RECENT MICROBIOLOGICAL ADVANCES FOR COMBATING SOIL POLLUTION

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Soil, an essential part of ecosystem, serves as the source and sink of the elements of life. Any adverse change in its composition will have direct implications on the life on earth. World's Soil Resources Report (SWSR) has identified soil pollution as one of the main soil threats affecting global soils thereby posing threat to foodsecurity as well as the human health. Soil pollution has deteriorated large areas of agricultural lands and has adversely affected soil biodiversity. The diversity of soil contaminants and their spread has achieved an alarming trend finely correlating with increased anthropogenic activities.

Soil remediation is a term used for various processes used to decontaminate soil. Several soil remediation methods are in practice ranging from physical, chemical and biological methods either to remove or minimize the adverse effects of soil pollutants. Soil remediation approaches are often dependent on soil type, composition, nature of contaminant, handling intensity, feasibility and cost etc. Among the several approaches, **bioremediation** offers cost-effective and eco-friendly approaches for soil clean-up. Bioremediation is defined as "use of biological processes to degrade, break down, transform, and/or essentially remove contaminants or impairments of quality from soil and water". Bioremediation is a natural process which relies on microorganisms and plants to metabolize contaminants using them as energy source as they carry out their normal life functions.

Depending on the type of organisms involved in bioremediation, it is broadly classified as phyto bioremediation and microbial bioremediation. However these can be used in combination with other remedial measures. **Phytoremediation** uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil. Most scientific and commercial interest in phytoremediation relies on phytoextraction (removing metals or organics from soils by accumulating them in the biomass) and phytodegradation (using plants to uptake, store and degrade organic pollutants), using selected plant species grown on contaminated soils. Microbial **bioremediation** involves the use of mainly microorganisms i.e. yeast, fungi or bacteria to clean up contaminated soil. Techniques rely on promoting the growth of specific microflora or microbial consortia that are indigenous or introduced to the contaminated sites that are able to perform desired activities. **Microbial** enrichment is done through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions. In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources.

Why Microbes?

- Wider adaptability: Microorganisms are extremely widespread occupying every possible niche even those considered extreme for any complex life forms. Microbial life is evidenced in extreme habitats from extremely dry and cold deserts in the Antarctic and deep into permafrost soils to geothermal and humid soils in volcanic areas, from extremely acid mines with sulfuric acid to high alkaline areas. Their ability to endure selective pressures of the environment have made them adaptable to new types of habitats created by anthropogenic activities, such as those polluted with heavy metals, radionuclides, and high concentrations of toxic xenobiotic compounds such as polychlorinated biphenyls, hydrocarbons and pesticides.

- Metabolic diversity: All metabolic reactions are mediated by enzymes belonging to groups of oxidoreductases, hydrolases, lyases, transferases, isomerases and ligases. Microorganisms are endowed with metabolic properties driven by a vast array of enzymes suited for the task of attacking recalcitrant substances. Many enzymes have a remarkably wide degradation capacity due to their nonspecific and specific substrate affinity. Microbial oxygenases have a broad substrate range and are active against a wide range of compounds, including halogenated organic compounds, that comprises the largest groups of environmental pollutants. Many microorganisms produce intra and extracellular laccases capable of catalyzing the oxidation of phenolic and aromatic substrates. Microbial lipases effectively degrades hydrocarbon from contaminated soil. (Karigar and Rao, 2011). Often microbes work in communities sharing complimentary metabolic steps resulting in enhanced degradation rates

Advantages of microbial bioremediation (Abatenh et al., 2017)

