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A REVIEW ON BIOREMEDIATION OF POLLUTANTS BY GENETICALLY ENGINEERED MICROORGANISMS

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Abstract: Bioremediation refers to a process where microorganisms such as bacteria are employed to remove the environmental pollutants. In Myco remediation Fungi and Algae and in phytoremediation plants are used. The conventional procedures like physical and chemical waste clean-up procedures are failed to remove hazardous compounds from the environment and however these methods cause harmful effects to the environment. In general chemicals are mostly used which are cost effective but don't perform adequately on polluted areas. Genetically Engineered Microbes [GEM] are produced by introducing a stronger protein into bacteria through biotechnology or genetic engineering to increase the desired trait. Genetically engineered microbes are worthy process that will benefit the environment as well as the human health.

Index Terms: **Bioremediation, Biostimulation, Phytoremediation, Genetically Modified Organisms, Environment Pollution.**

I. INTRODUCTION

Environmental pollution is the major cause for damage of human, animal and plant health. This problem is not solved by conventional methods, physical and chemical approaches are time consuming and ineffective in removing pollutants (Ahmed *et al.*,2017). The methods are mentioned in Table-1.

Table-1: Different techniques employed to solve environmental pollution

S.No	Techniques employed to solve environmental pollution
1.	Reduction and oxidation (evaporation)
2.	Electrochemical treatment (physiochemical treatment)
3.	Solidifications (incineration)
4.	Lagooing (landfill deposition)
5.	Treatment of biological molecule (using novel enzyme systems)
6.	Reverse osmosis (landfill deposition)

The control of the pollutants, by genetically engineered microbes is called bioremediation. Thus, Bioremediation is the process of removing pollutants in the environment by microorganisms which decomposes the materials into liquid and gas form.

Bioremediation is an advanced technology for the remediation of contaminated sites which involves Employing plants or microorganisms to treat environmental contaminants (Sharma *et al.*, 2018). Based on the knowledge of physiochemical characteristics like structure, phenotypic potential and function of 'Genetically Engineered Microrganisms' (GEM). Microorganisms have capacity to degrade Pollutants and can survive in contaminated sites because of their metabolic capabilities.

However, GEM is not widely allowed to release into the environment and bioremediation has not been commercialized. For bioremediation, the Scientists could assure the Safe use of GEM through the use of technical safeguards and proper regulatory procedures, such as carrying out proper risk assessments and monitoring.

It is possible to employ green plants and soil-associated beneficial bacteria to degrade and detoxify contaminants in polluted soil and water/wastewaters through a process known as phytoremediation (Zdarta *et al.*,2018). This method removes metal contaminants from polluted surfaces. If metals collected from polluted locations could be utilized to extract useable forms of commercially viable metals, it could be marketed and produce cash (Bilal *et al.*, 2018). Burning plant biomass can generate energy and the land can then be reclaimed for sustainable farming and conservation.

Table-2: Advantages of Bioremediation

S.No	Type of organism used	Name of the technique	Advantages of Bioremediation using GEO
1.	Bacteria	Bioremediation	• Relatively cheap • Eco-friendly • Socially acceptable technology (Bilal et al., 2018).
2.	Algae/ Fungi	Myco remediation	
3.	Plants	Phyto remediation	

1.1 History

Bioremediation exists in the world since around 600 BC, this process is supposed to have involved the ancient Roman's utilization of microorganisms to treat their waste Water. Bioremediation was recognized as the first commercial Application in 1972 for cleaning up spills of gasoline, diesel, heavy Metals and other easily degraded petroleum products Tional Research Council 1993 (Ahmed *et al.*, 2018, Stadlmair *et al.*,2018).

1.2 Types of Bioremediation

On the basis of removal and transportation of waste for treatment, bioremediation is of two different types.

