

# The importance of biotic and abiotic soil properties in the bioremediation of crude oil-contaminated soil

## Važnost biotički i abiotičkih svojstava tla u bioremedijaciji naftom onečišćenog tla

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### ABSTRACT

Soil is a non-renewable resource essential for food production, ecosystem services, and climate regulation, yet it is increasingly threatened by contamination. Crude oil and its derivatives (petroleum hydrocarbons, PHs) are complex mixtures of compounds that dissolve poorly in water and strongly adsorb to soil particles, reducing their bioavailability and making remediation challenging. Bioremediation, particularly through bioaugmentation and biostimulation, is recognized as a safe, cost-effective, and environmentally sustainable strategy for restoring contaminated sites. This technology exploits the metabolic diversity of microorganisms capable of biodegrading hydrocarbons. Indigenous microbial communities, naturally adapted to PHs contamination, often exhibit superior degradation efficiency due to the production of hydrocarbon-degrading enzymes, biosurfactants that increase pollutant availability, and tolerance to hydrocarbon toxicity. However, their activity is strongly influenced by abiotic soil properties such as texture, pH, temperature, salinity, nutrient availability, moisture, and oxygen content, all of which regulate microbial growth and metabolic activity. Biosurfactant-producing bacteria are of particular interest because they simultaneously degrade hydrocarbons and enhance their bioavailability, reducing the need for synthetic surfactants and lowering environmental risks and costs. Understanding the interactions between indigenous microbial communities, contamination characteristics, and soil physicochemical properties is therefore critical for designing effective, site-specific remediation strategies. This review summarizes current knowledge on soil properties important for bioremediation, highlights key bacterial strains adapted to PH contamination, and discusses how these insights can guide the development of efficient and sustainable approaches for the restoration of petroleum-contaminated soils.

**Keywords:** contamination, petroleum hydrocarbons, bacterial strains, soil properties, bioremediation

### SAŽETAK

Tlo je neobnovljiv resurs od velikog značaja, ključan za proizvodnju hrane, očuvanje ekosustava i regulaciju klime, a danas je sve više ugroženo zbog onečišćenja. Sirova nafta i njezini derivati složene su smjese spojeva slabo topljive u vodi, a vrlo često snažno vezane na čestice tla, što značajno smanjuje njihovu biodostupnost i usporava potupke pročišćavanja. Tehnologija bioremedijacije, uključujući i bioaugmentaciju i biostimulaciju, prepoznata je kao sigurna, jednostavno te ekonomski i ekološki prihvatljiva strategija za pročišćavanje onečišćenoga tla. Ova tehnologija koristi metabolički potencijal mikroorganizama sposobnih za razgradnju naftnih ugljikovodika. Autohtone mikrobne zajednice prilagođene onečišćenju, često pokazuju veću učinkovitost zahvaljujući proizvodnji enzima koji sudjeluju u razgradnji ugljikovodika i proizvodnji biosurfaktanata koji povećavaju dostupnost onečišćenja, ali i zbog tolerancije na toksičnost

ugljkovodika. Međutim, njihova učinkovitost uvelike ovisi o abiotičkim svojstvima tla, poput teksture, pH vrijednosti, temperature, saliniteta, dostupnosti hranjivih tvari, vlage i sadržaja kisika, koji imaju veliki utjecaj na rast i metaboličku aktivnost mikroorganizama. Bakterije koje proizvode biosurfaktante posebno su zanimljive jer istovremeno razgrađuju ugljikovodike i povećavaju njihovu biodostupnost, čime se smanjuje potreba za sintetskim surfaktantima te smanjuju okolišni rizici i troškovi. Razumijevanje međudjelovanja između autohtonih mikrobnih zajednica, karakteristika onečišćenja i fizikalno-kemijskih svojstava tla ključno je za dizajniranje učinkovitih i lokaciji prilagođenih strategija sanacije. Ovaj pregledni rad sažima postojeća saznanja o svojstvima tla važnima za bioremedijaciju, ističe ključne bakterijske sojeve prilagođene onečišćenju naftnim ugljikovodicima te raspravlja na koji način ta saznanja mogu doprinijeti razvoju učinkovitih i održivih pristupa obnovi tla onečišćenog naftnim ugljikovodicima.

