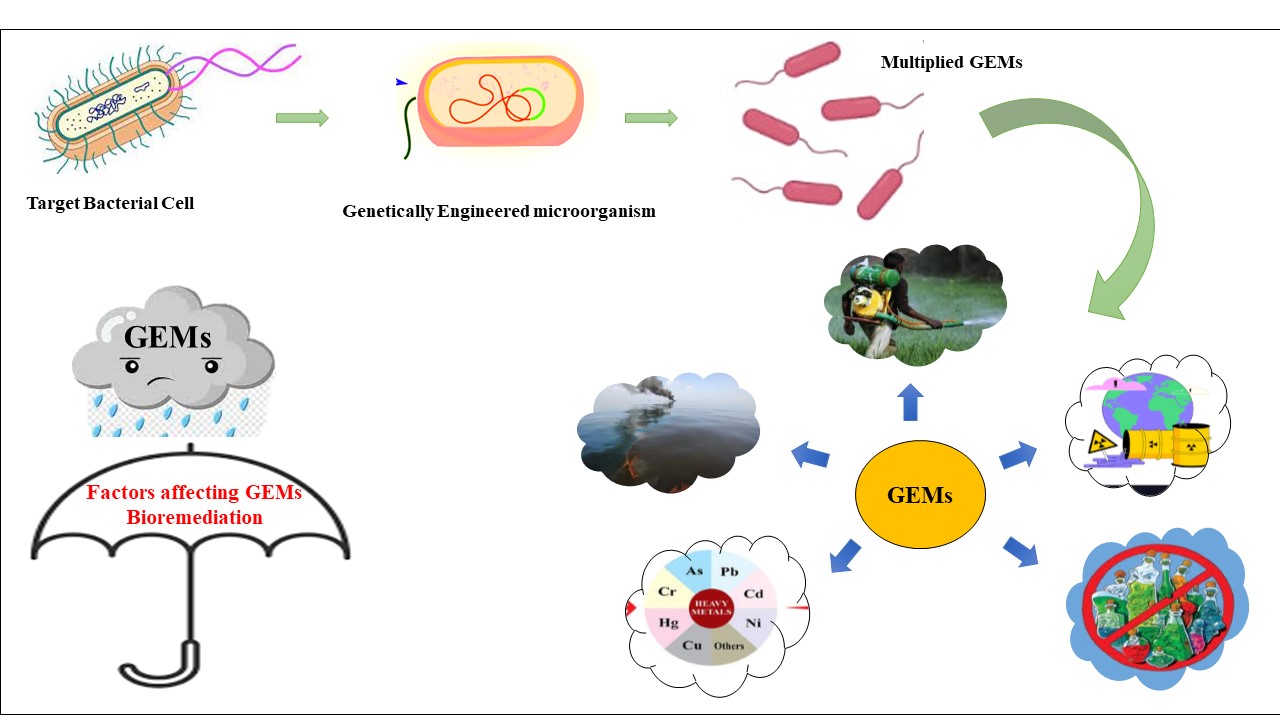
**GENETICALLY ENGINEERED ORGANISMS AND THEIR APPLICATION IN BIOREMEDIATION**

**Edward InpentCampal1, Nallaiah Hemamalini2, Shanmugam Sudarshan1, Sathiyanesan Subanesam1**

**1TNJFU-Dr. MGR. Fisheries College and Research Institute, Thalainayeru, Nagapattinam - 614 712, Tamil Nadu. India.**

**2TNJFU-Fisheries College and Research Institute, Thoothukudi – 628008, Tamil Nadu. India**

**Graphical Abstract**

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**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.

**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 biotechologies 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 break down 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.

**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 bio-remedial 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].

**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 | *Pseudomonas fluorescens* | *Pseudomonas fluorescens* HK44 | [26] |
| 2 | *Mesorhizobium huakuii* | *Mesorhizobium huakuii sub sp.* Rengei B3 | [24] |
| 3 | *Pseudomonas putida* | *Pseudomonas putida* KT2440 | [27] |
| 4 | *E. coli* | *E. coli* JM10 | [28] |
| 5 | *Sphingomonas* | *Sphingomonas desiccabilis* | [29] |
| 6 | *Ralstonia eutropha* | *Ralstonia eutropha* CH34 | [24] |
| 7 | *Achromobacter* | *Achromobacter sp* AO22 | [30] |
| 8 | *Spingomonas paucimobilis* | *Spingomonas paucimobilis* 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] |

**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].

1. **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].

