been classified as the pollutants of main concern by EPA. In order to decontaminate toxic waste sites in the USA, the expenses are around one trillion USD using traditional waste disposal methods such as land filling and incineration [2].

2. Environmental Biotechnology

Biotechnology can be used to evaluate the well being of ecosystems, transform pollutants into harmless substances, generate biodegradable materials from renewable sources and develop environmentally safe manufacturing and disposal processesthus contributing towards sustainable industrial development. Wide range of tools is being provided for industry to improve cost and reduce the environmental impacts of textile, paper,pulp and chemical manufacturing industries.Also the environmental performance has improved in comparison to that which is normally achievedbyusing conventional chemical technologies. Biotechnology can become an important tool to reduce interdependence ofeconomic growth,degradation of the environment and the quality of life. It takes advantage of appropriately qualified living organisms and employs genetic engineering to improve the effectiveness and cost, which are key factors in the future widespread utilization of organisms to reduce the environmental load of toxic substances.

In view of the urgent need of an efficient environmental biotechnological process, a technique called bioremediation has been devised by the researchers, which is an emerging tool for the rehabilitation of areas fouled by pollutants or otherwise damaged through ecosystem mismanagement. Bioremediation is regarded to be an effective and in the mean time an economic method for the decontamination of environment.

3. Bioremediation

The term of bioremediation consists of two parts: “bios” means life and “to remediate” means to solve a problem i.e. to use biological organisms to solve environmental problems like contamination ingroundwater or soil. Bioremediation is the use of living microorganisms to degrade environmental pollutants or to inhibit pollution thus restoring the original natural surroundings [3].It is a means of cleaning up contaminated terrestrial, aqueous or both types of environments. By exploiting the diverse metabolic abilities of microorganisms,the contaminants can be convertedinto harmless products by mineralization, conversion into microbial biomass or generation of carbon (IV) oxide and water[4,5].

Bioremediation and biodegradation should not be considered as same but are entirely two different processes. Biodegradation is only one of the mechanisms applied in the process ofbioremediation since some of contaminants are biodegradable and only some of microorganisms can degrade a fraction of contaminants. Therefore, to study the biodegrading potential of microorganisms would be of much significance.For at least a century, the microbes have been used for the treatment and transformation of waste products, bioremediation is considered as an eco-friendly technology for the decontamination of polluted environment. A most widely usedapplication of this technology ismicrobiological decontamination of municipal waste water under controlled conditions so that depending upon the metabolic activities of microorganisms, different systems of activated sludge and fixed films are applied in waste water treatment facilities [6].

According to USEPA, bioremediation is a spontaneous practice in which microbiological processes are used to degrade contaminants to non toxic levels, thereby remediating or eliminating environmental contamination' [7, 8]. The last 15 years have seenan increase in the types of contaminants to which bioremediation is being applied including solvents, explosives, polycyclic aromatic hydrocarbon related polychlorinated biphenyls(PCBs)[9].

Bioremediation techniques depend on having the right micro-organisms in the right place with the right environmental condition for degradation to occur. The right micro organisms are those bacteria and fungi which possess the physiological and metabolic capability to degrade the contaminants[10].Already bacteria with natural abilities to digest certain chemicals are being used to cleanup industrial sites[11]. By means of genetic engineering, biotechnology has brought about rapid production of bacteria. The use of organisms

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for the removal of contamination is basedon the concept that all organisms could remove substances from the environment for their own growth and metabolism[12-17]. Studies indicated *Saccharomyces cerevisiae*has been found to be an effective andinexpensive biosorbent in removing heavy metals from foodstuffs. It has good sorption characteristics for several heavy metals.Growth of *S. cerevisiae* is not affected by decreasing the nutrients present in the media[18,19].
On the basis of place where wastes are removed, there are primarily two ways of bioremediation based upon the place of waste removal-

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3.1 In Situ Bioremediation

It is applied for the degradation of contaminants in saturated soils and ground water. It is a superior method to clean contaminated environments since it is cheaper and uses harmless microbial organisms to degrade the chemicals. It involves either stimulation of indigenous or naturally occurring microbial populations (by feeding them nutrients and oxygen to increase their metabolic activity) or introduction of certain engineered micro-organisms to the site of contamination.

