

# GENETICALLY ENGINEERED ORGANISMS AND THEIR APPLICATION IN BIOREMEDIATION

## Abstract

Domestic and industrial pollution has been eliminated in the modern environment through physical and chemical means. However, these methods are pricy and harmful to the environment. The development of genetically modified microbes (GEMs) for bioremediation pollution abatement was prompted by the growing environmental contamination. Pollutants like oil spills, camphor, hexane, naphthalene, toluene, octane, and halo benzoates have been broken down using GEMs such bacteria, fungi, and algae. These genetically altered bacteria are quicker to adapt to different contaminants as co-metabolizers or substrates than wild strains, making them more effective than those. Engineered microorganisms can offer a more cost-effective and secure substitute for conventional methods.

**Keywords:** Genetically Engineered Microbes (GEMs), Bioremediation, Heavy metals, Dyes, Pollution.

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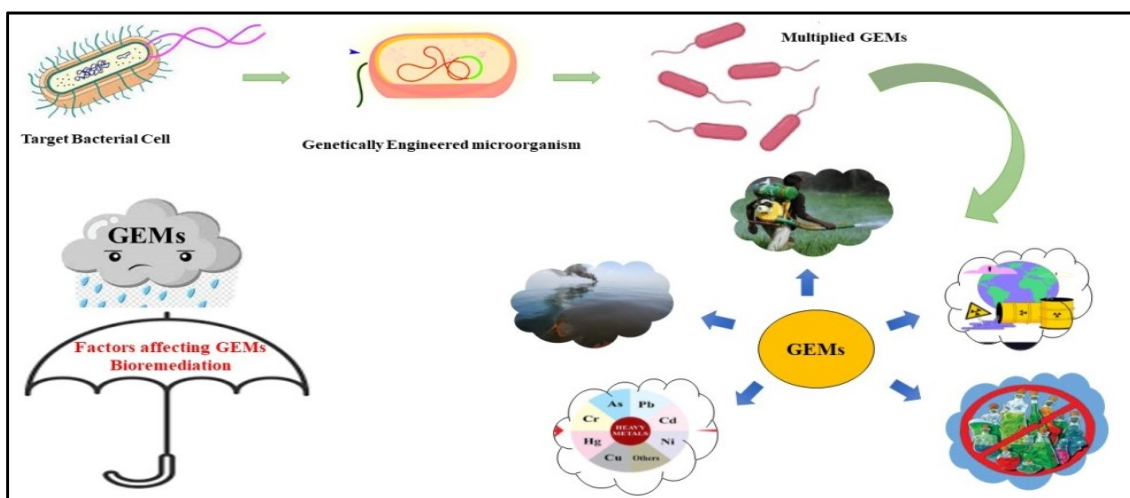
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## Graphical Abstract



## I. INTRODUCTION

Any undesired chemical released into the environment is called a pollutant or contamination. These toxins cause pollution, harming ecosystems, animals, and other living things. We have seen unprecedented levels of the industrial revolution and fast population expansion in recent decades, which have not only increased living standards and our environment [1][2]. The pollutants discharged into the environment through various processes are widely dispersed and extensively incorporated into the ecosystem. The bioremediation procedure is complicated due to the widespread distribution of contaminants.

According to Daar[3], bioremediation is one of the top 10 biotechnologies to improve the global health. Large quantities of harmful chemicals are unintentionally released into the environment, such as when pesticides are applied, or intentionally, such as when oil spills occur. Microbial bioremediation is an environmentally benign method to cure toxic environmental conditions in the context of continuously rising pollution levels. This is far superior to the traditional approaches because it preserves the ecosystem's equilibrium and does not change the natural microenvironment. Recent advances in novel methods integrating multidisciplinary techniques using genetically altered microbes for better bioremediation potential have been proposed to tackle this enduring environmental challenge.