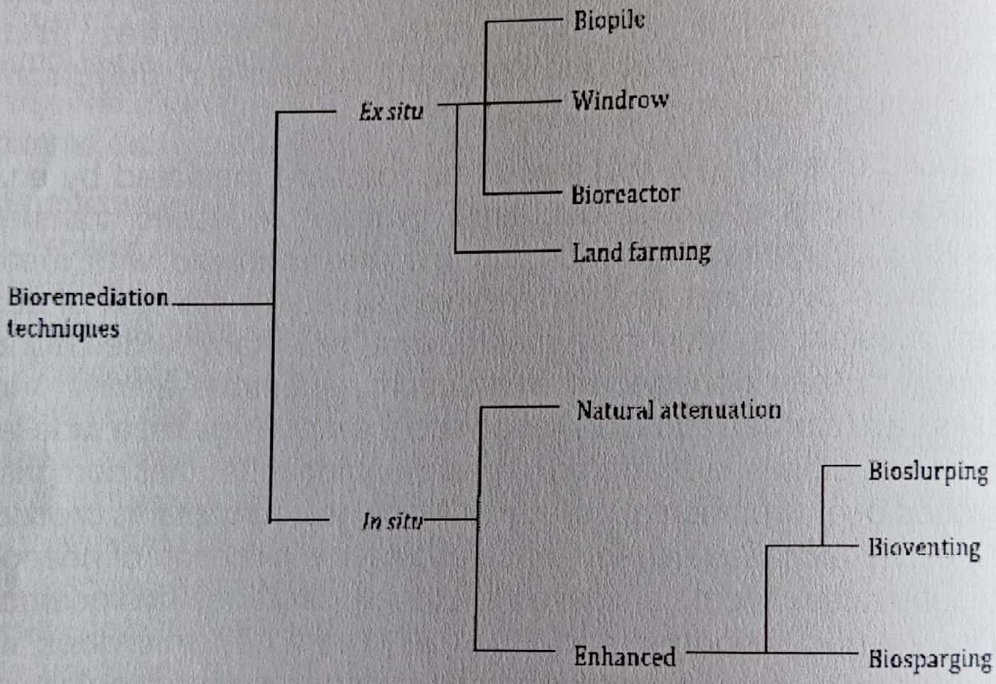
- Natural process and an acceptable waste treatment process for contaminated material such as soil.
- Requires very less effort and can often be carried out on site
- Cost effective process in comparison with conventional methods (technologies) used for clean-up of hazardous waste.
- Helps in complete destruction of the pollutants, many being transformed to harmless products
- It does not use any dangerous chemicals.
- Eco-friendly and sustainable.

Disdvantages of microbial bioremediation

- Limited to biodegradable compounds that. Not all compounds are susceptible to rapid and complete degradation. Biological processes are often highly specific.
- Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation.

Bioremediation techniques: (Vidali et al., 2001; Azubuike et al., 2016)

There are two basic types of bioremediation based on treatment site.



Source: Azubuike et al., 2016

In Situ Bioremediation

1. Treating polluted substances at the site of pollution.
2. Does not require any excavation, hence little or no disturbance to soil structure.
3. Uses harmless microorganisms (with chemotactic affinity to pollutants) to eliminate the chemical contaminations.
4. status of electron acceptor, moisture content, nutrient availability, pH and temperature are among the important factors to be considered for successful treatment
5. in situ microbial bioremediation techniques might be enhanced (bioventing, biosparging), while others might proceed without any form of enhancement

(intrinsic bioremediation or natural attenuation).

- Bioventing: Involves controlled stimulation of airflow by delivering oxygen to unsaturated zone to increase indigenous microbial activity. Amendment with nutrients and moisture is done to encourage microbial activity thereby accelerating microbial transformation of pollutants to a harmless state. Used in restoring sites polluted with light spilled petroleum products
- Biosparging: Combines vacuum-enhanced pumping, soil vapour extraction and bioventing to achieve soil bioremediation. It is used to remediate soils contaminated with volatile and semi-volatile organic compounds
- Intrinsic bioremediation (natural attenuation): Involves passive remediation of polluted sites, without any external intervention. The process relies on both microbial aerobic and anaerobic processes to biodegrade polluting substances including those that are recalcitrant. This is successfully used to treat chlorinated solvents, dyes, heavy metals, and hydrocarbons polluted sites
- Engineered in situ bioremediation: performed through the introduction of certain microorganisms to a contamination site. Improved physico-chemical conditions are provided to support enrichment and bioactivity the exogenously amended microorganisms.