1. *In Situ* Bioremediation

2. *Ex Situ* Bioremediation

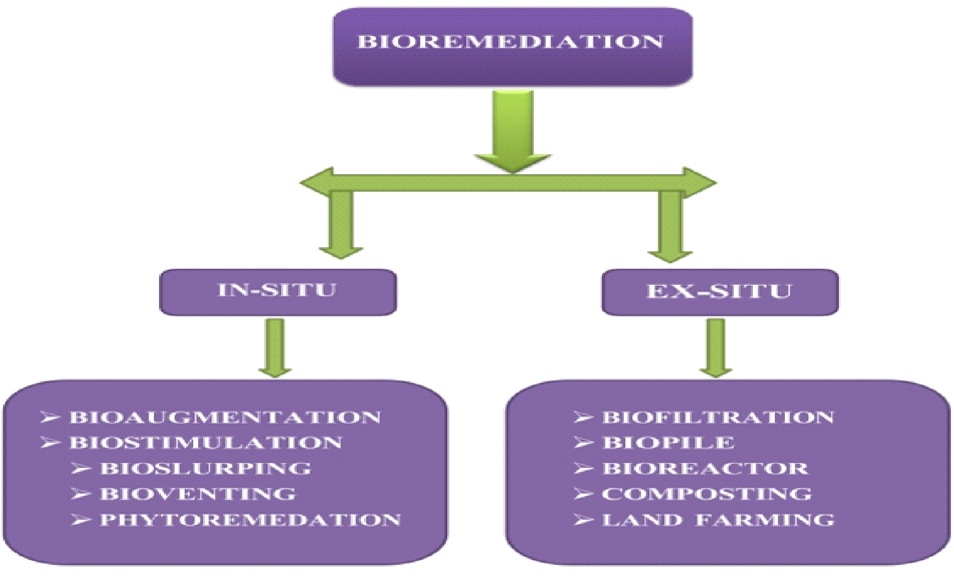

Figure 1: Types of Bioremediation

1.2 a. *In Situ* Bioremediation:

In situ remediation is a cleanup method where biological agents like microbes are used to directly treat contaminants on-site, transforming them into less harmful substances. The major type of *in situ* bioremediation is

- **Intrinsic Bioremediation**

Intrinsic bioremediation also called natural attenuation, is a treatment of polluted sites through naturally occurring microbial population without any human intervention (Hohener and Ponsin 2014; Sui and Li *et al*., 2011). It uses microorganisms to naturally break down environmental pollutants into less toxic substances. It requires monitoring of the role of native microorganisms in eliminating contaminants via tests performed at field sites or on site-derived samples of soil, sediment, or water (Frutos *et al*., 2010). The process is favoured by environmental factors such as pH concentration, temperature and nutrient availability. It is cost effective, takes longer time to reach the target level of pollutant concentration. Engineered bioremediation or accelerated bioremediation is the advanced process of application of engineered systems to stimulate microbial activity for remediation of environmental pollutants (Thome *et al*.,2014). It is time consuming and liable process but is ineffective and reactive with metal contaminants that are mixed with organic compounds (Bilal *et al*.,2019).

In Situ Bioremediation Techniques

1.Bioaugmentation
2.Biostimulation
3.Bioslurping
4.Bioventing
5.Phytoremediation

i.Bio augmentation:

Is the process of addition of microbial culture i.e., strains of natural or genetically engineered bacteria to degrade specific soil and groundwater contaminants. They have been added to the contaminated site to supplement indigenous microflora and speed up biodegradation (Philp and Atlas, 2005; M'rassi *et al*.,2015). Microorganisms are either isolated from polluted sites or genetically modified to cleanup contaminants like chlorinated contaminants and petroleum hydrocarbon *etc*.

ii. Bio-stimulation:

It involves adding nutrients and electron acceptors like phosphorous, nitrogen, oxygen or carbon to the environment to stimulate the growth of existing microorganisms that help clean up petroleum pollutants in soil (Catania *et al*.,2015; Adetute *et al*., 2015).

iii.Bio-slurping:

This process is also called multi-phase extraction. Contaminated soils are remediated in situ through aerobic bioremediation, utilizing bioventing and vacuum enhanced free-product recovery, which extracts light, non-aqueous phase liquids (LNAPLs) from the capillary fringe (Gidarakos and Aivalitoi *et al*., 2007).

