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BIOREMEDIATION: ECO-FRIENDLY BIOLOGICAL PROCESS FOR SUSTAINABLE ECOSYSTEM

Environmental Science

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ABSTRACT

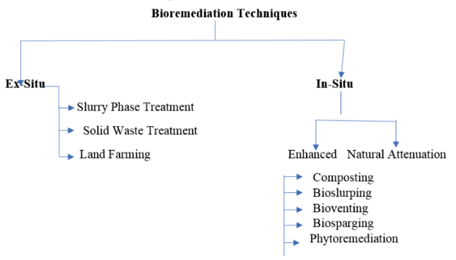
The role of micro-organisms is much studied and analyzed aspect of today's time. With the development in the field science and technology, especially microbiology and biotechnology has enabled humans to combat the increasing rate of synthetic contaminants by using biological agents, commonly known as bioremediation. Bioremediation is a technique in which living micro- organisms are used to degrade the environmental contaminants into less toxic forms by naturally occurring bacteria, fungi or plants and plays an important role in removing pollutants which are hazardous to human health and/or the environment. The technology involves Bio stimulation (stimulating viable native microbial population), Bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), Biosorption (dead microbial biomass), phytoremediation (plants) and Rhizoremediation (plant and microbe interaction). The micro-organisms may be indigenous to a contaminated area or they may be transferred to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. This is invaluable in finding solutions for several problems which mankind is encountering in maintaining the quality of the environment. Microbes play a very important role in human and animal health, genetic engineering, environmental protection and municipal and industrial waste water treatment. The extent of environmental bioremediation encompasses inorganic viz. arsenic, chromium, mercury, cyanide etc. and organics viz. Hydrocarbons, petroleum, pesticides etc. The use of micro-organisms have enabled feasible and cost-effective responses which would otherwise be impossible via straight forward chemical or physical engineering methods. It is an eco-friendly biological process employed in remediation of environmental problems (such as, pollution, contamination, etc.) using biological system or organisms. The present paper deals with the importance of Bio-remediation in combating environmental pollutants for healthy living.

KEYWORDS

INTRODUCTION

Globalization and industrialization has been the major concern now, in terms of pollutants production. This has compelled the technology to work in solving the problem of waste generation by biodegradable means. Micro-organisms like bacteria and fungi are the main role playes. Bioremediation technique is a positive attribute. Bioremediation is a waste management technique, as we all know, uses biological micro-organisms like yeast, fungi or bacteria to naturally break down complex hazardous substances into less toxic/harmless products (inorganic salts, CO_2 & H_20) as these will not degrade the environment by hemselves. This treatment process is cost effective and natural process. The technique is widely used to treat organics, fuels or solvents. Bioremediation is a better alternative as almost no residual treatment is required in treatment as there is complete breakdown the contaminant as compared to physical/chemical treatment. Most of the challenges associated with biodegradation and bioremediation of polluting substances [1] are easily solved by Mutochthonous (indigenous) microorganisms present in polluted environments provided that medium for their growth and metabolism is favourable. The process of pollutant removal depends primarily on the nature of the pollutant which includes: greenhouse gases, heavy metals, hydrocarbons agrochemicals, chlorinated compounds, dyes, nuclear waste, plastics and sewage.

A proper culture media is required for treatment with micro-organisms. Optimal levels of water, oxygen, heat and nutrients is a necessity of healthy micro-organisms growth. Process can be aerobic or anaerobic. There are basically two types of microbes used. Those already living (native) at the given site are known as Indigenous micro-organisms. Their growth can be stimulated by providing optimum conditions of soil temperature, humidity, oxygen, and nutrient content. On the other hand, Exogenous micro-organisms are those which are added to digest contaminants that are not present in the soil at the site. A proper selection of micro-organisms is to be done and added for bioremediation to occur on spot.



Bioremediation using microbes can be broadly done as:

- **In-Situ Bioremediation**: In-situ bioremediation is considered to be an extension of the goal that the microorganisms used in nature for billions of years It holds a upper hand in bioremediation biological treatment process. The process does not require excavation and transportation of soils which makes the process cost effective. Large volume of soil can be treated at the same time especially permaeable sandy soil. The process causes less contamination and dust production as compared to ex-situ techniques. However, the method is not effective in clays/highly layered subsurface environments as oxygen cannot be evenly distributed throughout the treatment area. This may result in delay to reach cleanup goal. Three different types of in situ bioremediation process are (i) in bioattenuation/Natural Attenuation, which depends on the natural process of degradation are (i) Biostimulation where intentional stimulation of degradation of chemicals is achieved by addition of water, nutrient, electron donors or acceptors, and (ii) Bioaugmentation chemicals is achieved by addition of water, nutrient, electron donors or acceptors, and (iii) Bioaugmentation where the microbial members with proven capabilities of degrading or transforming the chemical pollutants are added [2].