Ex situ bioremediation:

1. These techniques involve excavating pollutants from polluted sites and subsequently transporting them to another site for treatment.

- Biopile: Involves above-ground piling of excavated polluted soil, followed by nutrient amendment, aeration and moistening to accelerate remediation by basically increasing microbial activities.
- Windrows: This method relies on periodic turning of piled polluted soil to bring uniformity in aeration, distribution of pollutants and nutrients to speed up degradation activities (assimilation, biotransformation and mineralization) of indigenous and/or transient hydrocarbonoclastic bacteria. Windrow composting is an aerobic and thermophillic process that involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes.
- Bioreactor:Contaminated soil in form of dry matter or slurry is treated in vessel (bioreactors) in which raw materials are converted to specific product(s) following series of biological reactions. Excellent control of bioprocess parameters (temperature, pH, agitation and aeration rates, substrate and inoculum concentrations) is one of the major advantages of bioreactor-based bioremediation.

- Land farming: contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded by stimulating indigenous biodegradative microorganisms thereby facilitating aerobic degradation. In general, the practice is limited to the treatment of superficial 10–35 cm of soil

Microorganisms involved in bioremediation process:

Microorganisms prove potent candidates for bioremediation as they function as natural decomposers in varied ecosystems. Ease of proliferation, generation rate, chemotaction and enzymatic potential make them ideal for bioremedial applications. Microrganisms for bioremediation should fulfill the following requirements (Alexander,1994)

a) Should possess effective enzymes for bioremediation

b) Should thrive and demonstrate bioactivity under polluted environment

c) Should be able to get access to the fixed/insoluble contaminant

Several microorganisms such as Corynebacteria, Mycobacteria, Pseudomonads, yeast act as bioemulsifiers and use oil hydrocarbons as source of carbon and energy. Many degrade synthetic compounds (xenobiotics) such as remnants of pesticides in agroecosystems. Fungi and anaerobic bacteria are known to degrade dye compounds. Microbial bioremediation is performed through different mechanisms such as biosorption, biodegradation, bioaccumulation and biotransformation of the contaminant molecules. Following table lists a few microbes with proven bioremediation potentials.

Table 1: Microorgansims for bioremediation (Source: Abaten et al., 2017, Cycon et al., 2017)

Microorganisms	Compound **Hydrocarbons**
Penecillium chrysogenum	Monocyclic aromatic hydrocarbons, benzene, toluene, xylene and phenol compounds
Pseudomonas putida, P. veronii, Achromobacter sp., Flavobacterium sp. , Acinetobacter sp.	Petrol, diesel, polycyclic aromatic hydrocarbons, toluene
Cyanobacteria, Bacillus licheniformis	naphthalene
	Oil
Fusarium sp.	oil
Aspergillus niger, Candida glabrata, Saccharomyces cerevisiae	Crude oil