Free product which is floating on the water table can be effectively removed through Bio-slurping, which is limited to 25 feet below the ground surface. Aerobic biodegradation is promoted through biosparging, where gas and gas-phase nutrients are injected into the saturated zoneunder pressure and is recommended for the aerobic degradation of lighter to heavier petroleum contaminants such as oils, diesel, gasoline, jet fuels *etc* (Kim *et al*.,2014).

iv. Bioventing:

In situ treatment commonly involves the supply of air and nutrients through wells to contaminated soil, stimulating indigenous microorganisms, particularly for the treatment of petroleum hydrocarbons contaminants in soil. Air is supplied to the unsaturated soil zone using a combination of pumps and blowers, which enables the continuous injection of low volumes of air (Philp and Atlas *et al*.,2005). Bioventing can be categorized as aerobic, anaerobic or co-metabolic depending on the amendments used. The slow removal of air and maintaining 5% oxygen in subsurface is generally practice for bioventing point.

v.Phytoremediation:

Plant and their products are used for the decontamination or stabilization of contaminants and metals from soil. Certain plant varieties have the ability to absorb heavy metals from the soil through their roots and concentrate them in their stems, shoots and leaves. These plants have genes that regulate the metal amount taken up from the soil by roots and deposited at other locations within the plant (Meagher *et al* 2000; Kuiper *et al.*, 2004; Lee *et al*.,2013).

Based on the underlying processes, applicability and contaminant types categorized into the following g types

- Phytodegradation
- Phytostimulation/ rhizodegradation
- Phytovolatilization
- Phytoextraction
- Phyto stabilization.

1.2 b. *Ex-Situ* Bioremediation :

Ex-situ bioremediation also known as off-site bioremediation, is a process where contaminated materials are transported to a separate location for treatment, utilizing microorganisms to breakdown and remove pollutants. These techniques are based on the type of contaminants, site of pollution, degree of pollution, and cost of treatment (Philp and Atlas.,2005).

Techniques in *ex situ* bioremediation

i. Biofiltration

ii. Biopile

iii. Bioreactor

iv. Composting

v. Land Farming

i.Biofiltration

Biofiltration is the biological treatment process of biodegradable waste which relies on biodegrading microbial populations (Whelan *et al.*, 2015). Contaminated air, particularly that polluted with volatile organic compounds, is purified through a process that utilizes various materials including bio-filters, bio-trickling filters, bio-scrubbers, conventional bio-filters to treat the contaminants. Biofilter is treatment bed consisting of compost, soil or peat media inside which the pollutants come into contact with microorganisms and eventually get biodegrade (Dias *et al*., 2015; Gomez and Sartaj 2014).

ii. Biopile

Biopiling is a remediation process where polluted soil is excavated, piled above ground and treated with nutrients and aeration to increase the microbial activity and break down contaminants (Whelan *et al*.,2015). This technique incorporates a comprehensive system including aeration, irrigation, nutrient and leachate collection systems, all integrated within a treatment bed.

Biopile is effective in reducing and limiting the volatilization of low molecular weight pollutants and they also facilitate the remediation of extremely polluted environments. It is a cost-effective approach which ensures effective biodegradation (Gomez and Sartaj ,2013).

iii.Bioreactor

Pollutants are fed into the bioreactor vessel, an engineered system designed to facilitate a series of biological reactions, where they undergo degradation, promoting the growth of biological mass (Mohan *et al*., 2004).

The 5 different operating mechanisms in bio reactor which includes: batch, fed-batch, sequencing batch, continuous and multistage (Chikere *et al*., 2012; Zangi - kotler *et al*.,2015).