Bioattenuation/ Natural Atenuation: By natural ways, the pollutants are transformed to less harmful forms or immobilized largely due to biodegradation by microorganisms [3], and to some extent by the reactions with naturally occurring chemicals and sorption on the geologic media. The process is contaminant specific and widely accepted as methods for treating fuel components (e.g., BTEX) [4], but is limited. The time required for natural attenuation varies considerably with site conditions. Many polluted sites may not require an aggressive approach to remediation which makes it efficient and cost effective [5; 6].

1) Biostimulation: In this process, bacteria is stimulated to initiate the remediation process. The contaminated soil is mixed with special nutrients substances along with other vital components .It stimulates the growth of microbes which quickly removes the contaminants in an efficient way. he speed of microbial activity for bioconversion of chemical pollutants mainly depends on the supply of carbon, nutrients(Nitrogen and Phosphorous), temperature, availability of Oxygen, soil pH, redox potential along with the type and concentration of organic pollutant itself [7]. To accelerate microbial degradation, nutrients in the form of fertilizers {water soluble (e.g., KNO_3, $NaNO_3$, NH3NO$_3$, K_2HPO_4 and $MgNH_4PO_4$) and slow releasers (e.g., customblen, IBDU, max-bac) are added [8]. For oil spill remediation, around 1–5% N by weight of oil with a ratio of N:P between 5 and 10:1 is used [9]. According to specific site/pollutant combinations, formulation of nutrient-treatment strategies are to be designed. Limitations of nutrients on microbial decomposition of organic matter and the possible ecological implications of these effects for carbon flow through natural ecosystems are well known . The 'ecological stoichiometry' is concerned with the supplies of nutrients, and their elemental stoichiometry relative to the nutritional demands of the cell's innate physiology. Smith et al. [10] applied the resource-ratio theory to hydrocarbon degradation and concluded that the changes in nitrogen and phosphorus supply ratios not only altered the biodegradation rates of hydrocarbons (hexadecane and phenanthrene) but also the microbial community composition significantly.

2) Bioaugmentation: The process of bioaugmentation is used where microorganisms are required to extract the contaminants like, municipal wastewater. It has a major drawback tha at it almost becomes impossible to control the growth of microorganisms in the process of removing the particular contaminant. Pre-exposure and subsequent re-exposure of a chemical pollutant enhances the metabolic potential of microorganisms [11].'Soil memory' refers to the phenomenon of retaining specific metabolic capacity after exposure which makes a significant contribution to the subsequent natural attenuation. Now, in a typical bioaugmentation approach, microorganisms are amended to a polluted site to hasten detoxification and/or degradation. Gilbert and Crowley [12] found that the repeated application of carvone-induced bacteria enhanced biodegradation of PCBs in soil. To improve efficiency of bioaugmentation, microorganisms of different physiological groups and of different divisions can also be brought together. Bender and Phillips [13] suggested the use of microbial mats which occur in nature as stratified communities of cyanobacteria and bacteria to remediate organic contaminants by degrading and completely mineralizing the contaminants.

3) Intrinsic Bioremediation: Most effective method for soil and water treatment is intrinsic bioremediation because have a high probability of maximum contaminants and toxins. The process is mainly used for underground petroleum tanks. In such place, it is difficult to detect a leakage and contaminants and toxins can find their way to enter through these leaks and contaminate the petrol. Thus, only microorganisms can remove the toxins and clean the tanks.

Enhanced In- Situ Bioremediation

1. **Composting And Addition of Composted Material**

Traditionally, the practice of composting is intended to reduce volume and water content of vegetable wastes, to destroy pathogens, and to remove odour-producing compounds. This technology is now effectively applied for treating polluted soil mainly by: (i) Composting of polluted soils for efficient degradation (ii) By addition of composted materials. Degradation of two herbicides, benthiocarb (S-4-chlorobenzyl diethylthiocarbamate) and MCPA (4-chloro-2-methylphenoxyacetic acid) in soil was very well done by composed material. [15]. Composting depends on various parameters like: type of contaminants and waste materials to be used for composting; temperature and soil/ waste amendment ratio [16].

