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Recent Advances and Prospects of Microbial Biosurfactant-Mediated Remediation of Engine Oil Pollution: A Comprehensive Review

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ABSTRACT

A major global concern is the widespread environmental destruction caused by hydrocarbons, especially from the dumping of spent engine oil. Hydrocarbons are a major source of pollution in the environment and have an impact on agriculture, aquatic life, and soil fertility. The necessity of resolving this issue is highlighted by the detrimental impact on soil biocenosis and the potential conversion of soils into technogenic deserts. Due to high costs and polluting byproducts, the conventional approach of treating contaminated soil, sediment, and water is unsustainable. However, bioremediation, which makes use of biological agents like fungi and bacteria, appears to be a more practical and affordable solution. Microbial biosurfactants present a possible solution for environmental restoration due to their less harmful nature compared to chemical surfactants. This review highlights the green and sustainable nature of microbial biosurfactants while examining their advancements, biotechnological potentials, and future possibilities for bioremediation. The review also looks at the genetic basis and economic viability of biosurfactants for bioremediation applications. Furthermore, the review emphasizes the need for more studies in overcoming the challenges of large-scale application of biological surfactants for bioremediation of pollution and environmental restoration. As partners in nature, these bacteria aid in the breakdown of hydrocarbons, highlighting the need for industry and the environment to coexist sustainably. As biosurfactants are less harmful to the environment than chemical surfactants, they are more in line with the global trend toward sustainable methods and the use of natural processes for ecological restoration.

INTRODUCTION

The pollution of the environment by petroleum hydrocarbons and their products is an ongoing global challenge with its attendant problems (Venkatraman et al. 2024). Intentional or accidental disposal of spent engine oil into the soils and waterways is one of the most notable environmental problems (Shehu et al. 2023), almost as widespread as crude oil pollution in many developing countries (Emoyan et al. 2020). Hydrocarbons, their derivatives, and waste products, including spent engine oil, have been identified as significant contributors to the menace of environmental pollution (Ahmad 2022) and mainly emanate from the automobile industry, including auto mechanic workshops (Muze et al. 2020). Hydrocarbons are thought to be among the most hazardous environmental contaminants because of their extreme toxicity and widespread occurrence in the biosphere (Umar et al. 2021). Aquatic and marine plants and animals have not been spared from the effects of these activities, which have also led to the contamination of agricultural soils. Hydrocarbons and their by-products rank second in terms of their detrimental effects, right behind radioactivity (Waters et al. 2018). Due to its tremendous adsorbing surface area, the soil is particularly impacted because of its ability to store huge quantities of pollutants. The chemical composition, structure, and qualities of many soils have been significantly affected by hydrocarbon pollution, which in turn impairs soil fertility and agronomic value. This adverse situation has detrimental effects on soil biocenosis (Sydorenko 2023). Oil spills have the potential to transform soils into typical technogenic deserts devoid of most biological life. Soils contaminated with hydrocarbons are unsuitable for agricultural purposes and can potentially contaminate ground and surface waters. Depending on the kind of soil, self-restoration can take a long time, 10 to 30 years or more (Liftshits et al. 2018).

The typical approach of treating contaminated soil, sediment, and water is proven to be unsustainable because of the huge cost and generation of contaminating byproducts (Da'ana et al. 2021). However, with the versatile capabilities of biological agents, such as bacteria, fungi, and other microorganisms or their enzymes, bioremediation has evolved as a sustainable, cost-effective, and natural method for restoring contaminated soil, surface water, and groundwater (Pande et al. 2020). To accelerate the breakdown and/or removal of inorganic and organic contaminants, microorganisms are cultivated in the presence of contaminated soil, sediment, or water samples. This is an emerging and rapidly growing green and sustainable biotechnological field (Kumar et al. 2018). For instance, a biomolecule by *Bacillus* sp. isolated from a water reservoir in Brazil had previously been studied by Korenblum and associates (Korenblum et al. 2012). Similarly, Joshi et al. (2016) synthesized, optimized, and characterized biosurfactant from a *Bacillus licheniformis* W16 strain isolated from soil samples collected near an oil well in Oman, while El-Sheshtawy et al. (2015) produced biological surfactant with *B. licheniformis* isolated from an Egyptian oil reservoir. These studies demonstrate the versatility of biosurfactant-producing bacteria, particularly *Bacillus* strains, from different geographic locations (Brazil, Oman, and Egypt). This geographic diversity highlights the adaptability of these microbes to varying environmental conditions associated with oil contamination. Their findings suggest that exploring microbial strains from different regions can provide insight into biosurfactant production and optimize their application for bioremediation in diverse ecosystems. In many developing such as Nigeria, there are a lot of oil-contaminated soils, and this has negative health, social, and economic effects since there is inadequate regulation of oil waste disposal, among other things (Adeola et al. 2022, Orisakwe 2021). This review seeks to synthesize information and discusses the advances, biotechnological potentials, and prospects of microbial biosurfactants as an important green and sustainable option for the bioremediation of spent engine oil-contaminated soils, the genetics of the biosurfactant-producing microbes and the economic viability of microbial biosurfactants for bioremediation processes.