1. **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.

1. **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].

1. **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].

1. **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].

**vii)** **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 utilisation 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** |
| **List of modified organisms for the degradation of heavy metal ions** | | | |
| *Deinococcus radiodurans* (radiation-resistant) | MerH, a newly incorporated ion transporter gene from the *M. marinum* strain | Mercury ion degradation | [6] |
| *Mesorhizobium huakuii* | *Arabidopsis thaliana*-derived PC-coding genes were inserted for transformation. | Cd2+ degradation | [37] |
| *E.coli* SE5000 strain | Nickel transporting system is expressed (products of nixA gene) | Degradation of Nickel in the aquatic system | [38] |
| **List of modified organisms for the degradation of dyes** | | | |
| *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] |
| **List of modified organisms for the degradation of organic xenobiotics** | | | |
| *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] |
| **Genetically engineered microorganisms for bioremediation of pesticides** | | | |
| *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] |
| **List of modified organisms for the degradation of organic pollutants** | | | |
| *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] |
| **List of genetically modified bacteria to remediate oil pollution** | | | |
| *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 fcb operon derived from *A. globifirmis* | 4- chlorobenzoate degradation | [52] |
| **Various GEMs for bioremediation of radioactive compounds** | | | |
| *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] |

**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.

**a. 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.

**b. 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.

**c. 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.

1. **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 15oC–20oC 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.

1. **Temperature**

The temperature impacts the degradation of the pollutant, particularly the hydrocarbon, both in situ and ex-situ [83]. Higher temperature ranges, such as 30oC to 40oC, have been found to accelerate soil biodegradation. This is also true for microorganisms living in aqueous or marine environments.

**iii)** **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:

1. The contaminant's capacity to biodegrade

2. Whether the contaminant's biodegradability happens spontaneously, that is, on its own

3. Environmental factors that are favourable for biodegradation to take place

4. 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.

**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.

**Reference:**

[1] Zhao, Q., Yue, S., Bilal,M., Hu, H.,Wang,W., Zhang, X., 2017a. Comparative genomic analysis of 26 Sphingomonas and Sphingobium strains: dissemination of bioremediation capabilities, biodegradation potential and horizontal gene transfer. Sci. Total Environ. 609, 1238–1247.

[2] Jacob, J.M., Karthik, C., Saratale, R.G., Kumar, S.S., Prabakar, D., Kadirvelu, K., Pugazhendhi, A., 2018. Biological approaches to tackle heavymetal pollution: a survey of literature. J. Environ. Manag. 217, 56–70.

[3] Daar AS, Thorsteinsdottir H, Martin DK, Smith AC, Nast S, Singer PA. Top ten biotechnologies for improving health in developing countries. Nature Genetics 2002;32(2):229–32.

[4] Dangi, A.K., Sharma, B., Hill, R.T., Shukla, P., 2019. Bioremediation through microbes: systems biology and metabolic engineering approach. Crit. Rev. Biotechnol. 39 (1), 79–98

[5] Singh U, Arora NK, Sachan P. Simultaneous biodegradation of phenol and cyanide present in coke-oven effluent using immobilized Pseudomonas putida and Pseudomonas stutzeri. Brazilian Journal of Microbiology 2018; 49: 38-44.

[6] Gupta V, Sengupta M, Prakash J, Tripathy BC. Basic and Applied Aspects of Biotechnology: Springer, 2016.

[7] Tropel, D., Van Der Meer, J.R., 2004. Bacterial transcriptional regulators for degradation pathways of aromatic compounds. Microbiol. Mol. Biol. Rev. 68 (3), 474–500.

[8] Zhao, Q., Bilal, M., Yue, S., Hu, H.,Wang,W., Zhang, X., 2017b. Identification of biphenyl 2, 3-dioxygenase and its catabolic role for phenazine degradation in Sphingobium yanoikuyae B1. J. Environ. Manag. 204, 494–501.