Bioreactors maintains suitable controlled environment for the optimum growth conditions that leads to the proliferation of microbial populations (Mustafa *et al*.,2015). Pollutants are fed either in the dry or slurry form (Mohan *et al*., 2007)

iv.Composting

Composting is the process of degradation and decaying or organic waste under favorable controlled conditions with the action waste degrading microorganisms (Piskonen *et al*., 2005). Composting is a self-heating, substrate-dense and solid phase treatment process (Philp and atlas 2005). Microbial population metabolize the organic waste and degrade it to volume by 50% reduction forming the end product called compost or humus (Nikolopoulou *et al*., 2013). Compost is a nutrient rich soil which is very useful in application to the crops and plants for their effective growth (Philp and Atlas., 2005, Paudyan *et al.*, 2008). The steps involved in the process include sorting and separating, size reduction, and digestion of the refuse (Volpe *et al*., 2012; Silva-Castro *et al*.,2015).

v.Land Farming

One of the simplest bioremediation processes of excavation of polluted soil transported to above the ground surface allowing aerobic biodegradation of pollutant by autochthonous microorganisms (Paudyn *et al*.,2008). The autochthonous microorganisms are stimulated by tilling process which involves nutrients amendments (nitrogen, phosphorous *etc*.) aeration process and irrigation (Silva-Castro *et al*., 2012, Cerqueira *et al*., 2014).

It is a cost-effective approach which requires minimal environment and energy for treatment of large volume of polluted soils (Besaltatpour *et al*.,2011, da silva *et al*,2012).

2.0 Environmental pollutants and biodegradation

Genetically modified organisms have been created to treat oil spills and break down certain plastics in polluted areas. Marine environments are greatly affected due to accidental and intentional oils spills during transportation mining and in periods of civil war (Chakdar *et al*, 2022). Xenobiotic compounds persist in ecosystem due to less bio availability and create toxic effects upon exposure to humans and other organisms (Xue *et al*., 2022). Radioactive waste reminds us of high energy radiation is discharged through nuclear power plants volcanic eruption and mining of Radioactive ores these cause great impact on human health.

A. Heavy metals

Trace concentrations of heavy metals are essential for some metabolic processes of living cells. However, elevated levels may have an adverse impact on aquatic, terrestrial organisms and on the environment as they cause dangerous morbidity and mortality (Liao *et al* 2013). Some metals can destruct living cells directly *eg:* mercury, cadmium, lead, and chromium some have indirect effects *eg:* zinc (corrosive) and arsenic (pollute catalysts) (Zehra *et al*., 2020).

Heavy metals can enter the living cells through air, water and food chains, which can alter the physical and chemical properties (Glick and patten 2022). Ecosystem balance is disrupted by heavy metal pollution, which reduces soil microbes that are essential for decomposing organic matter and supporting crop growth, indirectly impacting the food chain, thereby, the world health organization (WHO) and the United States Environmental Protection Agency (USEPA) assigned the acceptable limit for different heavy metals in water as represented in table.

Genetically engineered microorganisms that can remove various heavy metals at once, or transgenic plants with similar capabilities represent a novel and promising approach to bioremediation (Joshi and Thagard 2013; Tellez *et al*.,2022).

S.No	Heavy metals contamination	Degrading Micro organisms
1.	Uranium, Copper, Nickel, Chromium	Pseudomonas aeruginosa, Aeromonas sp

2.	Lead, Chromium, Cadmium	Aerococcus, Rhodo pseudomonas sp.
3.	Chromium	Pseudomonas putida
4.	Mercury, Nickel, Lead	Saccharomyces cerevisiae
5.	Cadmium	Aspergillus fumigatus, Cladosporium sp
6.	Mercury	Pseudochlorococcum typicum, spirogyra hyaline

Table-3: Some of the heavy metals and their degrading microbes (Bala *et al*., 2022)

B. Xenobiotic compounds

Xenobiotic compounds persist in ecosystem due to less availability and create toxic effects to humans and other organisms (Imam *et al* 2022). These are synthetic compounds used in dye, pharma industries, pesticides manufacturing, explosives and other industrial chemicals (Rathore *et al*.,2022). Environmental xenobiotics include pesticides, polycyclic aromatic hydrocarbons, pharmaceutical active compounds, personal-care products, phenolics, chlorinated compounds. These compounds have been released into ecosystem due to anthropogenic activities and improper waste disposal, cannot be readily degraded and have harmful effects on humans and the natural ecosystem (Magan *et al*.,2022). Their toxicity results in unprecedented health hazards and risks to environmental safety and security (Puri *et al*.,2022).