In situ bioremediation is superior method for elimination of the pollutants from contaminated environments because it saves transportation costs and uses harmless microorganisms which have a positive chemotactic affinity toward contaminants. This characteristic increases the probability of bioremediation in close points where bioremediants have not been distributedas well asit causes least distraction of the contaminated area. Another advantage of in situ bioremediation is the probability of simultaneous treatment of soil and groundwater. However, in situ bioremediation is more time-consuming as compared to other remedial methods.A changed seasonal variation in the microbial activity has been observed because of the direct exposure to the varyingand uncontrollable environmental factors. The use of additives may cause additional problems. The yield of bioremediation is determined by the kind of waste materials, if wastes could provide the required nutrients and energy, only then microorganisms would be able to bioremediate otherwisethe loss of bioactivity may be compensated through stimulation of native microorganisms.

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

(i) Intrinsic bioremediation-This type of in situ bioremediation is carried out without direct microbial amendment and through intermediation in ecological conditions of the contaminated region and the fortification of the natural populations and the metabolic activities of indigenous or naturally existing microfauna by improving nutritional and ventilation conditions.

(ii) Engineered bioremediation-It is performed through the introduction of certain microorganisms to a contamination site. As the conditions of contamination sites are most often unfavorable for the establishment and bioactivity of the exogenously amended microorganisms, therefore like intrinsic bioremediation, the environment here is changed in a manner to providesuperiorphysico-chemical conditions. Nutrients like nitrogen, phosphorus, oxygen and electron acceptors are required to boost microbial growth.

Studies on cyanide resistant *Streptomyces tritici* D5 to treat the sago industry effluents have shown that the best organic pollutant reduction occurred at 35 °C and 45 °C. Also dried biomass of *S. tritici* D5 contained 69.56% of total protein as single cell protein (SCP) in 30 days of growth [20].The molecular size distribution studies of control and bacterial-treated samples from pulp and paper mill effluents revealed the degradation of high and medium molecular mass compounds. The results also established the high potential of *Bacillus cereus* as an important applicant for color removal[21].

3.2 Ex Situ Bioremediation

The process of bioremediation here takes place somewhere out from contamination site, and therefore requires transportation of contaminated soil or pumping of groundwater to the site of bioremediation. This technique has more disadvantages than advantages.

Depending on the state of the contaminant in the step of bioremediation, ex situ bioremediation is classified as:

(i) Solid phase system - Solid-phase soil bioremediation operates by three processes namely land-farming, soil biopiling, composting and biofilter. It is used for bioremediation of organic, challenging domestic and industrial wastes, municipal solid wastes and sewage sludge.

(ii) Slurry phase systems -Slurry phase bioremediation is a rather more rapid process as compared to other treatment processes. Here a large tank called bioreactor is used to mix contaminated soil with water and other additives which are then intermingled in solid-liquid suspension to bring the native microorganisms in close contact with soil contaminants. Nutrients, oxygen levels and other conditions in the bioreactor are so adjusted such that an optimal environment for microbial bioremediation is provided. After the process is completed, water is removedand the solid wastes are either disposed off or processed in order todecontaminate the left over pollutants.

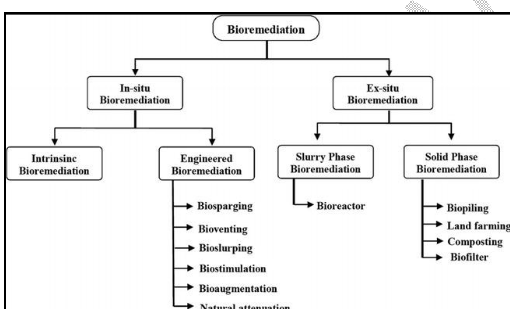

Figure-1 - Bioremediation types and techniques

4. Bioremediation Techniques

Numeroustechniques/procedures are applied for bioremediation [22].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. This improves microbial activities which stimulate theremoval of pollutants from those sites [23].Soil permeability and pollutant biodegradability are the two major factors on which the efficiency of biosparging depends.Biosparging, in general, has been used in treating water bodies and aquifers polluted with kerosene and diesel.