[9] Rittmann BE, Hausner M, Loffler F et al (2006) A vista for microbial ecology and environmental biotechnology. Environ Sci Technol 40:1096–1103

[10] Eapen S, Singh S, D’Souza S (2007) Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol Adv 25:442–451

[11] Macek T, Kotrba P, Svatos A et al (2007) Novel roles for genetically modified plants in environmental protection. Trends Biotechnol 26:146–152

[12] Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333

[13] Panz K, Miksch K (2012) Phytoremediation od explosive (TNT, RDX, HMX) by wild-type and transgenic plants. J Environ Manag 113:85–92

[14] Ezezika OC, Singer PA (2010) Genetically engineered oil-eating microbes for bioremediation: prospectus and regulatory challenges. Technol Soc 32:331–335

[15] Yadav A, Chowdhary P, Kaithwas G, Bharagava R. Toxic metals in environment, threats on ecosystem and bioremediation approaches. Handbook of metalmicrobe interactions and bioremediation. CRC Press, Taylor & Francis Group, Boca Raton 2017; 813.

[16] Vidali M (2001) Bioremediation: an overview. Pure Appl Chem 73:1163–1172

[17] Silva E, Fialho AM, Sa-Correia I et al (2004) Combined bioaugmentation and biostimulation to cleanup soil contamined with high concentrations of atrazine. Environ Sci Technol 15–38:632–637

[18] Li Y, Li B (2011) Study on fungi–bacteria consortium bioremediation of petroleum contaminated mangrove sediments amended with mixed biosurfactants. Adv Mat Res 183–185:1163–1167

[19] Liu L, Bilal M, Duan X, Iqba HMN. Mitigation of environmental pollution by genetically engineered bacteria - Current challenges and future perspectives. Sci Total Environ. 2019; 667:444-454.

[20] Peeters C, De Canck E, Cnockaert M, et al. Comparative Genomics of Pandoraea, a Genus Enriched in Xenobiotic Biodegradation and Metabolism. Front Microbiol. 2019; 10: 2556.

[21] Saccomanno M, Hussain S, O'Connor NK, et al. Biodegradation of pentafluorosulfanyl substituted aminophenol in Pseudomonas spp. Biodegradation. 2018; 29(3):259–270.

[22] Ghanian M, Ghoochani OM, Kitterlin M, Jahangiry S, Zarafshani K, Van Passel S, et al. Attitudes of agricultural experts toward genetically modified crops: A case study in Southwest Iran. Science and engineering ethics 2016; 22: 509-524.

[23] Kamthan A, Chaudhuri A, Kamthan M, Datta A. Genetically modified (GM) crops: milestones and new advances in crop improvement. Theoretical and applied genetics 2016; 129: 1639- 1655.

[24] Singh JS, Abhilash P, Singh H, Singh RP, Singh D. Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 2011; 480: 1-9.

[25] Strauss SH, Sax JK. Ending event-based regulation of GMO crops. Nature biotechnology 2016; 34: 474.

[26] Trögl J, Chauhan A, Ripp S, Layton AC, Kuncová G, Sayler GS. Pseudomonas fluorescens HK44: lessons learned from a model whole-cell bioreporter with a broad application history. Sensors 2012; 12: 1544-1571.

[27] Graf N, Altenbuchner J. Genetic engineering of Pseudomonas putida KT2440 for rapid and highyield production of vanillin from ferulic acid. Applied microbiology and biotechnology 2014; 98: 137-149.

[28] Jin R, Yang H, Zhang A, Wang J, Liu G. Bioaugmentation on decolorization of CI Direct Blue 71 by using genetically engineered strain Escherichia coli JM109 (pGEX-AZR). Journal of hazardous materials 2009; 163: 1123-1128.

[29] Chen J, Qin J, Zhu Y-G, de Lorenzo V, Rosen BP. Engineering the soil bacterium Pseudomonas putida for arsenic methylation. Applied and environmental microbiology 2013; 79: 4493- 4495.

[30] Ng SP, Palombo EA, Bhave M. Identification of a copper-responsive promoter and development of a copper biosensor in the soil bacterium Achromobacter sp. AO22. World Journal of Microbiology and Biotechnology 2012; 28: 2221-2228.

[31] Lan WS, Lu TK, Qin ZF, Shi XJ, Wang JJ, Hu YF, et al. Genetically modified microorganism Spingomonas paucimobilis UT26 for simultaneously degradation of methyl-parathion and γ- hexachlorocyclohexane. Ecotoxicology 2014; 23: 840-850.