Microorganisms play an essential role in the bioconversion and breakdown of xenobiotics in the environment. Bioremediation involves the metabolic capacities of organisms in the pollutant removal. It is the most suitable and promising technology now a days (Malik *et al*., 2022). Microbial remediation of xenobiotic compounds is regarded as a superficial, efficient, economical. A wide range of microorganisms consume organic pollutants as carbon or nitrogen supplements to maintain their developmental activities (Lindell *et al* 2022).

Most of the aerobic xenobiotics degrading bacterial strains use xenobiotics as the carbon and energy source and they are serves as models for studying the adaptation and evolution of bacteria in the environment. (Rathore *et al*., 2022 and Malik *et al*.,2022). Recent genome analyses of bacterial strains that degrade xenobiotics have strongly suggested mobile genetic elements played important roles in the recruitment of the genes. The importance of microbial symbiosis for xenobiotic degradation has also been suggested some novel concepts in the field of microbial ecology (Nolte *et al*., 2022).

C. Radioactive Compounds

Radioactive waste a high energy radiation discharged through nuclear explosions testing of nuclear weapon accidents at nuclear power plants, volcanic eruption and mining of Radioactive ores that cause great impact on human health (Hu *et al* 2022).

Radioactive waste in the environment can have long lasting effects due to its radioactive properties and slow decay rate (Rafeeq *et al* 2022). These particles can occur in various forms, such as oxides, coprecipitates, organic or inorganic complexes depending on their source and release mechanisms. Generally, they found in oxidized form, so more soluble in water and more mobile. Unlike organic pollutants, which can be broken down, inorganic contaminants must be either transformed into a stable form or extracted from the environment.

Through the common metabolic process of reduction radioactive elements like plutonium or uranium in the target waste can be precipitated to make them easier to collect and dispose (Lloyd *et al*., 2001). Microorganisms can inactivate certain radionuclides by adding electrons which converts them from a soluble to insoluble state. The precipitated radionuclide is still radioactive, but it is not transported into solutions, i.e., ground water, as easily as its soluble counterpart (Fujimoto and morita *et al*., 2006)

In toxic environments, oxygen is used as the electron acceptor in microbial respiration, resulting in the generation of H2O. However, microbes use alternative electron acceptors, including radioactive elements, for respiration when growing in a toxic environment (Lloyd and Macskie.,2000). Oxidized forms of a radioactive elements are reduced by becoming electron acceptors for microbes using anaerobic respiration (Peretrukhin *et al*., 1996).

Direct, indirect and genetically bioengineered reduction

1.Direct Reduction

In anaerobic respiration, when an organism utilizes the oxidized form as an electron acceptor the direct reduction occurs. For example, the Microbes Geobacter metallireducens GS15 and Shewanella oneidensis reduce oxidized soluble plutonium Pu (VI/V) to the reduced insoluble form Pu (IV) (Wildung *et al*., 2000).

2.Indirect Reduction

It occurs when a non-radioactive element is utilized by a microorganism as an electron acceptor. The reduced element from this anaerobic respiration provides electrons for the further reduction of a radioactive element *eg*: Uranium was reduced by ferric Iron by G. metallireducens and S. oneidensis via anaerobic growth. Insoluble radionuclides are easier to dispose of as waste, because the total volume of the waste is reduced.

3.Genetically Engineered Bioreduction

Deinococcus radiodurans can be genetically engineered to metabolize radioactive waste,. The organism consists of potent antioxidants and an enhanced DNA repair mechanisms it acts as radioactive resistant. For example, the microbe has been engineered to express the Escherichia coli merA gene, which encodes a mercuric ion reductase (Premuzic and colleagues., 1985). The organism can convert toxic mercuric to elemental mercury which is lesser harm and easy disposal by expressing reductase.