4.2 Bioventing

Bioventinginvolves drawing oxygen through the infected medium to stimulate the growth and activity of microbes.It is a common in situ treatment method in which air and nutrients are supplied to contaminated soil through wells to stimulate the indigenous bacteria.The air flow rate here is lowand the amount of oxygen sufficient for the biodegradation is providedso that volatilization and release of contaminants to the atmosphere are minimized. This technique works for simple hydrocarbons and can be usedwhere the contamination is deep under the surface. In many soils the effective oxygen diffusion, for desirable rates of bioremediation, extend to a range of only a few centimeters to about 30 cm into the soil, though depths of 60 cm andeven greater have been successfully treated in some cases [24]. This technique has acquiredrecognition among other in-situ bioremediation techniques [16].

Both biospargingand bioventing systems are combined with various treatment approaches such as permeable reactive barrier and soil vaporextraction technologies[25].

4.3 Bioslurping

To accomplish soil and ground water remediation, this technique combines vacuum-enhanced pumping, soil vapor extraction and bioventing for an indirect supply of oxygen and stimulation of contaminant biodegradation [17]. This technique is designed for products recovery from remediating capillary, light non-aqueous phase liquids (LNAPLs), unsaturated and saturated zones. It is also usedfor remediation of soils contaminated with volatile and semi-volatile organic compounds. This method uses a "slurp" that spreads into the free product layer which pulls up liquids from this layer. LNAPLs are transported by pumping machine to the surface by upward movement, where theygetseparated from air and water. Moisture in thesoillimits air permeability and declines oxygen transfer rate thus reducing microbial activities. Although this technique is not suitable for low permeable soil remediation, but due to less amount of ground water, minimum storage, treatment and disposal costs, it is considered a cost effective operation procedure.

4.4 Biostimulation

It involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of different types of rate limiting nutrients and electron acceptors such as phosphorus, nitrogen, oxygen or molasses.Biostimulants are addedto severely polluted sites to stimulate the existing bacteria to degrade the hazardous and toxic contaminants.

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4.5 Bioaugmentation

In insitu processes, the most frequently used method is the addition of microbial cultures to a contaminated medium. Two factors limit the use of added bacterial cultures in treatment of a land unit-

(a) Foreign cultures rarely compete appropriately with a native population to develop and strengthen beneficial population levels

(b) Most soils havingprolonged exposure to eco-friendly waste develop indigenous microorganisms that are effective degraders if the land treatment unit is properly managed [24]. Studies have demonstrated that bioaugmentation of autochthonous fungal species might enhance the bioremediation of aged hydrocarbon pollution and overcome the vastly described inconveniences of white-rot fungi's use for the remediation of aged-industrially-polluted soils enriched in heavy hydrocarbons. Cytochrome P450 and fungal laccases may play a very relevant role in the fungal bioremediation of oil hydrocarbons, without increasing the ecotoxicological risk. Melanized fungi, such as the bioaugmented *Ulocladium* sp., have also been associated to hydrocarbon biodegradation due to the oxidative enzymatic machinery required for the biosynthesis of melanin [26].

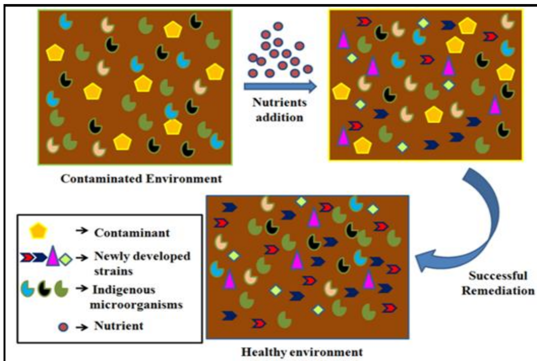

Figure-2 Bioaugmentation

4.6 Natural attenuation

It is the application of engineering biotechnology principles to soil and groundwater systems as natural bioreactors to transform or immobilize contamination to less toxic or less bioavailable form.Natural attenuation relies on natural processesto decrease or "attenuate" concentrations ofcontaminants in soil and groundwater. Scientistsmonitor these conditions to make sure naturalattenuation is working. Monitoring typically involves collecting soil and groundwater samples to analyzethem for the presence of contaminants and other sitecharacteristics. The entire process is called "monitorednatural attenuation" (MNA). Natural attenuation happens at most contaminated sites but the right conditions must exist underground to clean sitesproperly and quickly. Regular monitoring mustbe conducted to ensure that MNA continues to work.

How Does It Work?