For the effective breakdown of dangerous pollutants of great concern, microbe-assisted bioremediation principally relies on the secretion of enzymes in the metabolic pathways [4]. An essential requirement for bioremediation is the sustained presence of the desired microorganism with the proper catabolic and anabolic capacity [5][6]. Numerous bacteria that can effectively break down xenobiotics and hazardous substances in the environment have been isolated or created. However, the actual use of such organisms in bioremediation has not yet advanced with the same speed as their development or other breakthroughs in the biotechnology field. Since genetically designed microorganisms have been proposed as the prerequisite for bioremediation since naturally existing bacteria, particularly xenobiotics, cannot breakdown all harmful compounds, the genetic engineering of microorganisms has evolved. Many effective engineering bacteria with enhanced pollutant degradation abilities were created with the development of recombinant DNA and genetic engineering technologies in microbial breeding, significantly increasing the degradation efficiency of pollutants [7][8]. Several techniques have been utilised to create engineered strains to hasten the process of environmental governance, in addition to screening strains by natural mutation or physicochemical mutagenesis: (1) finding and cloning highly efficient degrading genes, (2) increasing the expression of enzymes with degradative functions in microorganisms, (3) expressing degradation genes for various pollutants in a recipient to create super-engineered bacteria, and (4) protoplast fusion by combining the advantages of both parents for degradative pollution. Furthermore, creating genetically modified bacteria with high degradation efficiency was made possible by discovering genes, degradation pathways, and mechanisms in bacteria [1].

Using standard technologies like iron exchange, precipitation-filtration, reverse osmosis, oxidation-reduction, and membrane separation, heavy metal removal from contaminated areas is highly challenging. Despite being slow, these methods still cause a significant buildup of toxicity in the environment. Conventional technologies, which concentrate on removing impurities rather than eliminating them, are costly, inefficient, and time-consuming. Bioremediation requires understanding physio-chemical traits, such as

structure, phenotypic potential, and function of genetically engineered organisms (GEO) interactions with the environment [9]. According to Eapen [10], Macek [11], Doty [12], and Panz[13], bioremediation utilising GEO is a relatively affordable, ecologically benign, and socially acceptable technology that may permanently eradicate waste. However, GEO for bioremediation has not been extensively approved for discharge into the environment or commercial use. Technical protections and appropriate regulatory practices, such as doing sufficient risk assessments and monitoring, could be used by scientists to ensure the use of GEO for bioremediation [14]. Natural bacteria cannot remove contamination from heavy metals like mercury, but genetically modified bacteria can. Eapen [10], Macek [11], Doty [12], and Panz[13] claim that the use of GEO in bioremediation is a relatively inexpensive, environmentally safe, and socially acceptable method that might eliminate waste. However, GEO for bioremediation has not received widespread approval for commercial use or discharge into the environment. Scientists could ensure the use of GEO for bioremediation by using technical safeguards and suitable regulatory processes, such as doing adequate risk evaluations and monitoring [14]. Heavy metal contamination, such as mercury, cannot be removed by natural bacteria, but genetically altered bacteria can do it.

## II. ADVANCEMENTS IN BIOREMEDIATION

Natural and wild microbial strains decompose waste and contaminants more slowly and with less power. Ex-situ and in-situ techniques are used for bioremediation. The ex-situ approach costs more and is less effective. Introducing genetically modified microbial strains into the polluted locations in situ is an economical and environmentally responsible strategy utilised for indirect reduction [15].

By introducing genetically altered microorganisms to boost the activity of insufficient native microbes, bioaugmentation can aid in the bioremediation of contaminated environments [16] [17] [18]. Microbes can build biological tolerance to any environmental toxin due to specialised jumping genes. These genetic changes include various techniques (such as replacement, hybridisation, and induced mutation) that alter the genetic makeup of microorganisms to produce the intended effects. While genetic engineering is one type of genetic modification that entails purposefully making a targeted change to a microbial gene sequence to accomplish a specific result [2].

GEMs, or genetically engineered microorganisms, are microorganisms (bacteria or fungi, including yeasts) that have undergone human genetic engineering utilising techniques from contemporary biotechnology. Genes are inserted into a single bacterium, giving GEMs the characteristics of several microorganisms [19]. GEMs can be utilised successfully for bioremediation purposes since bioremediation is a process that makes it easier to destroy and eliminate environmental pollutants utilising microorganisms or their enzymes. Ananda Chakrabarty, an Indian-born scientist and genetic engineer, developed the first genetically edited microorganism in 1971. The United States Supreme Court approved the patent in 1980. The bacteria, which belonged to the *Pseudomonas* genus, could dissolve the components of crude oil. Chakrabarty demonstrated that four strains of the widespread *Pseudomonas* bacteria possessed the enzymes necessary to degrade various hydrocarbons. He initially discovered that the genes for oil-degrading enzymes were found on extra-chromosomal components known as plasmids rather than the microorganism's chromosome. He created a strain of *Pseudomonas* using these plasmids combined.