[32] Yang C, Yu H, Jiang H, Qiao C, Liu R. An engineered microorganism can simultaneously detoxify cadmium, chlorpyrifos, and γ‐hexachlorocyclohexane. Journal of Basic Microbiology 2016; 56: 820-826.

[33] Sangwan N, Verma H, Kumar R, Negi V, Lax S, Khurana P, et al. Reconstructing an ancestral genotype of two hexachlorocyclohexane-degrading Sphingobium species using metagenomic sequence data. The Isme Journal 2013; 8: 398.

[34] Li Q, Wu Y-J. A safety type genetically engineered bacterium with red fluorescence which can be used to degrade organophosphorus pesticides. International Journal of Environmental Science and Technology 2014; 11: 891-898.

[35] Barman DN, Haque MA, Islam SMA, Yun HD, Kim MK. Cloning and expression of ophB gene encoding organophosphorus hydrolase from endophytic Pseudomonas sp. BF1-3 degrades organophosphorus pesticide chlorpyrifos. Ecotoxicology and environmental safety 2014; 108: 135-141.

[36] Huang K, Chen C, Shen Q, Rosen BP, Zhao F-J. Genetically engineering Bacillus subtilis with a heat-resistant arsenite methyltransferase for bioremediation of arsenic-contaminated organic waste. Appl. Environ. Microbiol. 2015; 81: 6718-6724.

[37] Porter SS, Chang PL, Conow CA, Dunham JP, Friesen ML. Association mapping reveals novel serpentine adaptation gene clusters in a population of symbiotic Mesorhizobium. The ISME journal 2017; 11: 248.

[38] Farnham KR, Dube DH. A semester‐long project‐oriented biochemistry laboratory based on Helicobacter pylori urease. Biochemistry and Molecular Biology Education 2015; 43: 333- 340.

[39] Mukkata K, Kantachote D, Wittayaweerasak B, Techkarnjanaruk S, Mallavarapu M, Naidu R. Distribution of mercury in shrimp ponds and volatilization of Hg by isolated resistant purple nonsulfur bacteria. Water, Air, & Soil Pollution 2015; 226: 148.

[40] Zhang R, Xu X, Chen W, Huang Q. Genetically engineered Pseudomonas putida X3 strain and its potential ability to bioremediate soil microcosms contaminated with methyl parathion and cadmium. Applied microbiology and biotechnology 2016; 100: 1987-1997.

[41] Kollah B, Patra AK, Mohanty SR. Aquatic microphylla Azolla: a perspective paradigm for sustainable agriculture, environment and global climate change. Environmental Science and Pollution Research 2016; 23: 4358-4369.

[42] Luo Q, Chen Y, Xia J, Wang KQ, Cai YJ, Liao XR, Guan ZB. Functional expression enhancement of Bacillus pumilus CotA-laccase mutant WLF through site-directed mutagenesis. Enzyme and Microbial Technology. 2018; 109, 11-19.

[43] Sudarshan, S., Bharti, V.S., Harikrishnan, S., Shukla, S.P. and RathiBhuvaneswari, G., 2022. Eco-toxicological effect of a commercial dye Rhodamine B on freshwater microalgae Chlorella vulgaris. *Archives of Microbiology*, *204*(10), p.658.

[44] Dubé, E., Shareck, F., Hurtubise, Y. et al. Homologous cloning, expression, and characterisation of a laccase from Streptomyces coelicolor and enzymatic decolourisation of an indigo dye. Appl Microbiol Biotechnol 2008; 79, 597–603.

[45] Godlewska EZ, Przystaś W, Sota EG. Decolourisation of Different Dyes by two Pseudomonas Strains Under Various Growth Conditions. Water Air Soil Pollut 2014; 225:1846.

[46] Sudarshan, S., Harikrishnan, S., RathiBhuvaneswari, G., Alamelu, V., Aanand, S., Rajasekar, A. and Govarthanan, M., 2023. Impact of textile dyes on human health and bioremediation of textile industry effluent using microorganisms: current status and future prospects. *Journal of Applied Microbiology*, *134*(2), p.lxac064.

[47] Wang J, Lu L, Feng F. Improving the Indigo Carmine Decolorization Ability of a Bacillus amyloliquefaciens Laccase by Site-Directed Mutagenesis. Catalysts 2017; 7(275), 1 -10.

[48] Bigley AN, Raushel FM. The evolution of phosphotriesterase for decontamination and detoxification of organophosphorus chemical warfare agents. Chem Biol Interact. 2019; 308:80–88.