Nature may work in five ways to clean the environment when it is contaminated with harmful chemicals-

i) Biodegradation occurs when very small organisms, known as "microbes," eat contaminantsand change them into small amounts of waterand gases during digestion. Microbes live insoil and groundwater and some microbes use contaminants for food and energy.

ii) Sorption causes contaminants to stick tosoil particles. Sorption does not destroy thecontaminants, but it keeps them from movingdeeper underground or from leaving the site withgroundwater flow.

iii) Dilution decreases the concentrations ofcontaminants as they move through and mix withclean groundwater.

iv) Evaporation causes some contaminants like gasoline and industrial solvents to change fromliquids to gases within the soil. If these gasesescape to the air at the ground surface, air willdilute them and sunlight may destroy them.

v) Chemical reactions with natural substancesunderground may convert contaminants intoless harmful forms.

For example, in low-oxygenenvironments underground, the highly toxic "Chromium 6" can be converted to a much lesstoxic and mobile form called "Chromium 3" whenit reacts with naturally occurring iron and water.MNA works best where the source of contamination has been removed. For instance, any waste buriedunderground must be dug up and disposed of properly or removed using other available cleanup methods. When the source is no longer present, natural processesmay be able to remove the remaining, smaller amountof contaminants in the soil or groundwater. The site ismonitored regularly to make sure that contaminantsattenuate fast enough to meet site cleanup objectivesand that contaminants are not spreading.

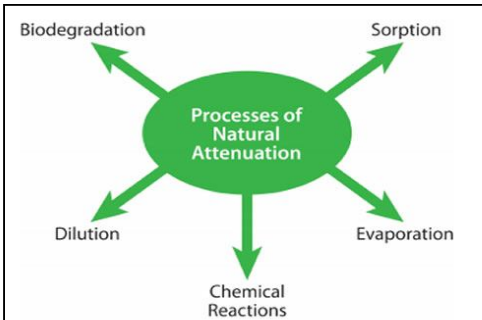

Figure-3 Natural attenuation

4.7 Bioreactors

It involves use of biological processes in a contained area for biological treatment of reasonably small quantities of waste. Bioreactor is used to treat slurries or liquids. Slurry or aqueous reactors are used for ex situ treatment of polluted soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the dispensation of any contaminated solid material like sludge, sediment, soil or water through an engineered containment system. A slurry bioreactor is an apparatus used to create a three-phase mixing condition to augment the bioremediation rate of soil-bound and water-soluble pollutants becausewater slurry of the contaminated soil and biomass (typically of indigenous microorganisms) is competent of degrading target contaminants. In general, in a bioreactor system the rate and extent of biodegradation is greater than in situ or in solid-phase systems as the contained environment is more manageable and therefore more controllable and predictable.

Regardless ofso many advantages of reactor systems, there are some disadvantages too. The contaminated soil requires pretreatment like mining/ stripping of the contaminant from the soil via soil washing or physical extraction (vacuum extraction) before being placed in a bioreactor [24].Soil and other materials contaminated with petroleum residues have been treated with the use of bioreactors [27, 28].

4.8 Biopiling

To improve bioremediation by microbial metabolic activities, dug polluted soil is piled above ground followed by aeration and nutrient amendment. This technique involves aeration, irrigation, nutrients, leachate collection and treatment bed systems. This technique is progressively being measured due to its valuable features besides being cost effective.Itpermits operative biodegradation conditions like temperature, pH, nutrient and aeration which are effectively controlled. Biopiling treats volatile low molecular weight pollutants andsuccessfully remediatesextremely cold polluted environments. This property of flexibility of biopile allows remediation time to be reduced[29-31].To increase contaminant availability and microbial activities, heating system can be integrated into biopile design thus increasing the biodegradation rate [32]. In addition, to enable better bioremediation, hot air can be injected into biopile design to deliver both air and heat in combination.Conversely,soil undergoing bioremediation may become dry due to heating of air which will inhibit microbial activities and promote volatilization rather than biodegradation [33].

4.9 Land farming

Land farming is a solid phase treatment system that can be applied as an ex situ or an in situ process in a soil treatment cell. It is a simple bioremediation technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The aim is to stimulate indigenous biodegradative microbes and enable aerobic degradation of contaminants. This practice is confined to the treatment of only superficial (10-35cm) layer of soil.Land farming is an excellent waste disposal alternative due to its ability to reduce monitoring and maintenance costs, clean-up liabilities etc.[24]. Spilled oil and wood-preserving wastes have been bioremediated by using this technique.[34, 35].