Pseudomonas cepacia, Bacillus cereus, Bacillus coagulans, Citrobacter koseri and Serratia ficaria	Diesel oil, crude oil
	Dyes
Bacillus subtilis	Oil-based paints
Pycnoporus sanguineous, Phanerochaete chrysosporium and Trametest rogii	industrial dyes
Bacillus spp. ETL-2012, Pseudomonas di-azo dye	Textile Dye (Remazol Black B), Sulfonated Reactive Red HE8B, RNB dye aeruginosa, Bacillus pumilusHKG212
Exiguobacterium indicum, Exiguobacterium aurantiacums, Bacillus cereus and Acinetobacter baumanii	azo dyes effluents
Bacillus firmus, Bacillus macerans, Staphylococcus aureus and Klebsiella oxytoca	vat dyes, Textile effluents
	Heavy metals
Saccharomyces cerevisiae	Heavy metals, lead, mercury and nickel
Pseudomonas fluorescens and	Fe2+, Zn2+, Pb2+, Mn2+ and Cu2+
Lysinibacillus sphaericus CBAM5	cobalt, copper, chromium and lead
Geobacter spp.	Fe (III), U (VI)
Bacillus safensis	Cadmium
Pseudomonas aeruginosa, Aeromonas sp.	U, Cu, Ni, Cr
	Pesticides
Bacillus, Staphylococcus	Endosulfan
Enterobacter	Chlorpyrifos
Pseudomonas putida, Acinetobacter sp., Arthrobacter sp.	Ridomil MZ 68 MG, Fitoraz WP 76, Decis 2.5 EC, malathion
Acinetobactor sp., Pseudomonas sp.,	chlorpyrifos and methyl parathion
Arthrobacter sp. DAT1	Atrazine
Catellibacterium caeni sp. novDCA-1T	Butachlor,Alachlor,Acetochlor,Propisochlor
Bacillus sp. DG-02	Bifenthrin,fenpropathrin, Cypermethrin,Cyfluthrin, cyhalothrin, deltamethrin

Recent advances in bioremediation

Pre-genomics scenario were greatly relying on culture dependent techniques that involved isolation, identification and screening of microbes with bioremediation potential, carried out in labs followed by field studies. Applicathat involved isolation, identification and screening of microbes with bioremediation potential, carried out in labs followed by field studies. Applications of genetically engineered microorganisms (GEM) in bioremediation received a great deal of attention. Due to regulatory risk assessment concerns, and the uncertainty of their practical impact and delivery under field conditions, the efforts remain confined to lab. Though a vast number of microorganisms were studied and explored for bioremediation, low success rate with bioaugmentation necessitates studies to understand the contribution of uncultivable counterparts to get deeper insights into their role in bioremediation.

Omics- approaches in microbial bioremediation:

With the advent of high throughput sequencing technologies and in silico analyses, advanced 'omics' tools such as genomics, transcriptomics and metabolomics are being increasingly utilized to design the strategies for bioremediation. 16S rRNA gene sequencing technologies and related molecular-based approaches like denaturing gel electrophoresis upgraded the field of microbial ecology studies by helping to profile complex microbial diversity, thus overcoming the biases inherent in culture based profiling. Metagenomics powered with next generation sequencing and computational tools have made a breakthrough in opening up the 'microbial black box' associated with the polluted environments. Sequence phylotyping proved valid information on microbial diversity. Functional metagenomics also offer a powerful tool to understand the functional aspects of gene pool operating in an environment. It also allows cloning and expression of genes from uncultivable microorganisms to screen for enzymatic activities thus aiding in discovery of novel genes and metabolism. Metagenomics based bioremediation approaches are emerging potent tools as they help in identifying key microbial processes and optimal community composition enabling mineralization of pollutants (Thomas et al., 2012). Databases also offer a rich stock of genes for construction of designer microbial strains for targeted approaches (Chandran and Das, 2011). Metatrancriptomics and proteomics approach helps to determine the expression of functional genomes henceforth useful in ascertaining the genes active in a particular environment. Proteomic approaches have revealed novel pathways operating in aerobic and anaerobic degradation of toxins, extending more possibilities in identification of novel enzymes (Dore et al., 2015). Microbial metabolomics study helps us to analyze the dynamic operation and functional aspects of microbial communities by exploring the role of low-molecular weight metabolites (Malla et al., 2018). Thus omics approaches present a remarkable tool in deciphering the mechanisms of bioremediational pathways. Integrated knowledge on 'Omics' would possibly aid successful execution of efficient bioremediational strategies by tracking responsible organisms.

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