[49] Undugoda LJS, Kannangara S, Sirisena DM. Genetic Basis of Naphthalene and Phenanthrene Degradation by Phyllosphere Bacterial Strains Alcaligenes faecalis and Alcaligenes sp. 11SO. J Bioremed Biodeg. 2016; 7(2), 1-5.

[50] Zuo Z, Gong T, Che Y, Liu R, Xu P, Jiang H, et al. Engineering Pseudomonas putida KT2440 for simultaneous degradation of organophosphates and pyrethroids and its application in bioremediation of soil. Biodegradation 2015; 26: 223-233.

[51] Mahmood I, Imadi SR, Shazadi K, Gul A, Hakeem KR. Effects of pesticides on environment. Plant, Soil and Microbes. Springer, 2016, pp. 253-269.

[52] Li C, Zhang C, Song G, Liu H, Sheng G, Ding Z, et al. Characterization of a protocatechuate catabolic gene cluster in Rhodococcus ruber OA1 involved in naphthalene degradation. Annals of microbiology 2016; 66: 469-478.

[53] Marihal A, Jagadeesh K. Plant–Microbe interaction: a potential tool for enhanced bioremediation. Plant Microbe Symbiosis: Fundamentals and Advances. Springer, 2013, pp. 395-410.

[54] Kuppusamy S, Thavamani P, Megharaj M, Naidu R. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by novel bacterial consortia tolerant to diverse physical settings– Assessments in liquid-and slurry-phase systems. International Biodeterioration & Biodegradation 2016; 108: 149-157.

[55] Kumar A, Sharma G, Naushad M, Thakur S. SPION/β-cyclodextrin core–shell nanostructures for oil spill remediation and organic pollutant removal from waste water. Chemical Engineering Journal 2015; 280: 175-187.

[56] Aybey A, Demirkan E. Inhibition of quorum sensing-controlled virulence factors in Pseudomonas aeruginosa by human serum paraoxonase. Journal of medical microbiology 2016; 65: 105-113.

[57] Kaushik G. Applied environmental biotechnology: Present scenario and future trends: Springer, 2016.

[58] McDonald R, Knox OG. Cold region bioremediation of hydrocarbon contaminated soils: do we know enough? ACS Publications, 2014.

[59] De Sanctis E, Monti S, Ripani M. Energy from Nuclear Fission. Energy from Nuclear Fission: An Introduction, Undergraduate Lecture Notes in Physics, ISBN 978-3-319-30649-0. Springer International Publishing Switzerland, 2016.

[60] Gogada R, Singh SS, Lunavat SK, Pamarthi MM, Rodrigue A, Vadivelu B, et al. Engineered Deinococcus radiodurans R1 with NiCoT genes for bioremoval of trace cobalt from spent decontamination solutions of nuclear power reactors. Applied microbiology and biotechnology 2015; 99: 9203-9213.

[61] Kotnik T, Frey W, Sack M, Meglič SH, Peterka M, Miklavčič D. Electroporation-based applications in biotechnology. Trends in biotechnology 2015; 33: 480-488.

[62] Choi MH, Jeong S-W, Shim HE, Yun S-J, Mushtaq S, Choi DS, et al. Efficient bioremediation of radioactive iodine using biogenic gold nanomaterial-containing radiation-resistant bacterium, Deinococcus radiodurans R1. Chemical Communications 2017; 53: 3937-3940.

[63] Sandrine, G., 2003. Pre-Columbian fishing strategies in Guadeloupe archipelago (FWI). In Presencia de la arqueoictiología en México/Presence of the archaeoichthyology in Mexico, Libro de Memorias de la 12a reunión del Grupo de Trabajo en Restos de Peces del Consejo Internacional para la Arqueozoología/Proceedings of the 12th meeting of the Fish Remains Working Group of the International Council for Archaeozoology, 4-12 Sept. 2003 (pp. 53-64). CONACULTA–INAH.

[64] Brim, H., Osborne, J. P., Kostandarithes, H. M., Fredrickson, J. K., Wackett, L. P., & Daly, M. J. (2006). Deinococcus radiodurans engineered for complete toluene degradation facilitates Cr (VI) reduction. Microbiology, 152(8), 2469-2477.