4.10 Composting

Composting is a technique that involves addingharmless organic substances such as manure or agricultural wastes to the contaminated soil. These organic materials support the growth of a rich microbial population at elevated temperature,a characteristic of composting [24].Typical compost temperatures range between 55-65°C. The rise in temperature is due to heat produced during the degradation of the organic material in the waste.

4.11Windrow composting

Initially the contaminated soils are excavated and screened to remove debris and large rocks [36, 37]. The soil is transported to a composting pad with a temporary arrangement to provide containment and shelter from extreme weather conditions. Various amendments like manure, alfalfa, straw, agricultural wastes and wood chips are used as bulking agents and as a carbon source supplement. Soil and amendments are layered into elongated piles known as windrows which are then mixed thoroughly by turning with a commercially available windrow turning machine. Moisture, pH, temperature and explosives concentration are monitored. After the completion of the composting period, the windrows are disassembled and the compost is sent to the final disposal area.

4.12 Biofilter

Biofiltration is a technology in which vapor-phase organic contaminantssuch as fuel hydrocarbons are passed through a soil bed where they sorb to the soil surfaces and are broken down by microorganisms in the soil. Specific strains of bacteria may be introduced into the filter to preferentially degrade specific compounds. The biofilter provides several advantages over conventional activated carbon adsorbers. Biofilters are self-regenerating, thus they maintain maximum adsorption capacity. The greatest advantage is that the contaminants are destroyed, not just separated.Biofiltration is used to treat vapor emissions from remediation systems including compost piles, to treat non-chlorinated VOCs and fuel hydrocarbons. Halogenated VOCs and semi-volatile organic compounds (SVOCs) also can be treated but the process may be less effective. Additionally, biofilters have been successfully used to control odors from compost piles. Its effectiveness is dependent upon the biodegradability of the contaminants.

Comment [a11]: Add references of biofilter technique

Besides these, some other techniques being used include Phytoremediation and mycoremediation.

5. Phytoremediation

Phytoremediation is the process of minimizing the toxicity of pollutants in the contaminated soils by physical, chemical, biological, microbiological and biochemical interactions of plants. Depending upon nature and amount of pollutant, several mechanisms such as extraction, degradation, filtration, accumulation, stabilization and volatilization are involved in phytoremediation. Removal of radionucleides and heavy metals is carried out by extraction, transformation and sequestration. Organic pollutants, hydrocarbons and chlorinated compounds are generally removed by degradation, rhizoremediation, stabilization and volatilization. For plants like willow and alfalfa, mineralization is alsofeasible[38, 39].

Mainattributes of plantsto act as phytoremediator include fibrous or tap root system depending on the depth of pollutant, above ground biomass, toxicity of pollutant to plant, plant existence and its capacity to adapt itself to leading environmental conditions.Growth rate of plants, regular site monitoring and time play a key role to attain the ideal level of cleanliness. Besides these factors, plants must be resistant to diseases and pests [40].Removal of pollutants by the process of phytoremediation includestheir uptake and translocation from roots to shoots. Their translocation and accumulation depends on transpiration and partitioning [41]. Plants have the abilities to absorb ionic compounds in the soil even at low concentrations through their root system. Plants extend their root system into the soil matrix and establish rhizosphere ecosystem to accumulate heavy metals and modulate their bioavailability, thereby reclaiming the polluted soil and stabilizing soil fertility [42-44].

Phytoremediationis advantageous since it is economically feasible, autotrophic system powered by solar energy.Therefore it is simple to manage and the cost of installation and maintenance is low.It is environment and eco-friendlyand can be applied over a large-scale field. It can also be easily disposed off, prevents erosion and metal leaching through stabilizing heavy metals thereby reducing the risk of spreading of contaminants.It can also improve soil fertility by releasing various organic matters into the soil [43,45,46].