MICROBIAL BIOSURFACTANTS AND THEIR PROPERTIES

Biosurfactants are biosurface-active agents which are produced by numerous microorganisms. Biosurfactants are exopolymeric substances (EPS) with amphipathic properties, produced outside of the cell or as part of cell membrane biomolecules by a variety of bacteria, fungi, and yeasts (Santos et al. 2016). The commonly used surfactants are chemically derived (Moldes et al. 2021), but their high persistence power, low degradation rate, and hazardous nature limit their applications (Alizadeh-Sani et al. 2018). Microbial (bio) surfactants hold numerous advantages over chemical surfactants, including greater selectivity, less toxicity, increased temperature tolerance, stability in pH change, and high salt tolerance (Sarubbo et al. 2022, Shekhar et al. 2015). Due to their numerous potential uses as wetting agents, emulsifiers, foaming agents, detergents, and dispersants, biosurfactants, which can be neutral or anionic, are becoming more and more valued in the commercial sector (Gaur et al. 2021). They can be used in many different industries, such as the food processing, cosmetics, petroleum, agricultural, and pharmaceutical sectors, so also in oil recovery, site management, and cleanup (Adetunji & Olaniran 2018, Araújo et al. 2019). Their potential as antiviral, antifungal, antibacterial, and anti-adhesive medicines against a variety of drug-resistant organisms has also been explored for a variety of biological uses (Alara & Alara 2024). Biosurfactants are high-efficiency molecules, they enhance the extraction of oil from the well (Enhanced Oil Recovery) and are frequently employed in hydrocarbon bioremediation research (Karlapudi et al. 2018).

CLASSIFICATION AND CHEMICAL NATURE OF BIOSURFACTANTS

The chemical composition and microbiological source are the main criteria for classifying biosurfactants. As depicted in (Fig. 1), microbial surfactants are categorized into high (including polymeric and particulate surfactants) and low (including phospholipids, lipopeptides, and glycolipids) molecular weight surfactants (Abo Elsoud 2021). Biosurfactants based on high molecular weight and low molecular weight include:

1. **Polymeric biosurfactants:** Lipomanan, alasan, liposan, and emulsan are among the most notable polymeric biosurfactants (Luft 2022). Nonetheless, at low concentrations, emulsan is thought to be an effective bioemulsifier for emulsifying hydrocarbon-water mixtures. The most -researched examples include emulsan and biodispersan, which are produced

by *Acinetobacter calcoaceticus* and contain a heteropolysaccharide moiety bonded covalently to fatty acids (Adetunji & Olaniran 2021). *Candida lipolytica* produces liposan, an emulsifier that is primarily composed of carbohydrates (83%) and proteins (17%). *Yarrowia lipolytica* also produces a similar type of glycoprotein complex (Shekhar et al. 2015).

2. **Glycolipids:** They contain lipids attached to a carbohydrate by a glycosidic bond. The carbohydrate moiety attached to aliphatic or hydroxy aliphatic acids through an ether or ester group makes up these widely researched and widely used biosurfactants (Saranraj et al. 2022). The carbohydrates domain consists of rhamnose, mannose, glucose, galactose, galactose sulfate, and glucuronic acid (Adetunji & Olaniran 2021). According to Chrzanowski et al. (2012), rhamnolipids, mannosyl erythritol lipids, trehalose lipids, cellobiolipids, and sophorolipids are the glycolipids that have been investigated the most.
3. **Phospholipids:** When cultured in n-alkane-rich conditions, bacteria create large amounts of phospholipid and fatty acid biosurfactants (Adetunji & Olaniran 2021). The length of the hydrocarbon chain is correlated with the amount of hydrophilic and lipophilic components in the surfactants (Aubry et al. 2020). The phosphatidyl ethanolamine-containing vesicles excreted by *Acinetobacter* sp. make micro-emulsions of alkane and water, while *Rhodococcus erythropolis* creates vesicles that lessen the interfacial tension of a hexadecane and water mixture (Karlapudi et al. 2018).
4. **Lipopeptides:** The most investigated lipopeptide is surfactin, which is synthesized by *Bacillus subtilis* ATCC 21332 (Chavarria-Quicaño et al. 2023). Lipopeptides consist of amino acids linked to the carboxyl and hydroxyl groups of a C_{14} acid via a lactone bond (Bhadra et al. 2023). It is considered the most potent biosurfactant with remarkable surface activity at low concentrations (Nadaf et al. 2021).
5. **Particulate biosurfactants:** A type of particulate biosurfactant that aids in microorganisms' absorption of alkanes is extracellular membrane vesicles by partitioning oil-water mixtures and forming micro-emulsions at the interface (Siddiqui et al. 2021). Vesicles made by *Acinetobacter* sp. are one example of this. They are composed of phospholipids, proteins, and lipopolysaccharides (Vijayakumar & Saravanan 2015).

BIOSURFACTANT PRODUCING MICROORGANISMS

Microorganisms have well been explored for biosurfactant production in diverse industries including food, medicine, cosmetics, agriculture, and environmental cleanup (Sunde & Borresen 2016). Compared to their chemical counterparts, biosurfactants provide several benefits, including reduced toxicity, biodegradability, and the possibility of producing them at a very cheap cost using renewable resources. According to reports, a large number of the microorganisms that produce biosurfactants are also capable of breaking down hydrocarbons (Singh et al. 2021). Nonetheless, research conducted in recent decades has demonstrated the impact of surfactants produced by microorganisms on increased oil recovery in addition to bioremediation (Gudina et al.

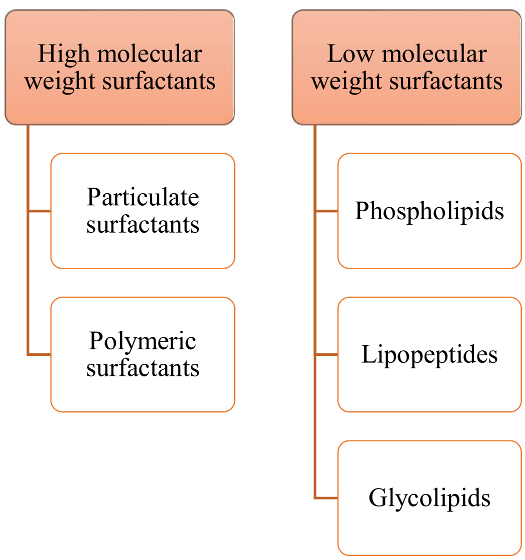

Fig. 1: Classification of biosurfactants based on molecular weight.