Bacteria can significantly degrade environmental pollutants [20]. Biphenyls, polychlorinated biphenyls (PCBs), nitroaromatics, chloroaromatics, polycyclic aromatics, and oil components are just a few of the pollutants that bacteria can break down [20]. These bacteria have been isolated to potentially use their metabolic capacity for bioremediation of polluted sites. Although some of the more stubborn and dangerous xenobiotic substances, such as highly nitrated and halogenated aromatic compounds, as well as some pesticides and explosives, are typically stable, chemically inert under natural conditions, and are not thought to have been effectively degraded by many microorganisms [21], some of these substances are still harmful to humans and animals. A significant barrier to efficient microbial biodegradation is the toxicity of specific organic contaminants to the existing microbial communities and the difficulties brought on by pollutant combinations. Due to these restrictions, bacterial strains with significant bioremediation potential above other microorganisms can now be artificially designed to have efficient catabolic pathways [19]. Composting electro-bioremediation, microbe-assisted phytoremediation, and other biostimulation and bioaugmentation-based methods fall within the category of bioremediation [22]. To improve the natural ability of microbes for remediation with cutting-edge scientific discoveries, genetic engineering is popular today. It has been noted that successful in situ bioremediation utilising genetically modified microbes require knowledge of biotechnology and ecology, field engineering approaches, and biochemical processes [19].

### III. GEMS FOR ENVIRONMENT RESCUE AGAINST POLLUTANTS

According to research by Jacob [2], bioremediation is the only method to effectively decontaminate polluted sites while being safer, cleaner, sustainable, and affordable. Therefore, selecting the right microbial strain is difficult regarding its potential, quick growth, nutrient reactions, and engineering. When selecting and engineering a specific bacterial strain, there are a few considerations to be made, such as the presence of genes for metal homeostasis, biodegradative enzymes, metal uptake, synthesis of metal chelators, genes for survival in biotic and abiotic stress conditions, etc. [23]. According to Singh [24] and Liu [19], recombinant DNA technology effectively transforms organisms (bacteria, fungi, etc.) into the form that is wanted. It employs a vector (phage, plasmid, or virus) into which the desired gene has been inserted, allowing gene expression in the selected host [6]. The technique improves the value of the bioremediation process. Reverse transcriptase, alkaline phosphatase, T4 polynucleotide kinase, host, S1 nuclease, Klenow fragment, exonuclease, linker, terminal deoxynucleotidyl transferase, and adaptor molecules are among the necessary tools [25].

**Table 1: Genetically engineered organisms are involved in various processes (Adapted from Pant et al., 2021)**

S.No	Bacteria	Genetically modified organism	References
1	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas fluorescens</i> HK44	[26]
2	<i>Mesorhizobium huakuii</i>	<i>Mesorhizobium huakuii</i> sub sp. Rengei B3	[24]
3	<i>Pseudomonas putida</i>	<i>Pseudomonas putida</i> KT2440	[27]
4	<i>E. coli</i>	<i>E. coli</i> JM10	[28]
5	<i>Sphingomonas</i>	<i>Sphingomonas desiccabilis</i>	[29]
6	<i>Ralstonia eutropha</i>	<i>Ralstonia eutropha</i> CH34	[24]

7	Achromobacter	Achromobactersp AO22	[30]
8	Spingomonaspaucimobilis	Spingomonaspaucimobilis UT26XEGM	[31]
10	Sphingobium japonicum	Sphingobium japonicum UT26	[32]
11	Sphingobium indicum	Sphingobium indicum B90A	[33]
12	Stenotrophomonas sp	Stenotrophomonas sp YC-1	[34]
13	Pseudomonas sp	Pseudomonas sp BF1-3	[35]
14	Bacillus subtilis	Bacillus subtilis 168 YCMarsM	[36]

