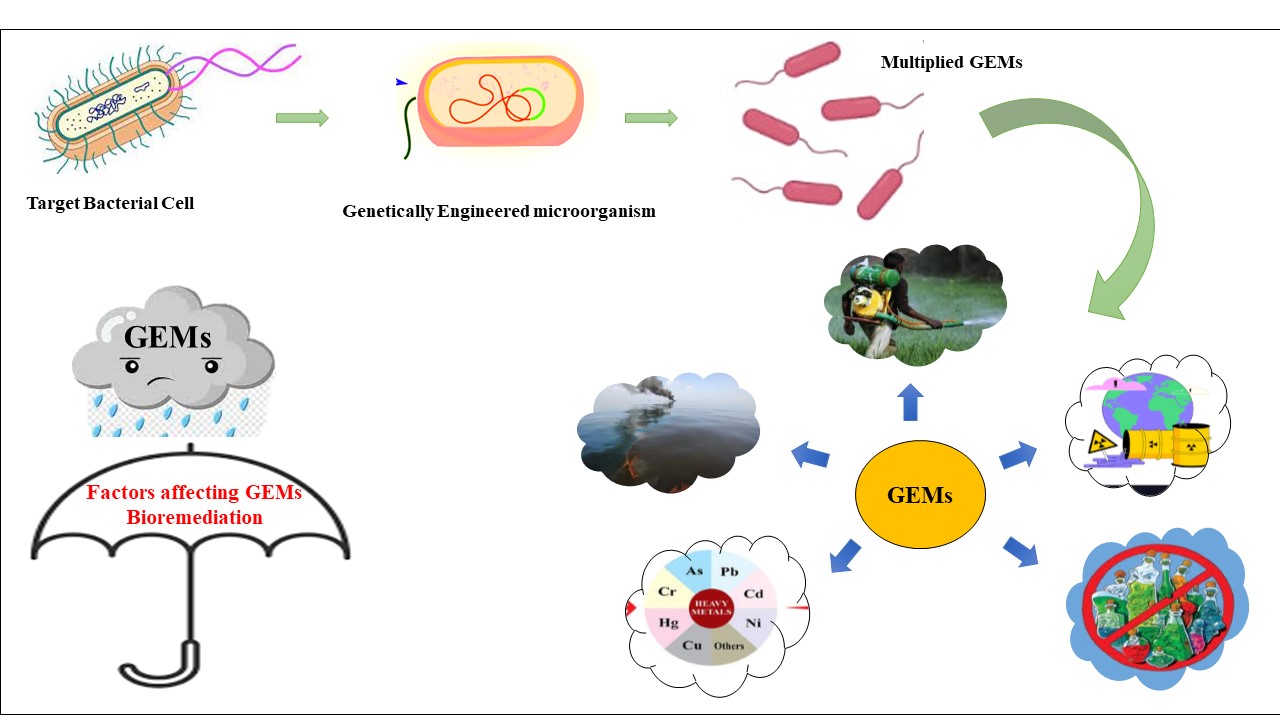
**GENETICALLY ENGINEERED ORGANISMS AND THEIR APPLICATION IN BIOREMEDIATION**

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**Graphical Abstract**

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**Abstract**

In the modern environment, domestic and industrial pollution has been cleaned up using physical and chemical methods. These techniques, however, are expensive and damaging to the environment. The increasing environmental contamination motivated the study of microorganisms and the creation of genetically modified microbes (GEMs) for bioremediation pollution abatement. Using GEMs such bacteria, fungi, and algae, pollutants like oil spills, camphor, hexane, naphthalene, toluene, octane, xylene, and halo benzoates have been broken down. These genetically modified microbes are more efficient than wild strains, quickly adapting to various pollutants as co-metabolizers or substrates. Engineered microbes can provide a safer and cheaper alternative to traditional approaches.

**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 (Zhao et al., 2017a; Jacob et al., 2018). 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.

One of the "Top 10 Biotechnologies to Improve Global Health" is bioremediation, according to Daar et al. (2002). 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 (Dangi et al., 2019). An essential requirement for bioremediation is the sustained presence of the desired microorganism with the proper catabolic and anabolic capacity (Singh et al., 2020; Gupta et al., 2020). 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 (Tropel and Van Der Meer, 2004; Zhao et al., 2017b). 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 (Zhao et al., 2017a).

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 (Rittmann et al., 2006). According to Eapen et al. (2007), Macek et al. (2007), Doty (2008), and Panz et al. (2012), 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 (Ezezika et al., 2010). Natural bacteria cannot remove contamination from heavy metals like mercury, but genetically modified bacteria can. Eapen et al. (2007), Macek et al. (2007), Doty (2008), and Panz et al. (2012) 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 (Ezezika et al., 2010). 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 (Yadav et al., 2017).

By introducing genetically altered microorganisms to boost the activity of insufficient native microbes, bioaugmentation can aid in the bioremediation of contaminated environments (Vidali et al., 2001; Silva et al., 2004; Li et al., 2011). 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 (Jacob et al., 2018).