2018, Nikolova & Gutierrez 2021). Certain bacteria, such as *Achromobacter xylosoxidans* DN002 and *B. licheniformis,* possess the ability to degrade aromatic hydrocarbon fractions (Eskandari et al. 2017). Likewise, other bacteria, like *Dietzia* spp. and *Geobacillus thermodenitrifican,* are capable of degrading particular alkanes (Xu et al. 2018). The microorganisms capable of breaking down hydrocarbons often use them as sources of energy and carbon for their growth and reproduction (Ivshina et al. 2024). To relieve the hydrocarbon-induced physiological stress in the bulk microbiological environment, indigenous bacteria eventually break down or metabolize petroleum hydrocarbons (Siddiqui et al. 2021, Tripathi et al. 2022). For instance, in the study of Patil et al. (2012) on the breakdown of engine oil in polluted soil, the authors isolated and identified versatile hydrocarbon-degrading bacteria strains, including *Bacillus* species*, Acinetobacter* species*, Micrococci* species, *Pseudomonas* species, and *Streptomyces* species. *Bacillus licheniformis*, an effective hydrocarbon-degrading strain with resistance to high salinity, alkalinity, and temperature, was also identified by Liu et al. (2016) from soils polluted by oil in the vicinity of Tianjin, China's Dagang Oilfield. Even with their more complex structures, the strain that was observed was able to degrade both long-chain and short-chain alkanes.

Microorganisms produce a range of compounds, including biosurfactants, which enable the transport of carbon sources into their cells, even when they are intractable, such as hydrocarbons (Banerjee et al. 2024). Ionic surfactants are released by certain bacteria, and they help to emulsify the CxHy (hydrocarbon with x carbon atoms and y hydrogen atoms) material present in the growth media (Unaeze 2020). Some examples of this class of biosurfactants are sophorolipids, which are produced by multiple *Torulopsis* species, and rhamnolipids, which are produced by various *Pseudomonas* species (Kumar et al. 2021). By generating lipopolysaccharides or nonionic surfactants within their cell wall, certain other bacteria can modify the structure of their cell wall. *Rhodococcus erythropolis*, different *Mycobacterium* spp., and *Arthrobacter* spp. are examples of this category, and they generate nonionic trehalose corynomycolates (Sulochana et al. 2021). *Acinetobacter* spp. create lipopolysaccharides such as emulsan (Sen et al. 2021), and *Bacillus subtilis* produces lipoproteins like surfactin (Choi et al. 2021).

Yeasts, including *Candida, Rhodotorula*, and *Saccharomyces,* have also been investigated for their potential to produce biosurfactants (Adebayo et al. 2021). Depending on the strain and method used, producing biosurfactants from yeasts might be a profitable endeavor. Moreover, yeasts are simple to grow. Relatively few fungi are known to produce biosurfactants (Mohapatra et al. 2022). Fungi such as *Candida bombicola, C. lipolytica, C. ishiwadae, Aspergillus ustus,* and *Trichosporon ashii* are a few of the fungi that have been explored for biosurfactant production (Abdel-Azeem et al. 2021). *Starmerella bombicola* has been reported to synthesize a biosurfactant known as sophorolipids (Rawat et al. 2020). It is well known that several of these can make biosurfactants from inexpensive raw materials. The primary class of biosurfactants that these strains produce are called sophorolipids, or glycolipids (Prasad et al. 2021). When *Candida lipolytica* grows on n-alkanes, it creates lipopolysaccharides that are linked to the cell wall (Rivaldi et al. 2018).

THE GENETICS OF BIOSURFACTANT PRODUCTION

Microbial surfactants are encoded by arrays of genes, while numerous production routes, operons, and enzymes involved in the synthesis have been identified (Markande et al. 2021). The core synthetic genes for the production of bacillibactin, fengycin, and surfactin in *Bacillus* sp. XT-2, a novel facultative-halophilic long-chain hydrocarbon degrader, was elucidated by Wang et al. (2022). To demonstrate how genetic engineering of strains that produce biosurfactant can lead to the creation of an affordable bioremediation system, Wu et al. (2019) developed a systematic genetic engineering approach in which 53 genes of *Bacillus subtilis* 168 were altered to produce surfactin biosurfactant. Their investigation involved five main stages to optimize surfactin production. Initially, they combined the entire *sfp* gene into *B. subtilis* 168 to activate the biosurfactant biosynthesis. In an attempt to lessen competition in the second stage, 3.8 percent of the targeted strain's entire genome, which controls the polyketide synthase pathways and biofilm formation, was deleted. The third phase involves the possible overexpression of self-resistance-associated proteins, which improves the cell tolerance to the surfactin biosurfactant. The supply of precursor branched-chain fatty acids was boosted in the fourth phase by the engineering of the branched-chain fatty acid biosynthesis pathway. Ultimately, they redirected Acetyl-CoA from the process of cell growth to the manufacture of surfactin by improving the transcription of *srfA*. Furthermore, Zhu et al. (2021) reported that there are four Open reading frames (ORFs) governing the genetic regulation of lipopeptides. These ORFs in the *srfA* operon directs the synthesis of surfactin, including *srfAA*, *srfAB*, *srfAC*, and *srfAD,* which are multi-enzyme synthase complexes responsible for the synthesis of surfactin. The biosurfactant titer in this experiment reached a maximum value of 12.8 g/l, demonstrating the enormous potential of genetic engineering techniques and the crucial role that genome sequencing plays in creating an ideal biosurfactantbase bioremediation system (Wu et al. 2019). Yasmin et al. (2022) in their study, reported that the major factor governing the biosynthesis of biosurfactants is the genetic makeup of the organisms producing these biosurfactants.