#### IV. BIOREMEDIATION OF COMPOUNDS

- 1. Heavy Metals:** Heavy metals like Cadmium (Cd), Nickel (Ni), Mercury (Hg), Cobalt (Co), Arsenic (As), Lead (Pb), and others are being released into the soil and water system at an alarming rate due to the rapid rise of industrialisation[37]. These heavy metals infiltrate the bodies of living things and interfere with their organs' physiological functions. Therefore, there is a pressing demand for heavy metal cleanup. However, genetically modified bacteria used in bioremediation is typically more environmentally benign. The technique of bioremediation has dramatically benefited from the use of genetically modified bacteria (Table 2). The genetically altered bacteria's metal detoxification machinery is essential to the entire metal detoxification process [38]. The dangerous heavy metal generated by numerous anthropogenic and natural processes that have received the most research is mercury. There is a study on the Hg reductase enzyme's detoxification of Hg into harmless Hg. According to Mukkata[39], this method is based on the clustered genes in an operon (mer). Tn5053 (Xanthomonas sp. W17), pKHL2 (Acinetobacter calcoaceticus JM83), p1258 (Staphylococcus aureus), transposon Tn21 (Shigella flexneri), and other forms of mer operons are among the many bacteria species that include them [24]. Research shows severe cadmium pollution in water sites [40]. The extreme toxicity of arsenic is another well-known characteristic[29]. By invading plants and absorbing minerals and water through root systems, these hazardous metals reach the food chain and pose severe risks to humans and ecosystems [41].
- 2. Dyes:** According to Luo [42], dyes and pigments are essential in various sectors, including textile, paper, pulp, etc. The dyes can inhibit the growth of aquatic flora and fauna, reducing the solubility of gases within the aquatic ecosystem [43]. These pollutants are directly or indirectly responsible for acute, mild, and chronic human diseases when they enter the body [44]. In addition, injuries to the reproductive, digestive, and central neurological systems are frequently recorded. In addition, certain dyes have been shown to cause cancer [45] [46]. Dyes are regarded as major environmental contaminants worldwide due to their excessive discharge in effluents and their well-known harmful effects on ecological balance and human health [47]. Applying genetically modified microorganisms gives a new path for creating a humane strategy, even though native bacteria have demonstrated the capacity for bioremediation of several dyes (Table 2) [45].
- 3. Xenobiotics:** Xenobiotics are manmade and artificial compounds with long shelf life in the ecosystem because of their intricate organic makeup. They are well-known as the main class of pollutants linked to chemical-induced toxicity in people and direct danger threats within a short exposure duration in the ecosystem [20]. The scope of bioremediation has expanded over conventional procedures due to the use of microbial

treatments with high specificity and efficiency for treating xenobiotics [48]. As indicated in Table 2, a more modern genetic engineering technology that creates microorganisms with improved degradative capabilities paves the way and offers prospects for detoxifying such persistent contaminants from the environment. Such designed microorganisms can be created using both in vitro and in vivo techniques, such as gene cloning and the strain-to-strain transfer of a complete plasmid [21]. According to Undugoda [49], the bacteria use various methods, such as oxidation, reduction, hydrolytic cleavage, dehalogenation, etc., to bioremediate organic xenobiotics from the polluted site.

4. **Pesticides:** According to Zuo [50], pesticides are chemical compounds that kill pests (plant or animal life), primarily organic pollutants. Due to the increased need for pesticides to sustain human well-being, pesticide manufacturing enterprises often produce complex chemical pesticide waste that could endanger the atmosphere [51]. Pesticides harm the ecosystem because of their high-water solubilities and soil stabilities [40]. Pesticides with half-lives between 100 and 200 days include dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), atrazine, and endosulfan. Pesticides are known to contribute 6.3% of volatile emissions into the environment. Contrarily, pesticides can quickly enter living things' tissues and cause bioaccumulation. As a result, microorganisms were employed in the situation, minimising soil pesticide contamination. The available species, however, could not significantly impact the bioremediation of pesticides [34]. In order to create modified organisms that break down pesticides (Table 2) and the wastes produced during the manufacture of pesticides, genetically engineering technologies were used [52].
5. **Organic compounds:** Since they produce toxic intermediates, several conventional strategies to remediate toxic wastes have been attempted for many years, but with slight effectiveness [29]. Microorganism use is a viable alternative with minimal adverse effects [53]. Numerous microbes have been developed to clean up these severely polluted locations because organic pollutants and heavy metals are often co-contaminated (Table 2). *Mycobacteria* sp., for instance, has lipophilic surfaces that can take up bound contaminants from soil and have catabolic efficiency towards PAHs up to 5 benzene rings, making it an excellent choice for remediating sites contaminated with polyaromatic hydrocarbons (PAHs) [54]. In addition to biological factors, the water and soil systems' physical characteristics, such as pH, temperature, water content, redox potential, solubility, co-contaminants, microbial communities, nutrients, organic matter, volatility, particle size, etc., can also support or promote bioremediation [29]. When used for cleanup, biosurfactants can sometimes increase the availability of organic contaminants [5]. These compounds, which have both hydrophilic and hydrophobic components, increase bioavailability by removing contaminants from surfaces or by improving the apparent solubility of the surfaces and by controlling the attachment and detachment of microbes to and from surfaces [54].
6. **Oil and its components:** Oil spill cleanup becomes necessary when petrochemical businesses leak liquid petroleum hydrocarbon into the atmosphere, primarily into maritime areas [55]. Oil spills in the ocean harm marine life, particularly filter feeders like clams and oysters and seabirds like seagulls and ducks. The type of microbial species, using fertilisers or inorganic nutrients, and aeration significantly impact how well these sites are remedied [52]. Additionally, it has been noted that removing salt from oil-polluted locations before the bioremediation process begins may shorten the time needed