**Ključne riječi:** onečišćenje, naftni ugljikovodici, bakterijski sojevi, svojstva tla, bioremedijacija

## INTRODUCTION

Soil is recognised as a fundamental, non-renewable resource that supports life on Earth as it is currently understood. It plays a critical role in the production of food and nutrient cycling, as well as in carbon sequestration, which mitigates the impacts of climate change. Beyond these functions, soil also serves as the foundation for industry and infrastructure development, making it essential for economic growth, but it is also a vital ecosystem offering habitats for plants, animals, and humans (Clunes et al., 2022; Lal et al., 2021). As energy demands continue to rise in both industry and daily life, crude oil remains a primary source of supply. Consequently, contamination of soil and water with oil and oil derivatives is a major environmental problem related to the oil industry (Koshlaf and Ball, 2017; Pandolfo et al., 2023). Significant soil degradation is currently being observed on a global scale, and the loss of clean and viable soil due to contamination is increasingly recognised as a major environmental problem. Once released into the environment, hydrocarbons interact with both the soil matrix and its living community, including microorganisms, plants, and animals. In addition to their toxic effects on plants, animals, and even humans, crude oil further disrupts soil ecosystems by altering their physical and chemical properties, thereby negatively affecting the overall functioning of the environment (Kisic et al., 2009). Today comprehensive efforts are being made to mitigate ecological and health risks and restore contaminated sites to their pre-contamination state (Clunes et al., 2022).

Bioremediation technology exploits microorganisms with metabolic pathways and mechanisms that enable

them to biodegrade hydrocarbons and today is recognised as a safe, cost-effective and environmentally friendly solution for soil contaminated with crude oil (Al-Dhabaan, 2019; Kisić et al., 2022; Ossai et al., 2020; Pandolfo et al., 2023). A specific microbial community with enzymes involved in hydrocarbon breakdown is crucial for successful bioremediation (Pandolfo et al., 2023). Microorganisms exposed to oil contamination have adapted and exhibit significantly higher biodegradation rates compared to those from uncontaminated environments (Łebkowska et al., 2011; Szulc et al., 2014). These conclusions have led to the development of bioaugmentation techniques, in which external microbial strains with specific enzymes are introduced into contaminated soil to enhance biodegradation rates and further optimize the bioremediation process (Ossai et al., 2020). The effectiveness of bioaugmentation relies on the ability of the introduced microbial strains to adapt to the specific biotic and abiotic conditions of the contaminated soil. For successful bioremediation, environmental factors such as nutrient availability, temperature, moisture, pH values and oxygen levels must be optimized to support microbial growth and metabolic activity (Aziz et al., 2024). The limited bioavailability of hydrocarbons to microorganisms is also a significant challenge. Since hydrocarbons are mostly poorly soluble in water and strongly bound to the soil matrix, microorganisms cannot effectively break down these contaminants (Pandolfo et al., 2023). To overcome these problems and to optimise environmental conditions, the biostimulation technique is often used. Biostimulation involves loosening the soil to

achieve homogenization and aeration, maintaining optimal soil moisture, adding nutrients, and applying surfactants to enhance pollutant bioavailability for bacteria (Ossai et al., 2020; Vidali, 2001).

In addition to microbial-based approaches, other biological technologies such as rhizoremediation and the use of soil fauna can further enhance bioremediation of contaminated soil. Plant-microbe interactions in the rhizosphere stimulate microbial activity through root exudates, while soil organisms such as earthworms not only contribute to contaminant degradation but also improve soil structure, aeration, and contaminant bioavailability, thereby indirectly supporting microbial degradation (Correa-García et al., 2018).

The bioremediation process involves complex interactions between soil, contaminants, and microorganisms. Consequently, the efficiency of the bioremediation of soil is influenced by many factors, such as soil properties, the bioavailability of contaminants to microorganisms, contaminant concentration, and the competition between bioaugmented microorganisms and the native microbial community (biostimulation) (Aziz et al., 2024; Bidja Abena et al., 2019a; Liu et al., 2020; Vidali, 2001). Because of that, to enhance biodegradation rates and further optimize the bioremediation process, biostimulation is often performed together with the bioaugmentation technique. Today bioremediation is recognised as a “green technology” and viable solution for the remediation of soil contaminated with PHs.

The aim of this article is not only to expand the understanding of microbial diversity in contaminated soils but also to further support the design of efficient bioremediation approaches, ultimately enhancing the remediation of petroleum-contaminated sites.