Some of the genes that have been reported to produce biosurfactants are depicted in (Table 1).

MICROBIAL SURFACTANTS FOR VARIOUS BIOTECHNOLOGICAL APPLICATIONS

Biosurfactants unique qualities and eco-friendliness make them useful in a variety of industries. They have been applied for different purposes in the following industries:

Oil Industry

According to Haider (2020), there was a 1.5% increase in global oil consumption in 2018 to 99.5 million barrels per day. It is anticipated that light and medium oils will become scarcer at the current rate of usage, increasing the need for heavy and extra-heavy oils (Sarubbo et al. 2022). In addition, it is anticipated that the world's oil supplies will run out in the next 40 to 45 years (Fenibo et al. 2019). According to Rawat et al. (2020) and Khademolhosseini et al. (2019), biosurfactants effectively mobilize immobile hydrocarbons by encouraging the decrease of surface tension between the oil and rock, this, in turn, lessens the capillary forces that impede the oil's passage through the rock pores.

Food Sector

The use of biosurfactants in food has gained attention recently due to consumers' growing interest in vegetarian and vegan food products, as well as sustainably produced components. (Hassoun et al. 2024). Some of these natural chemicals have low toxicity and can be utilized to improve formulations by changing their texture or viscosity and inhibiting the growth of some harmful microbes (Ribeiro et al. 2020). This increases food's shelf life, quality, and safety. Microorganisms utilized for biosurfactants in the food industry include *Saccharomyces cerevisiae*, *Meyerozyma guilliermondii, C. lipolytica, C. utilis, Starmerella bombicola,* and *Candida sphaerica* (Thraeib et al. 2022).

Detergent Industry

The detergent market encompasses personal care, household, and heavy industrial cleaning solutions. The most commonly used chemical surfactants are usually derived from petrochemicals. These often pose a significant risk to aquatic life (Chirani et al. 2021). Biosurfactants are increasingly emerging as a viable commercial substitute for these artificially produced surfactants (Celik et al. 2021). This circumstance has prompted a search for environmentally friendly goods, such as detergents that degrade efficiently through microbial breakdown and are made of straight-chain organic molecules (Farias et al. 2021). One of the main qualities of biosurfactants in this industry is their capacity to emulsify, which is essential for detergent activity. Other

Table 1: Some of the reported biosurfactant-producing genes.

Genes	
rhlA, *rhlB*, and *rhlC* genes	These genes are frequently identified in *Pseudomonas aeruginosa* and are involved in the formation of rhamnolipid biosurfactants (Shatilla et al. 2020). While *rhlB* and *rhlC* are involved in the production of the final rhamnolipid structure (Zhao et al. 2021), *rhlA* encodes the enzyme responsible for the synthesis of the precursor molecule (Wittgens et al. 2017).
Sophorolipid genes	Yeasts, especially *Candida bombicola*, produce sophorolipids, which are glycolipid biosurfactants (Qazi et al. 2022). *SL1*, *SL2*, and *SL3* are among the genes involved in sophorolipid production (Park et al. 2022). Enzymes involved in the synthesis of sophorose, the building block of sophorolipids, are encoded by these genes (Liu et al. 2020).
Alasan genes	Alasan genes encode a biosurfactant generated by *Acinetobacter species* (Saranraj et al. 2022). The production of alasan involves the *alsA* and *alsB* genes. According to Dabbagh et al. (2020), and *alsB* is involved in the precursor's transportation and acylation to create the final alasan molecule (Dabbagh et al. 2020).
Emulsan genes	*Acinetobacter calcoaceticus* produces the glycolipid biosurfactant emulsan (Pirog et al. 2021). The genes *emuC*, *emuD*, and *emuA* are involved in emulsan biosynthesis. These genes are in charge of the synthesis and assembly of emulsan on the surface of bacteria (Segovia et al. 2021).
Surfactin operon	*Bacillus subtilis* and other *Bacillus* species produce the lipopeptide biosurfactant surfactin, also known as the surfactin operon (Li et al. 2021). There are several genes in the surfactin operon, including *srfA*, *srfB*, *srfC*, *srfD*, and *srfE* (Kashif et al. 2022). The synthesis, transport, and regulation of surfactin production are carried out by these genes (Muller et al. 2021).
Mannosylerythritol lipids (MELs) genes	MELs are glycolipid biosurfactants produced by yeast-like fungi such as *Pseudozyma* spp. (Nouri et al. 2023). The biosynthesis of MELs involves several genes, including *MEL1*, *MEL2*, and *MEL3* (Perdomo et al. 2020). These genes encode enzymes responsible for the synthesis and modification of MELs (Yamamoto et al. 2022).

than this, others resembling commercial detergents may find application in the laundry and detergent industries (Drakontis & Amin 2020). By comparing a lipopeptide from *B. subtilis* SPB1 to commercial detergents, Bouassida et al. (2018) found that the latter was less effective in reducing stains from coffee and vegetable oil. Likewise, Fei et al. (2020) discovered that *B. subtilis* HSO121 surfactin has the same applications as chemical surfactants but also has the benefits of low toxicity and no irritation, as well as good wetting capacity and emulsifying activity, high compatibility, stability, biodegradability, and foaming capacity.