[56]. Thus, bioremediation promises to treat such oil spills more quickly [57]. Genetically engineered organisms are a superior alternative since they are changed with the created machinery and oil-degrading enzymes, making them more efficient in a shorter time. Microbes like algae, bacteria, and fungi are less effective. The remediation and degradation of the hazardous chemicals found in crude oil have also been improved by genetically modified bacteria (Table 2). Despite some successful occurrences, bioremediation is seen in cold climates as a lengthy process for clearing oil from the sites, especially in polar locations (arctic and sub-arctic regions) [58].

**7. Radioactive Compounds:** Low-level radioactive waste is created by several nuclear enterprises, research institutions, universities, hospitals, etc. These wastes build up and cause radiological pollution [59]. The bioremediation process is typically made more difficult by organic pollutants, heavy metals, and other contaminants in radioactive waste. Given that normal creatures cannot clean up these extremely polluted places, it presents a chance for genetically modified organisms [60]. The biotransformation of such contaminants into an insoluble form that precipitates is a component of the bioremediation of radioactive substances [61]. The utilization of genetically modified organisms, which are more resilient and have a higher chance of surviving in highly radioactive waste sites than wild bacteria, is necessary for the bioremediation of radioactive substances (Table 2). According to Choi [62], no such papers describe the capacity of wild-type microbe strains to break down or transform radioactive compounds into less dangerous forms. The bacterium *Deinococcus radiodurans*, which is the most radioresistant organism found to date, has undergone genetic modification to enable it to digest radioactive iodine (>99%) and ionic mercury from highly radioactive nuclear wastes [60]. Therefore, genetically modified bacteria may be the quickest and safest method to transform or destroy radioactive materials. Engineering microbes to produce enzymes for industry by offering active regions that reduce the reaction's activation energy, enzymes serve as biological catalysts for converting substrates into products [63]. For decades, several investigations have established microbial-associated enzymes' biodegradation of hazardous chemicals (Table 2). Incorporating cutting-edge methods like functional genomics, proteomics, metabolomics, and recombinant DNA technology into industrial microbiology has a significant positive impact on both the quality and quantity of industrial enzyme production [64]. The various classes of industrial enzymes, including laccases, peroxidases (lignin peroxidases, manganese peroxidases, and versatile peroxidases), and hydrolytic enzymes (lipases, cellulases, and proteases), demonstrate their affinity towards environmental pollutants [65]. Future sources for addressing environmental challenges related to pollutants could include these biotechnology-based eco-friendly, economically viable facilities [66].

**Table 2: List of modified organisms for the degradation of various contaminants**

Modified organisms	Modified gene expression	Applications	References
<b>List of modified organisms for the degradation of heavy metal ions</b>			
<i>Deinococcus radiodurans</i> (radiation-resistant)	MerH, a newly incorporated ion transporter gene from the <i>M. marinum</i> strain	Mercury ion degradation	[6]