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 (Liu et al., 2019). 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 (Peeters et al., 2019). Biphenyls, polychlorinated biphenyls (PCBs), nitroaromatics, chloroaromatics, polycyclic aromatics, and oil components are just a few of the pollutants that bacteria can break down (Peeters et al., 2019). 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 (Saccomanno et al., 2018), 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 (Liu et al., 2019). Composting electro-bioremediation, microbe-assisted phytoremediation, and other biostimulation and bioaugmentation-based methods fall within the category of bioremediation (Ghanian et al., 2016). 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 (Liu et al., 2019).

**GEMs for environment rescue against pollutants:**

According to research by Jacob et al. (2018), 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. (Kamthan et al., 2016). According to Singh et al. (2011) and Liu et al. (2019), 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 (Gupta et al., 2016). 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 (Strauss and Sax, 2016).

**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 | (Trögl et al., 2012) |
| 2 | *Mesorhizobium huakuii* | *Mesorhizobium huakuii sub sp.* Rengei B3 | (Singh et al., 2011) |
| 3 | *Pseudomonas putida* | *Pseudomonas putida* KT2440 | (Graf and Altenbuchner, 2014) |
| 4 | *E. coli* | *E. coli* JM10 | (Jin et al., 2009) |
| 5 | *Sphingomonas* | *Sphingomonas desiccabilis* | (Chen et al., 2013) |
| 6 | *Ralstonia eutropha* | *Ralstonia eutropha* CH34 | (Singh et al., 2011) |
| 7 | *Achromobacter* | *Achromobacter sp* AO22 | (Ng et al., 2012) |
| 8 | *Spingomonas paucimobilis* | *Spingomonas paucimobilis* UT26XEGM | (Lan et al., 2014) |
| 10 | *Sphingobium japonicum* | *Sphingobium japonicum* UT26 | (Yang et al., 2016) |
| 11 | *Sphingobium indicum* | *Sphingobium indicum* B90A | (Sangwan et al., 2013) |
| 12 | *Stenotrophomonas sp* | *Stenotrophomonas sp* YC-1 | (Liu et al., 2014) |
| 13 | *Pseudomonas sp* | *Pseudomonas sp* BF1-3 | (Barman et al., 2014) |
| 14 | *Bacillus subtilis* | *Bacillus subtilis* 168 YCMarsM | (Huang et al., 2015) |

**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 (Porter et al., 2017). 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 (Farnham et al., 2015). 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 et al. (2015), 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 (Singh et al., 2011). Research shows severe cadmium pollution in water sites (Zhang et al., 2016). The extreme toxicity of arsenic is another well-known characteristic (Chen et al., 2013). 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 (Kollah et al., 2016).

1. **Dyes**

According to Luo et al. (2018), 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 (Sudarshan et al., 2022). These pollutants are directly or indirectly responsible for acute, mild, and chronic human diseases when they enter the body (Dubé et al., 2008). In addition, injuries to the reproductive, digestive, and central neurological systems are frequently recorded. In addition, certain dyes have been shown to cause cancer (Godlewska et al., 2014; Sudarshan et al., 2023). 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 (Wang et al., 2017). 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) (Godlewska et al., 2014).

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 (Peeters et al., 2019). The scope of bioremediation has expanded over conventional procedures due to the use of microbial treatments with high specificity and efficiency for treating xenobiotics (Bigley et al., 2019). 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 (Saccomanno et al., 2018). According to Undugoda et al. (2016), 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 et al. (2015), 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 (Mahmood et al., 2016). Pesticides harm the ecosystem because of their high-water solubilities and soil stabilities (Zhang et al., 2016). 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 (Li and Wu, 2014). 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 (Li et al., 2016).

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 (Chen et al., 2016). Microorganism use is a viable alternative with minimal adverse effects (Marihal and Jagadeesh, 2013). 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) (Kuppusamy et al., 2016). 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 (Chen et al., 2016). When used for cleanup, biosurfactants can sometimes increase the availability of organic contaminants (Singh et al., 2018). 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 (Kuppusamy et al., 2016).

1. **Oil and its components**

Oil spill cleanup becomes necessary when petrochemical businesses leak liquid petroleum hydrocarbon into the atmosphere, primarily into maritime areas (Kumar et al., 2015). 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 (Li et al., 2016). Additionally, it has been noted that removing salt from oil-polluted locations before the bioremediation process begins may shorten the time needed (Aybey et al.,2016). Thus, bioremediation promises to treat such oil spills more quickly (Kaushik, 2016). 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) (McDonald and Knox, 2014).

**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 (De Sanctis et al., 2016). 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 (Gogada et al., 2015). The biotransformation of such contaminants into an insoluble form that precipitates is a component of the bioremediation of radioactive substances (Kotnik et al., 2015). 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 et al. (2017), 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 (Gogada et al., 2015). 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 (Sandrine et al., 2003). 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 (Brim et al., 2006). 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 (Karigar et al., 2011). Future sources for addressing environmental challenges related to pollutants could include these biotechnology-based eco-friendly, economically viable facilities (Kiyono et al., 2006).