Cosmetic Industry

The development of products with more renewable and natural active ingredients is the global trend in the cosmetics business to reduce or eliminate the use of synthetic raw materials (Goyal & Jerold 2023). Besides affecting people and animals, chemical surfactants can impact soils and groundwater, harming the environment. The cosmetic industry can benefit from the properties of microbial biosurfactants, such as their antimicrobial, skin surface moisturizing, and low toxicity (Karnwal et al. 2023). These properties can potentially replace chemical surfactants in current pharmaceutical formulations for personal skincare and cosmetics.

Nanotechnology

In nanotechnology, biosurfactants are mostly used because of their ability to function as stabilizers and reducing agents, particularly for silver particles, which makes them useful for the creation of nanoparticles (Vecino et al. 2021). This is because there is a growing demand for "green" substitutes for the chemical processes currently in use (Duehnen et al. 2020). Therefore, biosurfactants represent a substitute that supports an effective, environmentally friendly procedure that requires no energy and doesn't include any hazardous substances (Joanna et al. 2018). With documented biological activity, certain microbes, like the bacterium *B. subtilis*, may synthesize gold and silver nanoparticles both intracellularly and extracellularly (Rane et al. 2017).

Agriculture

The adaptable characteristics of biosurfactants additionally render them suitable for use in agriculture, primarily as a substitute for synthetic surfactants in pesticide and agrochemical formulations (Gayathiri et al. 2022). This has encouraged the growth of "green chemistry" in this industry in response to the need to lessen or eliminate the harmful effects that excessive use of chemical compounds has on the environment and human health (Köhl et al. 2019).

BIOREMEDIATION STRATEGIES

Bioremediation techniques have advanced over the last 20 years, with the ultimate aim being the inexpensive and environmentally friendly restoration of damaged environments (Landa-Acuña et al. 2020). Different bioremediation approaches, as shown in (Fig. 2) have been developed and studied. However, no particular bioremediation strategy can be regarded as the most effective for restoring damaged habitats due to the nature and diversity of contaminants (Bala et al. 2022). Most issues about the biodegradation and bioremediation of polluting substances can be resolved by native microorganisms that are common in contaminated locations, provided that the environment is favorable to their growth and metabolism. (Verma & Jaiswal 2016). Among the main benefits of bioremediation over chemical and physical remediation techniques are its cost- and environmentally-friendly characteristics. Bioremediation strategies are generally classified as ex-situ and in-situ, as depicted in Fig. 2.

Ex-situ Bioremediation

Ex-situ bioremediation techniques involve removing pollutants from contaminated sites and moving them to another location for remediation (Butnariu & Butu 2020). Excavated contaminated soils are spread out on the ground and cleaned with natural microorganisms. The evaluation of ex-situ bioremediation techniques usually takes into account the cost of treatment, the kind and concentration of pollutants, the level of pollution, the location of the polluted site, and its geology (Azubike et al. 2016).

In-situ Bioremediation

When bioremediation is carried out at the original site of pollution, it is referred to as in situ bioremediation (Fragkou et al. 2021). The concept of in situ bioremediation is mostly utilized to address groundwater and soil contamination (Marcon et al. 2021). Since the process does not involve excavation, the soil structure is either a little disturbed or not at all (Zeng & Hausmann 2022). Since excavation processes do not incur additional costs, the desired cost of these methods should be lower than that of ex-situ bioremediation methods (Muhammad et al. 2024). The expense of developing and setting up some advanced equipment on the site to increase microbial activity during bioremediation, however, can be a major issue (Azubike et al. 2016).

APPLICATION OF BIOSURFACTANT-PRODUCING MICROORGANISMS FOR IN-SITU AND EX-SITU DEGRADATION OF HYDROCARBON AND ITS PRODUCTS

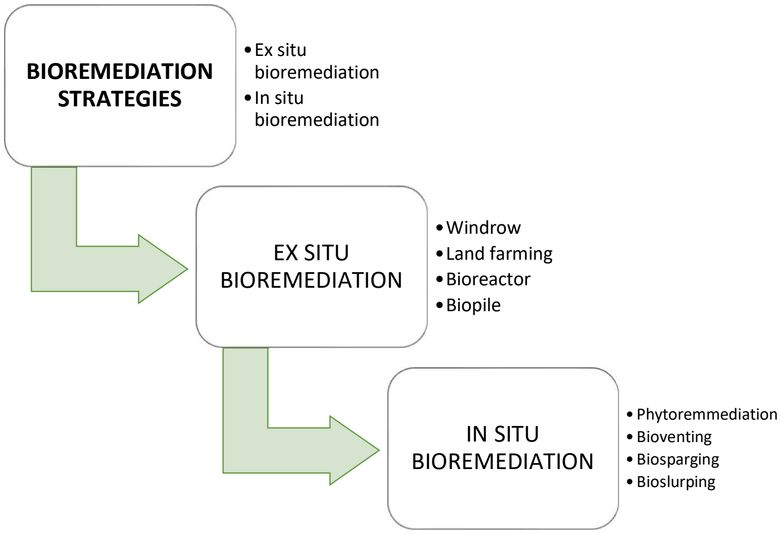

Fig. 2: Bioremediation strategies.