The accomplishment of any phytoremediation method primarily depends on improving the remediation potentials of inhabitant plants growing in polluted sites by bioaugmentation with either endogenous or exogenous plants. Studies have shown that some precious metals can bioaccumulate in some plants and can be recovered after remediation, a process known as phytomining. Research evidence showed that phytoremediation of heavy metals has become one of the developmental hotspots [47]. Recent discovery of hyperaccumulator plants for internal growth and developmental mechanisms of plants indicates evolution of research on phytoremediation of heavy metal-polluted environments [48,49]. Researchers have studied the seed germination and seedling growth characteristics of plants that are resistant to heavy metal stress and showed involvement ofmetallothioneins and phytochelatins in the mechanisms of resisting the stress induced by heavy metals [50,51].Also, the addition of citric acid and chromium significantly enhanced shoot and root length, fresh and dry weight, chlorophyll and carotenoid levels and 2,2-diphenyl-1-picryl-hydrazyl-hydrate activity in *Phaseolus calaratus* L. [52].

6. Mycoremediation

Mycoremediation is a form of bioremediation in which fungi-based remediation methods are used to decontaminate the environment [53]. Fungi have been proven to be a cheap, effective and environmentally sound way for removing a wide array of contaminants from damaged environments or waste water. These contaminants include heavy metals, organic pollutants, textiledyes,leather tanning chemicals and wastewater, petroleum fuels, polycyclic aromatic hydrocarbons, pharmaceuticals and personal care products, pesticides and herbicidesin land, fresh water, and marine environments [54].

The byproducts of the remediation can be valuable materials themselves, such as laccase enzymes, edible or medicinal mushroomsmaking the remediation process even more profitable [53,55]. Some fungi are useful in the biodegradation of contaminants in extremely cold or radioactive environments where traditional remediation methods prove too costly or are unusable due to the extreme conditions. An ecofriendly and bio-based approach for bioremediation are enzymes as well. Microorganisms exposed to contaminated sites and specific pollutants are attractive sources for the isolation of active enzymes against those pollutants.3,5,6-trichloro-2-pyridinol(TCP) is a major metabolite of the insecticide chlorpyrifos andis dangerous to human and animal health.TCP-degrading enzymes and chlorpyrifos-degrading enzymes in the cow rumen microbiome is an instance for this statement [56].Mycoremediation can even be used for fire management with the encapsulation method. This process consists of using fungal spores coated with agarose in a pellet form. This pellet is introduced to a substrate in the burnt forest, breaking down the toxins in the environment and stimulating growth [57].

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7. Sustainable Remediation of SOCs via Genetically Modified Biological Agents

The detection and degradation of organic chemicals produced during paper production are enhanced by researchers using sophisticated techniques. Biotechnological intervention by means of synthetic and systems biology for producing genetically modified organisms specifically for potential degradation of synthetic organic compounds (SOCs) is being given due consideration. Degradation of these compounds is based upon the secretion of enzymes by microorganisms, mainly bacteria and fungi that participate in various metabolic pathways. The conventional physicochemical bioremediation methods (*in situ* and *ex situ*)) are ineffective for degradation and removal of fresh emerged compounds [58,59].With the advancement in the field of genetic engineering and recombinant DNA technology, many genetically modified microorganisms were constructed by using diverse techniques for the remediation of synthetic organic compounds [60]. The engineered strains of *Escherichia coli*were effectively used for phenol degradation [61].Degradation of organophosphates, carbamates and pyrethroids was achieved by engineering *Pseudomonas putida*[62].

Oleophilic microorganisms, considered as cost-effective, eco-friendly and an ultimate natural mechanism for maintaining ecosystem and environmental sustainability, are used for degradation and cleaning of petroleum based pollutants from terrestrial and aquatic systems. [63,64].The general and widely used process for the aerobic degradation of petroleum hydrocarbon using microorganisms is shown in Fig. 4[65].In this process, specific anaerobic and aerobic enzymes as well as emulsifiers and biosurfactants are used at a certain condition, to attach microbial cells to the substrates.

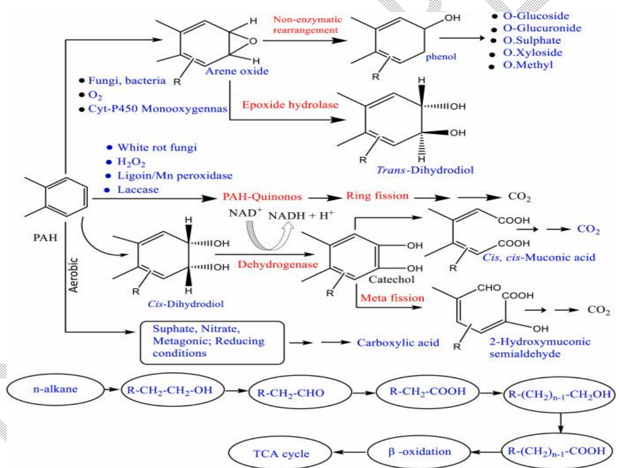

Figure-4. Graphical flowchart for PHs and alkanes degradation using Microbial degradation adapted from Koshlaf and Ball, 2017.