**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 new ion transporter gene incorporation from the *M. marinum* strain | Degrades ionic mercury | (Gupta and Walther, 2016) |
| *Mesorhizobium huakuii* | Transformed with genes that code for PCs from Arabidopsis thaliana | Capable of degrading Cd2+ | (Porter et al., 2017) |
| *E.coli* SE5000 strain | Express nickel transporting system (products of nixA gene) | Degrades nickel from aqueous system | (Farnham and Dube, 2015) |
| **List of modified organisms for the degradation of dyes** | | | |
| *Pseudomonas fluorescens* strain | Sz6 and SDz3 gene expression | Decolourisation of different dyes and decrease of phyto- and zoo toxicity | (Godlewska et al., 2014) |
| *Bacillus amyloliquefaciens* D501G variant | Site-directed mutagenesis | Enhanced stability and catalytic efficiency against indigo carmine. | (Wang et al., 2017) |
| *Streptomyces lividans* strain | Homologous expression | Enhances thermostability and catalytic efficiency against indigo carmine | (Dubé et al., 2008) |
| **List of modified organisms for the degradation of organic xenobiotics** | | | |
| *Pseudomonas diminuta* | Recombinant expression | Activation of phosphotriesterase enzyme for decontamination of the organophosphorus | (Bigley and Raushel 2019) |
| *Alcaligenes sp.* | DMFase gene expression | Enhanced microbial activity and phytoremediation activation compound production | (Hussain et al., 2018) |
| *Pandoraea sp.* | Lactane gene expression | It enhanced xenobiotic biodegradation by the production of various primary metabolites. | (Peeters et al., 2019) |
| **Genetically engineered microorganisms for bioremediation of pesticides** | | | |
| *Escherichia coli* | pL-DsRed–pL-OPH and Pds plasmid insertion | Degrade Organo Phosphorus pesticides | (Li and Wu, 2014) |
| *Moraxella sp.* | Contains surface-expressed organophosphorus hydrolase | Degradation of p- nitrophenol (PNP) and organophosphorus pesticides | (Schüürmann et al., 2014) |
| *Sphingomonas sp.* CDS1 | Insertion of methyl parathion hydrolase gene(mpd) into the chromosome | Degrade methyl parathion and carbofuran | (Jiang et al., 2007) |
| **List of modified organisms for the degradation of organic pollutants** | | | |
| *P. putida* KT2442 | Modified pathway | Degrade toluene/benzoate | (Khan et al., 2016) |
| *Comamonas Testosteroni* VP44 | Modified substrate specificity | Degrade o-, p- monochloride- biphenyls | (Marihal and Jagadeesh, 2013 |
| P. pseudo-alcaligenes KF707-D2 | Modified substrate specificity | Degrade TCE, toluene, benzene and flourobiphenyl | (Chen et al., 2016) |
| **List of genetically modified bacteria to remediate oil pollution** | | | |
| *Pseudomonas putida* | Presence of NAH and XYL plasmid with a hybrid plasmid (from CAM and OCT) | Degrade crude oil | (Nagata et al., 2014) |
| *Pseudomonas fluorescens* HK44 | Contains pUTK21 plasmid containing nah gene | Degrade Naphthalene | (Kawasaki et al., 2012) |
| *Rhodococcus sp.* RHA1 | Contains pPC3 with fcb operon from *A. globifirmis* | Degrade 4- chlorobenzoate | (Li et al., 2016) |
| **Various GEMs for bioremediation of radioactive compounds** | | | |
| *Deinococcus radiodurans* | Plasmids increase resistance to more radioactive elements like merH locus incorporation. | Degrades radioactive compounds like toluene | (Gogada et al., 2015) |
| *D. geothermalis* | Plasmids from *D. radiodurans* are induced | Degrades Fe(III) nitrilotriacetic acid, Hg(II), U(VI), and Cr(VI) | (Kotnik et al., 2015) |
| *Deinococcus radiodurans* R1 | Plasmids are used to increase resistance to some more radioactive elements. | Removal of radioactive iodine (>99%) | (Choi et al., 2017) |

**Factors affecting GEMs-assisted bioremediation**

Guthrie and Pfaender (1998) 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. (Mohan et al., 2006). Interactions between the substrate and the pollutants impact their degradation rate as well (Wang et al., 2007). 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 (Mathur et al., 2010). 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 (Adams et al., 2015). 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 (Abuhamed et al., 2004). BTEX chemicals demonstrated an inhibiting effect (Mathur and Majumder, 2010) 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 (Van Hamme et al., 2003). 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 et al. (2015), 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 (Sihag et al., 2014). The mineralisation of petroleum hydrocarbons benefits from neutral pH. According to current research, even a temperature range of 15oC–20oC may impact biodegradation (Mueller et al., 1989). 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 et al. (1998) 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 (Margesin and Schinner, 2001). 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 (Sihag et al., 2014). 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 (Coates et al., 1996).

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 (Adriaens and Hickey, 1993). 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 type 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 (Singh et al., 2011). 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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