Some bacteria like *Dietzia* spp., *Oleispira antarctica*, *Pseudomonas* spp. (Ibrar et al. 2022), *Geobacillus thermodenitrifican* and *Bacillus licheniformis* can degrade aromatic or resinous hydrocarbon components. These bacteria use the hydrocarbons as energy and carbon sources for growth and reproduction, as well as to relieve physiological stress brought on by hydrocarbons in the microbial bulk environment; indigenous bacteria eventually break down or metabolize hydrocarbons (Kleindienst et al. 2015). Hydrocarbon-contaminated soil is cleaned up using a variety of recognized bacterial strains that function as effective biodegraders. Bioremediation is carried out by microorganisms, either indigenous or foreign (Jabbar et al. 2022). The availability of nutrients can increase the activity of native microorganisms, even as the introduction of exogenous bacteria to the contaminated site promotes bioremediation (Liu et al. 2020a). The most crucial stage in the biodegradation of hydrocarbons, according to Shi et al. (2019), is the formation of the interface between substrates and bacteria that break down petroleum. Presently, there are three methods to develop this interface:

1. In an aqueous solution, microbial organisms dissolve and absorb petroleum hydrocarbons (Ambaye et al. 2023).
2. Microbial cells directly absorb large hydrocarbon molecules. (Pandolfo et al. 2023).
3. Small hydrocarbon particles that are encapsulated, pseudo-soluble, or quasi-soluble interact with microbial cells for absorption (Ejaz et al. 2021).

Contrarily, bacterial cell hydrophobicity influences how well they attach to petroleum hydrocarbons (Kebede et al. 2021). Thus, for the bioremediation process to be more effective, the bacterial cells must be highly hydrophobic, which calls for the use of biosurfactants to facilitate the effective interaction of the bacteria with the petroleum hydrocarbons (Zahed et al. 2022). According to Chandra et al. (2013), in aerobic catabolism, the hydrocarbon skeleton appears to be modified by the addition of one or two hydroxyl groups. This seems to be the initial step in the process. The primary enzymatic response during the early intracellular breakdown of organic pollutants is the process of incorporating and activating oxygen (Mbachu 2020), oxygenase and peroxidase-catalyzed, making it an oxidative reaction (Cárdenas-Moreno et al. 2023, Chandra et al. 2013).

The addition of one oxygen atom and two hydroxyl groups to hydrocarbons, respectively, is catalyzed by monooxygenases and dioxygenases. Peripheral degradation pathways transform organic contaminants into metabolites of vital intermediate breakdown, like the tricarboxylic acid cycle (Fuentes et al. 2014). The major precursor metabolites include acetyl-CoA, succinate, and pyruvate, which are used in cell biomass synthesis (Kim et al. 2021) and gluconeogenesis to produce sugars required for subsequent biochemical activities involved in growth and development. (Scholtes & Giguère 2022). Therefore, by oxidizing these substrates, the bacterium can live in an environment with low nutrients (Wang et al. 2020). However, sulfate and nitrate function as terminal electron acceptors during anaerobic degradation (Su et al. 2023), which is carried out by coupling hydrocarbons to carbon dioxide (CO_2) or fumarate (Sah et al. 2022). However, the anaerobic degradation of petroleum hydrocarbons proceeds more slowly than aerobic microbial catabolism (Wartell et al. 2021).

There are only a few types of bacteria that can break down a broad range of hydrocarbons (Ławniczak et al. 2020). For example, *Dietzia* spp. DQ-12-45-1b uses n-alkanes (C_6-C_{40}) (Feknous et al. 2019) and other substances as its only carbon source, and *Achromobacter xylosoxidans* DN002 breaks down a range of monoaromatic and polyaromatic hydrocarbons (Gaur et al. 2022, Ma et al. 2015). However, only a small portion of petroleum hydrocarbon components can be metabolized by the majority of bacterial species, rendering others inaccessible (Varjani 2017, Xu et al. 2018). This is because various native bacterial species have unique catalytic enzymes (Xu et al. 2024). Hence, the cooperation of various functional bacteria is necessary to accomplish the effective bioremediation of petroleum hydrocarbon-contaminated soils (Dombrowski et al. 2016). Varjani et al. (2015) provided evidence to bolster the aforementioned claims when they stated that breaking down 3% v/v crude oil at a rate of 83.49% is the halotolerant Hydrocarbon Utilizing Bacterial Consortium (HUBC), composed of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Ochrobactrum* spp. (Siddiqui et al. 2021).

In a field study by Szulc et al. (2014) in the course of treating soil contaminated with diesel oil for 365 days, an 89% biodegradation efficiency by an artificial consortium made up of *Alcaligenes xylosoxidans*, *Aeromonas hydrophila*, *Pseudomonas fluorescens In*, *Gordonia* spp., *Rhodococcus In equi*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, and *Xanthomonas* spp. was confirmed (Szulc et al. 2014, Xu et al. 2018). These genes work in concert to achieve pollution purification because the bacterial consortium possesses a variety of catabolic genes (Gurav et al. 2017). As a result, the synergistic effects of a variety of catabolic genes within a bacterial consortium are advantageous in attaining the purification of contaminants (Albers et al. 2018). Due to the following factors, a bacterial consortium consisting of strains of *Mycobacterium, novosphingobium, Mycobacterium ochrobactrum* (Laothamteep et al. 2021), and a *Bacillus* strain demonstrated synergistic pyrene degradation (Zhang et al. 2022). Two *Mycobacterium* bacteria started the process of pyrene breakdown, whereas the *Bacillus* strain increased the bioavailability of pyrene by generating biosurfactants. Pyrene intermediates were successfully removed by *Mycobacterium ochrobactrum* and *Mycobacterium novosphingobium* (Wanapaisan et al. 2018). However, because of the complexity of the hydrocarbon components, it has been necessary to create a genetically modified bacteria or a minimally functional bacterial consortium for hydrocarbon bioremediation. (Dvorak et al. 2017).