Aerobic transformation, involves attack on the petroleum hydrocarbons by oxygenase enzyme followed by the cleavage of the ring. The cations of peroxides can also oxidize polycyclic aromatic hydrocarbons into phenolic and quinone derivatives using fungi [65].The enzymes use the molecular oxygen produced from the aerobic transformation process for the oxidation of the substrate of polycyclic aromatic hydrocarbons to hydroxylation rings and other forms of intermediate oxidized products [66].Degradation of phenanthrene andfluorene through an anaerobic bio-transformation method coupled withsulfate-reducing bacteria was conducted. Results revealed the formation of molybdate,indicating that phenanthrene and fluorene are bio-transformed viahydration and hydrolysis processes followed by decarboxylation withp-cresol and phenol formation [67].Hence, the formation of these metabolites canbe considered as a new route of biotransformation of phenanthrene and fluorene. Research studies indicatethat most petroleum hydrocarbons' degradation mechanism modes are not fully understood and more research and investigationsare needed in future, taking into account the complexity of thefield scale, as shown in Fig.5

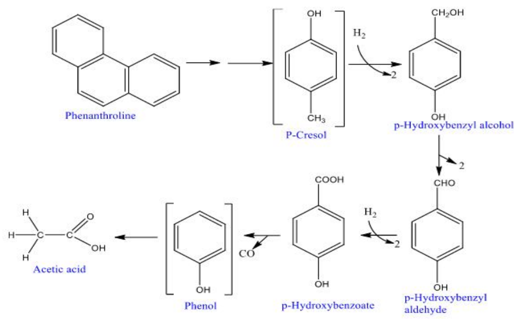

Figure-5. Biotransformation route of phenanthrene using anaerobic and sulfate-reducing bacteria adapted from Tsai et al 2009.

The list of some dormant microorganisms which can degrade petroleum hydrocarbon is shown in Table1.

Table 1. Summary of some potential petroleum hydrocarbon-degrading bacteria and fungi.

Microorganism	**Compound**	**Removal Rate**	**Reference**
Aspergillus species	Crude Oil	59-87.7% of crudeoil recovery	[68]
Aspergillus ocharaceus CBAI 849	Benzo[a]pyrene	76.6-99.7% bioremediation from saline environments	[69]
Penicillium sp. RMA1 and RMA2	Crude oil	Degradation achieved by RMA1-57% and RMA2-55%	[70]
Aspergillus species RFC-1	Different PHs	60.3-97.4% removal of PHs from aqueous environments	[71]
Ochobactrum species	Crude oil	83.49% degradation of crude oil equivalent to 3% v/v	[72]
Candida tropicalis	Diesel	Degradation of 83% diesel	[73]
Pseudomonas species	Petrol, diesel and waste engine oil	Degradation achieved petrol-76%, diesel-83% and waste engine oil-69%	[74]
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	[75]

8. Nanotechnology and Enzyme Technology

The combination of enzymes with nanotechnology is of great importance for making the nanomaterials less noxious to the environment. When hydrocarbons and enzyme molecules are present along with nanomaterials, they minimize their cell interaction through stearic hindrance and decrease the surface energy [76]. In view of the fact that enzymes are eco-friendly and provide a supplementary uniqueness of catalysis, nanomaterials are more adaptable and efficient in bioremediation and green energy production. The applicability of nanoparticles for large-scale remediation of petroleum hydrocarbons from polluted soil is a matter of concern due to their recalcitrant nature and stability of manufactured nanoparticles [77]. It is essential to understand the environmental fatein a better way before they are used on a broad scale [78].

The pollutant substrate can recognize different microbial enzymes under optimum conditions of pH, temperature, contact time, concentration etc. to efficiently transform them into other rather harmless products. The most representative enzymes involved in bioremediation include cytochrome P450s, laccases, dehalogenases, dehydrogenases, hydrolases, proteases and lipases which have shown promising potential degradation of polymers, aromatic hydrocarbons, halogenated & agrochemical compounds, dyes, detergents etc. favored by mechanisms such as oxidation, reduction, elimination and ring-opening.