[65] Karigar CS, Rao SS. Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. Enzyme Research. 2011. 1 – 11. Doi.org/10.4061/2011/805187.

[66] Kiyono M and Hou HP. Genetic Engineering of Bacteria for Environmental Remediation of Mercury. Journal of health science 2006; 52(3):199-204.

[67] Hussain I, Aleti G, Naidu R, et al. Microbe and plant assisted-remediation of organic xenobiotics and its enhancement by genetically modified organisms and recombinant technology: A review. Sci Total Environ. 2018; 628-629:1582–1599.

[68] Schüürmann J, Quehl P, Festel G, Jose J. Bacterial whole-cell biocatalysts by surface display of enzymes: toward industrial application. Applied microbiology and biotechnology 2014; 98: 8031-8046.

[69] Jiang J, Zhang R, Li R, Gu J-D, Li S. Simultaneous biodegradation of methyl parathion and carbofuran by a genetically engineered microorganism constructed by mini-Tn5 transposon. Biodegradation 2007; 18: 403.

[70] Khan, A. M., Wick, L. Y., Harms, H., & Thullner, M. (2016). Biodegradation of vapor-phase toluene in unsaturated porous media: Column experiments. Environmental Pollution, 211, 325-331.

[71] Nagata Y, Tabata M, Ohhata S, Tsuda M. Appearance and evolution of γ- hexachlorocyclohexane-degrading bacteria. Biodegradative Bacteria. Springer, 2014, pp. 19- 41.

[72] Kawasaki A, Watson ER, Kertesz MA. Indirect effects of polycyclic aromatic hydrocarbon contamination on microbial communities in legume and grass rhizospheres. Plant and soil 2012; 358: 169-182.

[73] Guthrie, E.A., Pfaender, F.K., 1998. Reduced pyrene bioavailability in microbially active soils. Environ. Sci. Technol. 32, 501508.

systems. *Global warming of 1.5° C.*

[74] Mohan, S.V., Kisa, T., Ohkuma, T., Kanaly, R.A., Shimizu, Y., 2006. Bioremediation technologies for treatment of PAH-contaminated soil and strategies to enhance process efficiency. Rev. Environ. Sci. Biotechnol. 5, 347-374.

[75] Wang, L., Barrington, S., Kim, J.W., 2007. Biodegradation of pentyl amine and aniline from petrochemical wastewater. J. Environ. Manage. 83, 191-197.

[76] Mathur, A.K., Majumder, C.B., 2010. Kinetics modelling of the biodegradation of benzene, toluene and phenol as single substrate and mixed substrate by using Pseudomonas putida. Chem. Biochem. Eng. Q. 24, 101-109.

[77] Adams, G.O., Fufeyin, P.T., Okoro, S.E., Ehinomen, I., 2015. Bioremediation, biostimulation and bioaugmentation: a review. Int. J. Environ. Biorem. Biodegrad. 3, 28-39.

[78] Abuhamed, T., Bayraktar, E., Mehmeto˘glu, T., Mehmeto˘glu, U¨., 2004. Kinetics model for the growth of Pseudomonas putida F1 during benzene, toluene and phenol biodegradation. Process Biochem. 39, 983-988.

[79] Van Hamme, J.D., Singh, A., Ward, O.P., 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Biol. Rev. 67, 503-549.

[80] Sihag, S., Pathak, H., Jaroli, D.P., 2014. Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. Int. J. Pure Appl. Biosci. 2, 185-202.

[81] Mueller, J.G., Chapman, P.J., Pritchard, P.H., 1989. Creosote-contaminated sites. Their potential for bioremediation. Environ. Sci. Technol. 23, 1197-1201.

[82] Stapleton, R.D., Savage, D.C., Sayler, G.S., Stacey, G., 1998. Biodegradation of aromatic hydrocarbons in an extremely acidic environment. Appl. Environ. Microbiol. 64, 4180-4184.

[83] Margesin, R., Schinner, F., 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl. Microbiol. Biotechnol. 56, 650-663.

[84] Coates, J.D., Anderson, R.T., Lovley, D.R., 1996. Oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. Appl. Environ. Microbiol. 62, 1099-1101.

[85] Adriaens, P., Hickey, W.J., 1993. In: Stone, D.L. (Ed.), Biotechnology for the Treatment of Hazardous Waste. Lewis Publishers, Ann Arbor, MI, pp. 97-120.