Mesorhizobium huakuii	Arabidopsis thaliana-derived PC-coding genes were inserted for transformation.	Cd <sup>2+</sup> degradation	[37]
E.coli SE5000 strain	Nickel transporting system is expressed (products of nixA gene)	Degradation of Nickel in the aquatic system	[38]
<b>List of modified organisms for the degradation of dyes</b>			
Pseudomonas fluorescens strain	Expression of Sz6 and SDz3 genes	Decolorization of various dyes and reduction of toxicity to plants and animals	[[45]]
Bacillus amyloliquefaciens D501G variant	Site-specific mutagenesis	Enhanced catalytic effectiveness and stability against indigo carmine.	[47]
Streptomyces lividans strain	Homologous expression	Increases the catalytic effectiveness and thermostability against indigo carmine	[44]
<b>List of modified organisms for the degradation of organic xenobiotics</b>			
Pseudomonas diminuta	Recombinant expression	Phosphotriesterase enzyme activation for organophosphorus detoxification	[48]
Alcaligenes sp.	Expression of DMFase gene	Increased microbial activity and synthesis of phytoremediation activation compounds	[67]
Pandoraea sp.	Expression of Lactane gene	By increasing the production of several primary metabolites, it improved xenobiotic biodegradation.	[20]
<b>Genetically engineered microorganisms for bioremediation of pesticides</b>			
Escherichia coli	pL-DsRed-pL-OPH and Pds plasmid insertion	Degradation of Organo Phosphorus pesticides	[34]
Moraxella sp.	Contains organophosphorus hydrolase that is surface-expressed	Degradation of organophosphorus insecticides and p-nitrophenol (PNP)	[68]
Sphingomonas sp. CDS1	Methyl parathion hydrolase gene insertion (mpd) into the chromosome	Degradation of methyl parathion and carbofuran	[69]



<b>List of modified organisms for the degradation of organic pollutants</b>			
P. putida KT2442	Pathway Modification	Degradation of toluene/benzoate	[70]
Comamonas Testosteroni VP44	Substrate specificity Modification	Degradation of o-, p-monochloride-biphenyls	[53]
P. pseudo-alcaligenes KF707-D2	Substrate specificity Modification	Degradation of TCE, toluene, benzene and flourobiphenyl	[29]
<b>List of genetically modified bacteria to remediate oil pollution</b>			
Pseudomonas putida	NAH and XYL plasmids are present together with a hybrid plasmid (from CAM and OCT).	Degradation of crude oil	[71]
Pseudomonas fluorescens HK44	pUTK21 plasmid harboring the nah gene is present.	Degradation of Naphthalene	[72]
Rhodococcus sp. RHA1	Contains pPC3 with fcboperon derived from A. globifirmis	4- chlorobenzoate degradation	[52]
<b>Various GEMs for bioremediation of radioactive compounds</b>			
Deinococcus radiodurans	Resistance to more radioactive substances, such as integration of the merH locus, is increased by plasmids	Radioactive substances, such as toluene, are degraded	[60]
D. geothermalis	D. radiodurans induced plasmids are used.	Degradation of Fe(III) nitrilotriacetic acid, Hg(II), U(VI), and Cr(VI)	[61]
Deinococcus radiodurans R1	For some highly radioactive elements, plasmids are employed to boost resistance.	Radioactive iodine removal (>99%)	[62]

## V. FACTORS AFFECTING GEMS-ASSISTED BIOREMEDIATION

Guthrie [73] assert that it is crucial to take these aspects into account in order to maximise the benefits of the bioremediation process. The toxic and hydrophobic properties of the hydrocarbons found in petrochemicals increase the process' complexity. Due to the diversity of microbial communities and the current environmental conditions, the complexities are further diversified. As the literature shows, the bioremediation process is constrained by several physical, chemical, and biological constraints. The effectiveness of the GEM-assisted bioremediation process is influenced by several factors, including pH, temperature, nutritional status, dissolved oxygen content, electron donors and acceptors, contaminant load, etc. [74]. Interactions between the substrate and the pollutants impact their degradation rate as well [75]. Interactions between substrates at various concentrations have a significant impact on bacterial metabolism. Due to their synergistic effects, catabolic

enzymes have been identified to accelerate the breakdown of specific contaminants. Beyond a particular concentration, the BTEX chemicals exhibit an inhibitory effect on microbial activity due to their intricate interactions [76]. The primary limiting elements affecting the degradation of petrochemical wastes will be highlighted in the following sections. The microbial bioremediation process depends on several variables for the best pollutant removal. Below is a discussion about them.