During composting, heat generated by microbial metabolism is trapped in the compost matrix and at this time, microbial decomposition and biomass formation occurs. The mixing of remediated soil with contaminated soil increases the efficiency of composting [17]. Microorganisms degrade pollutants into innocuous compounds and transform pollutants into less toxic substances and/or lock up the chemical pollutants within the organic matrix. Humic material which accumulates with an increase in stability of the compost is known to act like a surfactant and plays an important role in releasing PAHs absorbed to the soil.

Composting also has both advantages and limitations. Addition of compost to contaminated soil for bioremediation makes it a sustainable technology since the biodegradable organic waste in the compost is being utilized for beneficial activity. Also, composting improves the soil structure, nutrient status and microbial activity. One of the limitation of composting technique is lack of sufficient knowledge about microorganisms involved during various stages of composting along with their fate.

2. Bio Slurping

This technique is a combination of vacuum-enhanced pumping, soil vapour extraction and bioventing. The method is used to remediate soil and groundwater pollutant by indirect provision of oxygen and stimulation of contaminant biodegradation [20]. The technique is well designed for free products recovery such as light non-aqueous phase liquids (LNAPLs). However, this technique is not suitable for remediating soil with low permeability but, it saves cost due to less amount of groundwater resulting from the operation thus minimizes storage, treatment and disposal costs [14]. Establishing a vacuum on a deep high permeable site and fluctuating water table, which could create saturated soil lenses that are difficult to aerate are amongst the major constraints in applying the technique.

3. Bioventing

Controlled stimulation of airflow by delivering oxygen to unsaturated (vadose) zone in order to increase bioremediation is done which increases activities of indigenous microbes. In bioventing, by adding suitable nutrients and moisture , bioremediation is enhanced with the ultimate goal to achieve microbial transformation of pollutants to a harmless state [14]. This technique has gained much popularity among other in-situ bioremediation techniques especially in restoring sites polluted with light spilled petroleum products [18]. Frutos et al. [19] reported the effectiveness of bioventing treatment in remediation of phenanthrene-contaminated soil and recorded > 93 % contaminant removal after 7 months. Airflow rates and air intervals are the basic parameters on which success of bioventing based bioremediation depends along with increase in number of air injection points as it helps to achieve uniform distribution of air. Bioventing can be used for anaerobic bioremediation process especially in treating vadose zone polluted with chlorinated compounds, which are recalcitrant under aerobic conditions.

4. Biosparging

The technique is very similar to bioventing in that air is injected into soil subsurface to stimulate microbial activities in order to promote pollutant removal from polluted sites. Here, air is injected at the saturated zone which causes upward movement of volatile organic compounds to the unsaturated zone to promote biodegradation. The effectiveness of biosparging depends mainly on soil permeability, which determines pollutant bioavailability to microorganisms and on pollutant biodegradability [14]. Biosparing is similar in operation with a closely related technique known as In-situ Air Sparging (IAS), which relies on high airflow rates to achieve pollutant volatilization, whereas biosparging promotes biodegradation. Kao et al. [23] showed that biosparging of benzene, toluene, ethylbenzene and xylene (BTEX)-contaminated aquifer plume resulted in a shift from anaerobic to aerobic conditions

5. Phytoremediation:

Plants are directly used to clean up or contain contaminants in the soil over the past 30 years. This method of bioremediation helps mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere. This is called phytoremediation. In general, plants can promote dissipation of organic pollutants by immobilization, removal, and promotion of microbial degradation. Some organic compounds are transported across plant membranes, released through leaves via evapotranspiration (phytovolatilization) or extracted, transported and accumulated in plant tissues (phytoextraction) or degraded via enzymatic processes (phytodegradation). Some of the non-volatile compounds are sequestered in planta and are less bioavailable (phytostabilization). With metallic contamination, the usual task is to accumulate metal in the residual plant parts that must be disposed of before or after incineration in a hazardous waste landfill site. However, mercury and selenium can be released as volatile elements directly from plants to the atmosphere. For some inorganic materials such as metals, biological processes have been used to reduce radioactivity and remove organic contaminants.