Furthermore, two other issues that require resolution are the guard of the altered bacteria and the stability of the community (Xu et al. 2018). Therefore, the results mentioned above imply that using bacterial consortia could be a rational and efficient method for successfully removing petroleum hydrocarbons from polluted areas (Lara-Moreno et al. 2021). According to da Silva et al. (2021), the commercially manufactured biosurfactant derived from *Starmerella bombicola* significantly decreased water's surface tension, indicating both a strong emulsification and dispersal capacity for hydrophobic chemicals and a lack of toxicity in the investigated conditions (Hossain et al. 2021). In both static and kinetic testing, the designed biosurfactant demonstrated exceptional performance in eliminating motor oil (Gielnik 2019) and promoting the biodegradation of the contaminant in various soil types (da Silva et al. 2021). Another study reported by Almeida et al. (2021) where the potassium sorbate-containing biosurfactant from *Candida tropicalis* UCP0996 in petroleum products decontamination displayed stability and promoted a high motor oil emulsification rate (over 90%) at almost all tested conditions

CASE STUDIES

Extreme conditions can be stable for biosurfactants because of their high surface activity, capacity to emulsify, and resistance to high temperatures and salt concentrations (Bami et al. 2022). According to Durval et al. 2020 and Huang et al. 2020 Serratia marcescens ZCF25 and Bacillus cereus UCP 1615, two microorganisms isolated from oily sludge, produce extremely stable lipopeptide-type biosurfactants that lower surface tension and can remediate oil spills. Ambust et al. (2021) demonstrated that Pseudomonas spp. SA3 produced a biosurfactant that enhances agricultural crop growth in oil-contaminated soil, with emulsification and surface tension reduction capacities of 43% and 34.5 (milli Newton per meter) mN m^{-1}, respectively. Studies have shown that when Serratia spp. are cultivated in spent vegetable oil, they produce a biosurfactant that increases the solubility of various contaminants, including tetrachloroethylene (TCE), perchloroethylene (PCE), naphthalene, toluene, and phenanthrene. These samples were taken from a petroleum-contaminated site (Mulligan 2014). In their study of oil degradation in oil-polluted soil, Patil et al. (2012) found that possible hydrocarbon-degrading bacteria included *Bacillus* species, *Acinetobacter* species, *Micrococci* species, *Pseudomonas* species, and *Streptomyces* species. *Bacillus licheniformis*, an effective hydrocarbon-degrading strain resistant to high salinity, alkalinity, and temperature, was identified by Liu et al. (2016) from severely oil-contaminated soil near the Dagang Oilfield in Tianjin, China. The strain has been reported to break down both long-chain and short-chain alkanes, even with their more intricate structures (Liu et al. 2016). It also secretes emplastic at high temperatures, which may be used as a surfactant to enhance the emulsifying action. Furthermore, Verma & colleagues, in 2006, evaluated the ability of three bacterial isolates from a contaminated site in Ankleshwar, India, to degrade oily sludge (Bahmani et al. 2020, Verma et al. 2006). Based on the gravimetric analysis, they found that *Acinetobacter* species, *Bacillus* species, and *Pseudomonas* species respectively degraded approximately 59%, 37%, and 35% of the oily sludge in five days at 30 ˚C (Swetha et al. 2020). Additionally, after five days, the *Bacillus* spp. were able to metabolize aromatics and components of oily sludge with a chain length of C12–C30 more effectively than the other two strains that had been found, according to the capillary gas chromatographic examination. Six naturally occurring oil-degrading bacterial species were identified by Jiji and Prabakaran (2020) as *Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Pseudomonas* spp., *Bacillus* spp. and *Staphylococcus aureus* from soil contaminated with petroleum in Kerala, India's Changanassery, Kottayam district (Jiji & Prabakaran 2020). Thermophilic strains of *P. aeruginosa* and *B. subtilis* that are native to North-East India are effective in biodegrading crude petroleum oil (Bharathi 2019). Similarly, published literature indicates that the most significant hydrocarbon-degrading bacterial genera in polluted soils are *Variovorax*, *Bacillus, Achromobacter, Rhodococcus, Nocardioides, Nocardia, Pseudomonas, Sphingomonas, Arthrobacter,* and some other unculturable bacterial clones (Jiji & Prabakaran 2020). According to Karlapudi et al. in 2018, *P. aeruginosa* isolated from oil-polluted seawater can produce biosurfactants and, after 28 days of incubation, break down nonadecanes, hexadecanes, octadecanes, and heptadecanes (Karlapudi et al. 2018). Furthermore, *P. aeruginosa* was also reported to efficiently decompose a variety of hydrocarbons, including virgin, tetradecane, and 2-methylnaphthalene (Li 2018). Furthermore, a great deal of research has been done on the capacity of many bacterial genera, including *Bacillus, Rhodococcus, Alcaligenes, Corynebacterium, Acinetobacter, and Pseudomonas,* to produce biosurfactants that lead to the effective breakdown of petroleum oil (Abbasian et al. 2016). *Acinetobacter haemolyticus* and the biosurfactant-producing strain *Pseudomonas* ML2 were introduced into soil polluted with hydrocarbons for a two-month incubation period to study the degradation of hydrocarbons. *Pseudomonas* ML2 and *Acinetobacter haemolyticus* were found to decrease hydrocarbon concentration by 11–71% and 39–71%, respectively (Karlapudi et al. 2018). According to a different study (Itrich et al. 2015), the oil degradation capacity of FinasolOSR-5 was increased when it was combined with trehalose-5, dicorynomycolates, a biosurfactant. This allowed for the complete elimination of volatile aromatic organic compounds from contaminated soil in a shorter time frame. These results demonstrated the amazing hydrocarbon-degrading capacity of bacterial cell-free biosurfactants (Karlapudi et al. 2018). From now on, the capacity of microorganisms to produce biosurfactants in conjunction with their capacity to bioremediate hydrocarbons might be utilized to accelerate the process of bioremediation in environments contaminated by hydrocarbons (Kebede et al. 2021). Some of the case studies on biosurfactant-producing microorganisms in bioremediation are shown in (Table 2).