D. Dye pollutants

When microalgae degrade dyes into carbon and nitrogen sources and later remove from the water, eutrophication in the environment is reduced. Many researchers have studied and documented the biodegradation of various dyes by many microalgae species like Chlorella, Scenedesmus and Aphanocapsa species. The process depends on the molecular geometry of the dye, algae species, and enzyme metabolism azo reductase. Microalgae can remove dye through biosorption, bioconversion or biodegradation. The treatment process for antibiotic removal from effluent of conventional biological waste water is a novel method (Bai *et al*., 2022). Glycosylated lactase glycoproteins have been revealed to have a significant role in industrial colors and phenols. The algal cells, by degrading these pigments and using them in development keep the aquatic environment from becoming too eutrophic. It was discovered that several Chlorella species could break the bond between the azo dye and the aromatic amines to produce simple organic molecules or CO2 as a byproduct. More than 90% of azo dyes were found to be broken down by Chlorella vulgaris activity.

Biodegradation of over 30 azo compounds has been proven by Chlorella pyrenoidosa, Chlorella vulgaris and its capacity to breakdown Reactive Blue 19 (RB19) and Remazol Brilliant Blue R (RBBR) in a range of aquatic environments. C. ellipta, C. kessleri and a variety of other species of cyanobacteria were capable of decomposing both mono and diazo tartrazine. For the removal of nutrients and pollutants from waste water and carbon dioxide from the atmosphere, phytoremediation makes use of microalgae and cyanobacteria (Ahmed *et al*., 2022). Eco- friendly, photoautotrophic bacteria like cyanobacteria are preferred for environmental clean-up because they are harmless, can thrive in non-arable land, and grow quickly (Ji, 2022). Blue-green algae use CO_2 as a source of energy, may produce algal biomass and increases O_2 concentrations. Biogas, biofuels and other valuable things may be produced using these methods. The enzymes excreted by algae and cyanobacteria may convert harmful substances into simpler and harmless, which degrades various contaminants. A review reported that Scenedesmus quadricauda could breakdown Reactive Blue 19 and Remazol Brilliant Blue R dye (RBBR) in a range of aquatic settings. Algae are versatile organisms that thrive in a various environment due to their photosynthetic nature. They play a crucial role in the nutrient cycling and oxygen generation. Recently, Scenedesmus quadricauda, a microalgae has emerged as a new arraival in used biosorption research (Siddiki *et al*., 2022).

E. Plastics

Today, plastics became necessity for the day-to-day life (Patel *et al*., 2022) and are low biodegradable. About 90% of the plastic waste is accumulated in the ocean without a proper waste management strategy Bacteria (Pseudomonas Aeruginosa) (Ogunbayo EX *et al.* 2019) and fungi (Aspergillus niger) species degrade polythene have evolved and their underlining mechanisms are to be uncovered (Debbarma *et al*.2017; Singh *et al*. 2021). In the plastic degradation, the initial steps were attachment of microbes on the plastic surfaces and biofilm.

3.0 Conclusion:

Studies on bioremediation provide evidence that it shows impact on environmental pollutants like heavy metals, xenobiotic compounds, radio active compounds, dye pollutants and plastics *etc*. Genetically engineered microorganisms (GEMs) are beneficial to the environment and humans. Biotechnology and genetic engineering offer safer and cost-effective methods for cleaning pollutants, surpassing conventional methods. GEMs may be a suitable and sustainable method for the bioremediation of contaminated sites.

More research should be done on the development of technologies using self-pollinated transgenic plants and creating infertile polyploidy strains. Discovery of many genes lead to creation of new engineering strains to degrade pollutants. A micro-eco system consists of two or more microorganisms is beneficial to achieve more effective benefit. In industrial areas, genes that can break down contaminants are useful as they produce new pollutants on a regular basis. Plasmids and protoplast fusion technology has shown results in the breeding of Biodegradation-engineered bacteria. Protoplast fusion technique creates recombinant strains of bacteria. In the environment, soil and water pollution may be reduced by using microbial metabolism to minimize the harmful contaminants. Genetic mutations like random mutagenesis by mutagens, site directed mutagenesis, PCR based methods, TA strategy and also gene transfer mechanisms like Molecular cloning, homologous recombination, horizontal gene transfer are also used in bioremediation process. The bioremediation of contaminated areas needs further high-throughput microbial technology study. Genetically engineered microbes are worthy process that will benefit the environment as well as the human health. Further we are interested to do more research in this aspect.

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