Dendroremediation,a type of phytoremediation using trees is useful in attenuating certain pollutants such as 2,4,6-trinitrotoluene and trichloroethylene from soil and groundwater [21].Phytostimulation of pollutant degradation by microorganisms in the rhizosphere or inside the plants also offers economic and environmental advantages as compared to other the conventional strategiesn. But, the disadvantage includes hydrophobicity and chemical stability of pollutants which influences the phytostabilization and the rates of degradation by the associated microorganisms [22]

- **Ex -situ Bioremediation:** Ex-situ techniques is faster, easier to control and are used to treat a wider range of contaminants and soil types than in-situ techniques. There is uniformity of treatment because of the ability to homogenize, screen, and continuously mix the soil. Ex-situ techniques include: slurry & solid phase bioremediation. Solid-phase soil treatment processes includes land farming, soil biopiles and composting. Slurry-phase soil treatment processes include the slurry phase bio-reactor. However, they require excavation of soils. There is also a problem of increased costs input and engineering for equipment. Also, there is risk of material handling along with worker exposure conditions.

1. **Slurry Phase:** A relatively rapid process as compared to other biological treatment processes. Contaminated soil is mixed with water and other additives in a large bio-reactor along with indigenous micro-organisms in contact with the contaminants. To this, nutrients and oxygen are also added at controlled conditions to ensure optimum environment uniform degradation the contaminants. On completion of the treatment, the water is removed from the solids and waste water is disposed or further treated if contamination still persists.

2. **Solid Phase Process:** Relatively simple to operate and maintain but requires large space and longer time of treatment than or slurry-phase processes. Used in treatment of soils in above-ground treatment area. The process is well equipped with collection systems which prevents any contaminant to escape the treatment. There is a control of moisture, heat, nutrients and oxygen to enhance biodegradation.

Table 1 Microorganisms studied for bioremediation function

Pollutants	Organisms	Function
2,4,6-Trinitrotoluene (TNT)	*Methanococcus* sp.	Biotransformation
Atrazine	*Pseudomonas* sp. (ADP)	Biodegradation
Chlorpyrifos	*Enterobacter* strain B-14	Biodegradation
Dibenzothiophene (DBT)	*Rhizobium meliloti*	Biodegradation
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	*Acetobacterium paludosum* *Clostridium acetobutylicum*	Biodegradation
PAHs	*Pseudomonas* sp., *Pycnoporus sanguineus*, *Coriolus versicolor*, *Pleurotus ostreatus*, *Fomitopsis palustris*, *Daedalea elegans*	Biodegradation
Phenanthrene, PAH	*Agrobacterium*, *Bacillus*, *Burkholderia*, *Pseudomonas*, and *Sphingomonas*	Biodegradation
Polychlorinated biphenyl (PCB)	*Rhodococcus erythropolis* TA421 *Rhizobium* sp.	Biodegradation
Polycyclic aromatic hydrocarbon (PAH)	Fungi	Biodegradation

Limitations Of Bioremediation

- Longer-term processes (than Chemical/Physical)
- Applicable to specific organic compounds only
- Does not deal with inorganic compounds
- Very difficult to confirm whether contaminants have been destroyed
- Biodegradability of highly chlorinated compounds is very low
- Chances are there that some compounds may be broken down into more toxic by-products (e.g., TCE to vinyl chloride) or PAHs to less degradable PAHs (carcinogens) which can make their way to ground water, if not controlled
- Sensitive to certain soil parameters also

Applications Of Bioremediation

1. Can be used to clean up both soil and water, either in situ or ex situ.
2. The microorganisms must be active and healthy for bioremediation to occur.
3. While contaminants are being broken down, intermediate products that are less/equally/more toxic than the original contaminants can be used for other purposes.
4. However, it is limited to organic contaminants (e.g. non-halogenated volatile & semi-volatile organics, fuels) but future prospects in other fields is high.
5. Strata must have med-high permeability (e.g. less effective in clays).
6. The specific technology used is dependent on several factors (e.g. the type of microorganisms present, the site conditions, the quantity and the toxicity of contaminant chemicals).

CONCLUSION

There are numerous applications including marine oil spills and clean up of pollution by chemicals. Bioremediation techniques has achieved new heights during past two decades in dealing effectively to restore polluted environments in an eco-friendly approach and at a very low cost. Bioremediation techniques have been successfully implemented to remediate soils/sludges and groundwater contaminated by petroleum hydrocarbons, solvents, pesticides, wood preservatives and other organic chemicals. The goal is to stimulate bioremediation microorganisms with nutrients and other chemicals that allow them to destroy contaminants. The bioremediation systems that are used today rely on microorganisms from contaminated sites and encourage them to work by enabling them to provide optimal amounts of nutrients and other chemicals necessary for their metabolism. The current bioremediation systems are therefore limited abilities of indigenous microbes. However, scientists are currently studying ways to extend sites contaminated with unsaturated microbes - including genetically modified microorganisms - specially adapted to the degradation of contaminants that relate to specific locations.

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