- 1. Concentration of the Contaminant:** The contaminant's concentration influences microbial activity. Bacterial degradation enzymes are prevented from being induced if the contaminant concentration is minimal. However, extremely high pollutant concentrations cause toxicity consequences [77]. The synergistic interactions between several contaminants enhance the catabolic enzymes' degradation rates. *Pseudomonas putida* growth rate was slower in batch culture at high substrate concentrations [78]. BTEX chemicals demonstrated an inhibiting effect [76] on the biodegradation process due to intricate microbiological interactions.
- 2. Nutrient Availability:** Microbes need calcium, potassium, phosphorus, nitrogen, and carbon to grow. Additionally, the relative concentrations of the nutrients that are readily available affect how quickly contaminants degrade. Excess nitrogen, potassium, and phosphorus in the degradation of hydrocarbons had a detrimental effect on biodegradation [79]. The bioavailability of organic contaminants, commonly referred to as accessibility to microorganisms, affects the biodegradation rate.
- 3. Characteristics of the Contaminated Site:** The parameters of the contaminated site considerably influence the GEM-assisted bioremediation process. The soil texture, permeability, pH, water-holding capacity, soil temperature, nutrient and oxygen content, and soil temperature all impact the microbial bioremediation process.
- 4. Ph:** The GEM-assisted bioremediation procedure requires an ideal pH connected to the pollutant at the contaminated site. According to Adams [77], the ideal pH range is between 6 and 8 (International Centre for Soil and Contaminated Sites, 2006). It should also be emphasised that bacteria can live in the polluted site's conditions and even break down polyaromatic hydrocarbons at high pH levels [80]. The mineralisation of petroleum hydrocarbons benefits from neutral pH. According to current research, even a temperature range of 15°C–20°C may impact biodegradation [81]. Higher temperatures have been found to increase the solubility of organic hydrocarbons in the medium, making petrochemical hydrocarbons more accessible to a little change in pH. Stapleton [82] found that in acidic conditions, certain fungi and acidophilic bacteria had a higher propensity for biodegradation.
- 5. Temperature:** The temperature impacts the degradation of the pollutant, particularly the hydrocarbon, both in situ and ex-situ [83]. Higher temperature ranges, such as 30°C to 40°C, have been found to accelerate soil biodegradation. This is also true for microorganisms living in aqueous or marine environments.
- 6. Oxygen Availability:** The bioremediation process is either aerobic or anaerobic, depending on oxygen availability. The function of monooxygenase and dioxygenase enzymes in the oxidation of the aromatic ring depends on the brief first steps of aerobic metabolism of PAH oxygen [80]. In their substituted forms, ferrous iron, nitrate, and

sulfate are required as electron acceptors during the anaerobic oxidation of aromatic molecules. However, the process causes the ecosystem to become contaminated by high phosphorus levels and ferrous ions. Petrochemical hydrocarbons undergo anaerobic decomposition, which raises pH and releases greenhouse gases like methane and nitrogen dioxide. Anaerobic processes are primarily responsible for bioremediation in buried marine sediments and aquifers [84].

The following elements influence biodegradation to varying degrees:

- The contaminant's capacity to biodegrade
- Whether the contaminant's biodegradability happens spontaneously, that is, on its own
- Environmental factors that are favourable for biodegradation to take place
- The sink for the waste in the case that biodegradation fails.

Higher levels of organic matter are seen in surface soils. This is because the variety of microorganisms and their composition is more significant. Contrarily, because of more diversity and a greater abundance of microorganisms, the organic matter content of subsurface soil and groundwater sediments is reduced [85]. With increasing depth, bacteria that can employ alternate electron acceptors have become more common than other microbial communities. Other elements affecting the extent to which microbial communities spread include temperature, moisture content, and dissolved oxygen. The selection of suitable microorganisms has a significant impact on the success of bioremediation. It should be emphasised that for a technology to be effective, the surrounding environment must also be supportive. Traditionally, incineration and the construction of landfills have been used to eliminate pollution. However, microbial-assisted bioremediation has taken its place.

## VI. CONCLUSION

The variety of microbial population needed to degrade the pollutants is one aspect that determines whether the bioremediation procedure is successful. (ii) The contaminants' accessibility for the microorganisms to act. (iii) Aspects of soil include pH, temperature, soil type, oxygen availability, and other nutrients. The field requirements and other complex conditions that can arise later are ignored in the engineering of bacteria [24]. These GEMs may include self-destruction mechanisms (vectors or suicide genes), or they could only function in the specific environment for which they were created. Therefore, in addition to bioremediation, specific parameters should be considered while creating any desired microorganism. These elements may influence the microflora existing in a particular habitat, including their capacity for horizontal gene transfer and their capacity for survival. Due to unknown concerns, the potential value of modified bacteria for bioremediation is not without risk when released into the environment.

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