LIMITATIONS OF MICROBIAL BIOSURFACTANT-MEDIATED REMEDIATION

Native bacteria are slow-growing and have low metabolic

Table 2: Case studies on biosurfactant-producing microorganisms in bioremediation.

Microorganisms	Category of Pollutant	Type of Biosurfactant	References
Serratia spp.	Hydrocarbon	Lipopeptide	Gidudu et al. (2020)
Paenibacillus spp. D9	Motor oil and diesel	Lipopeptide	Jimoh & Lin (2020)
P. aeruginosa	Crude oil	Rhamnolipids	Karlapudi et al. (2018)
Serratia spp.	Petroleum	Serrawettin	Sah et al. (2022)
Pseudomonas spp.	Oil	Rhamnolipids	Ambust et al. (2021)
B. cereus UCP 1615 and *S. marcescens* ZCF25	Oil	Lipopeptide	Durval et al. (2020)
B. stratospheric strain FLU	Motor oil	Lipopeptide	Nogueria Felix et al. (2019)
S. marcescens	Burned motor oil	Lipopeptide	Araújo et al. (2019)
Pseudomonas ML2	Hydrocarbon	Rhamnolipids	Karlapudi et al. (2018)
B. cereus, P. aeruginosa, B. subtilis and *S. aureus*	Petroleum	Lipopeptides, rhamnolipids and surfactin	Jiji and Prabakaran (2020)
Achromobacter spp. A-8	Petroleum	Not specified	Deng et al. (2020)
Wickerhamomyces anomalous	Crude oil	Lipopeptide	Souza et al. (2018)
Starmerella bombicola	Motor oil	Commercial biosurfactants	Da Silva et al. (2021)

activity because they are difficult to domesticate, which makes decontamination a laborious and ineffective process. Moreover, polycyclic aromatic hydrocarbons (PAHs) and alkanes with carbon chains that are longer and shorter (C_{10}, C_{20}–C_{40}) are difficult to break down (Paniagua-Michel & Banat 2024). Therefore, in the oil-contaminated site, hydrocarbon-degrading bacteria cannot remove all oil components. (Sun et al. 2022). However, hydrocarbon molecules are difficult to break down and stick to soil particles. As a result, one of the major issues limiting biodegradation in the environment is the minimal availability of oil contaminants to microbes (Sah et al. 2022). There is a need for researchers to employ a variety of tactics, including biostimulation techniques, which involve adding nutrients to promote microbial growth and activity, bioaugmentation, hydraulic control, genetic engineering of the microbe, and several other techniques to address these problems. The goal of these strategies is to increase the hydrocarbon's accessibility to microorganisms that break them down, which will help overcome the obstacles presented by local bacteria that develop slowly and their inability to break down particular hydrocarbon components.

FUTURE PROSPECTS

The economics of producing biosurfactants has been the subject of a great deal of research over the past 20 years, but achieving commercial success in comparison to synthetic alternatives still presents a difficulty (Rawat et al. 2020). There are still some areas that require research and a variety of approaches that can be used to boost the industrial production of these biomolecules. Production costs could be greatly decreased, for example, by producing biosurfactants in non-sterile environments. It is necessary to conduct more thorough research on the use of unprocessed and fortified waste substrates as well as the co-production of biosurfactants with other commercially viable products, particularly in large fermentation vessels. Future developments regarding the genes that produce biosurfactants have great potential to transform oil-contaminated site bioremediation techniques. Unlocking the complexities of these genes offers a way to improve the productivity and selectivity of biosurfactant synthesis, increasing their influence on environmental cleaning as our knowledge of microbial genetics expands. Investigating the genetic variety of bacteria that produce biosurfactants is one fascinating direction. The range of surfactant types that can be used for bioremediation can be increased by identifying and analyzing novel biosurfactant genes from various bacterial strains. This variability could be the key to creating customized solutions for particular kinds of oil contamination or environmental circumstances, enabling a more accurate and successful cleanup strategy.

CONCLUSIONS

In conclusion, the thorough investigation of the activities of biosurfactant-producing bacteria and their potential for bioremediation in oil-contaminated sites opens a promising new arena for environmental restoration. Investigating the activities of bacteria that produce biosurfactants and their potential for bioremediation at oil-contaminated locations throws up an exciting world of opportunities for hydrocarbon degradation. By delving into the complex methods these microbes use, this review has shed light on how they help lessen the ecological effects of contamination and oil spills. These fascinating microbes come out as nature's partners in restoring our ecosystems because they can break down hydrocarbons and improve oil solubility. Their elaborate relationship with pollutants demonstrates the fine equilibrium that exists in the microbial world and provides a glimmer of hope for a peaceful and sustainable cohabitation between industry and the environment. In addition, biosurfactants have greater advantages for the environment over chemical surfactants that are typically employed in oil-contaminated site remediation response because of their environmentally benign nature. Contrasting dramatically with the possible ecological impact connected with manufactured surfactants is the natural, biological process of microorganisms producing biosurfactants. This facet corresponds with the worldwide transition towards sustainable methodologies and emphasizes the need to utilize nature's inbuilt processes for ecological restoration.

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