Table-2 Microbial enzymes involved in bioremediation and their function-

S.No.	Enzymes	Function	Reference
1	Cytochrome P450	Synthesis and metabolism of various molecules and chemicals within cells oxidize steroids, fatty acids and xenobiotics	[79]
2	Laccase	Ring cleavage in aromatic compounds and reduce one molecule of oxygen in water and produce free radicals	[80]
3	Dehalogenase	Cleaves the carbon-halogen bond and eliminates halogens	[81,82]
4	Dehydrogenase	Oxidizing organic compounds and generating energy	[83,84]
5	Hydrolase	Degradation of fats and proteins	[85]
6	Protease	Degradation of proteins like keratin, casein, etc., leather dehairing and wastewater treatment	[86]
7	Lipase	Catalyzes the hydrolysis of mono, di, and triglycerides into fatty acids and glycerol. Also catalyze the esterification and transesterification reactions.	[87]

9. Advantages 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 generallynontoxic.
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 threats to human health and the environment.
3. It is cost effective in comparison to other conventional methods used for the treatment of oil contaminated sites. It transformsthe toxic& hazardous compounds into less harmful products, ensuring their complete degradation and disposal.

10. Limitations and Concerns

Bioremediation is limited to those compounds that are biodegradable. Availability of metabolically active microbial populations, appropriate environmental growth conditions, nutrient levels and contaminants are keyfactors necessary for its success.In some cases, the products of biodegradation may be more toxic or persistent in the environment than the parent compound. To scale up bioremediation process from batch and pilot scale studies, applicable to large scale field operations is quite tedious.Newinvestigations and research is needed to enhance the development of modern engineer bioremediation technologies suitable for sites with composite combinations of contaminants. It is uncertain to say that remediation is altogethercompleted as there is no conventional definition of clean. As a result, performance assessment of bioremediation is complicated and difficult since there is no acceptable endpoint for bioremediation treatments. Low temperatures may slow or stop degradation. Moisture levels, pH, temperature, and other filter conditions such as fungi growth should be monitored to maintain high removal efficiencies. Until recently, biofilters were not designed to treat chlorinated compounds. However, recent demonstrations have suggested that they can be used to remove these compounds as well. The accumulation of excess bacteria may plug filters, requiring periodic mechanical cleaning of the filter. There isa potential for the release of fugitive fungi.

11. Conclusion and Perspectives

Recent research advancements on biochar modification (physical, chemical and biological) opens greater opportunity to form tailored biochar with improved surface properties than their native forms for offering better removal efficiencies of important and potentially severe organic pollutants such as antibiotics and pesticides. Microbial interactions with biochar not only improve the bioremediation level further but also degrade the pollutants from the environment and open up better environmental and socio-economic prospects. Application of green, cost-effective and sustainable biochar for remediation of environmentally potential organic pollutants offers economical treatment methods as well as safe environment [88].Pesticides cause toxicity in human by inhibiting Acetylcholinestrase (AChE) enzyme. Bacterial mediated pesticide degradation is a sustainable and eco-friendly approach in pesticide-contaminated soils for a sustainable environment and resilient agriculture. High throughput next-generation sequencing and *in silico* analysis allow us to identify and discern the members and characteristics of core microbiomes at the contaminated site [89].

It can be concluded that with the rising population of the planet, the science and technology of bioremediation is going to become a prerequisite of today's modern life. Fortunately considering the youth of this interdisciplinary science, there has been significant progress in the field. The diversity of bioremediants, the range and variety of available techniques, different types of substrates used by bioremediants in both terrestrial and aqueous habitatsappear as good signs of this well-promising science and technology. Hence bioremediation will undoubtedly be used for a number of applications in varying environments. Fungi as a huge group of unicellular,filamentous microorganisms of great biodiversity, isolated from unlike environments are fairly considered as aneffective group of bioremediants.Keeping in view the recent advances in genetic and metabolic engineering, it seems that fungi will have an extra share in the bioremediation of pollutants and wastes.

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Based upon studies, investigations and available data, it is anticipated that bioremediation would expand to more precise scientific branches in the times to come to quicklyrespond to thechallenges of present and future world.

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