## SOIL

### *Formation and properties*

Soil is a top layer of the Earth's crust defined as a heterogeneous mixture of weathered rocks, nutrients, living organisms, organic matter, water, and air, which serves as a medium for the growth of plants (Kalev and Toor,

2018). The initial step in soil formation is the physical, chemical and biological weathering of the rocks, which is the reason for the similarity in chemical composition between the Earth's crust and soil. The result of the weathering process is the formation of parent material which is in soil formation process the state of the soil system at the time zero. Together with climate, biosphere, relief and time, parent material is one of the five soil forming factors (Kalev and Toor, 2018). Soil forming processes are highly complex and dynamic, involving numerous chemical and biological reactions. These processes include the addition or loss of water, minerals, and organic matter in the soil, the transformation of mineral and organic materials within the soil, and the transfer or translocation of energy and matter. The soil is formed through the interaction of soil forming factors and soil forming processes and because it can be formed from diverse parent materials and under various environmental conditions, there exists a remarkable diversity of soils worldwide.

### *The significance and degradation*

Soil formation is also a very slow process, with the formation rate typically ranging from 0.01 to 0.1 mm/year (Clunes et al., 2022). Therefore, compared to a human lifetime, soil is often called a non-renewable natural resource. Soil has many specific functions and performs a leading role in ecological sustainability, climate change mitigation, carbon sequestration, ecosystem services, land use and planning, groundwater protection and food production (Dazzi and Lo Papa, 2022; Lehmann et al., 2020). Furthermore, soil is regarded as a valuable natural resource that plays a crucial role in shaping ecosystems and is essential for sustaining life on the planet; however, widespread soil degradation is presently being observed worldwide. Soil is under constant threat from ongoing land use changes, sealing, compaction, waterlogging, erosion, loss of organic matter and biodiversity, chemical contamination, salinization, and nutrient mismanagement (Clunes et al., 2022; Lehmann et al., 2020). Soil contaminated with PHs has a negative impact on biodiversity and ecosystem preservation, additionally contributing to soil degradation. This problem is now

recognised as critical, and extensive efforts are being made to develop novel, more effective, and economically viable technologies for the remediation and restoration of contaminated sites to their original state. It is also crucial to adopt a comprehensive legal framework for soil protection to ensure food security, clean water, and the preservation of biodiversity and ecosystems.

## BIOTIC SOIL PROPERTIES IMPORTANT FOR BIOREMEDIATION

### *Indigenous microbial community*

Microbial remediation technology utilizes the metabolic diversity of microorganisms to transform, reduce, or eliminate PH contamination in the environment. Effective biodegradation of diverse hydrocarbons (HCs) requires the synergistic action of microbial consortia, since individual bacterial strains possess enzymes capable of degrading specific types of hydrocarbons (Figure 1) (Bidja Abena et al., 2019a; Varjani, 2017). This synergistic interaction among diverse microbiological communities is responsible for the comprehensive biodegradation of complex hydrocarbon mixtures (Bidja Abena et al., 2019b; Nwankwegu and Onwosi, 2017; Varjani, 2017).

Crude oil contamination renders soil toxic and disrupts its physicochemical properties, making it inhospitable to most microorganisms. Despite these extreme conditions, some bacterial strains have developed adaptive mechanisms that allow them to survive, grow, and even proliferate in contaminated environments. These adaptations involve activating DNA repair systems as well as producing quorum-sensing molecules and biofilms. Furthermore, adapted bacteria can emulsify and metabolize hydrocarbons while regulating efflux pumps and membrane channels to maintain optimal intracellular hydrocarbon concentrations (Pandolfo et al., 2023). By utilizing these adaptive strategies, microbial communities become more resilient and metabolically efficient, enabling them to degrade hydrocarbons more effectively than microorganisms in uncontaminated soils (Koshlaf et al., 2016; Szulc et al., 2014).

Understanding native microbial communities capable of surviving in oil-contaminated environments is essential for improving bioremediation efforts. In recent years, research has increasingly focused on how these indigenous bacterial strains adapt to and persist in petroleum-contaminated soils. The isolation and identification of these strains is especially important, as they hold considerable potential for application in bioaugmentation strategies (Table 1). Multiple studies have shown that autochthonous microbial communities can achieve high biodegradation rates and serve as effective agents for bioaugmentation (Abdelhaleem et al., 2019; Varjani et al., 2020; Varjani and Upasani, 2019). This high efficiency is largely attributed to their adaptive mechanisms, including the production of hydrocarbon-degrading enzymes, biosurfactants that enhance hydrocarbon bioavailability, and resilience to hydrocarbon toxicity, all of which enable these microorganisms to survive, proliferate, and efficiently metabolize complex hydrocarbon mixtures. Additionally, being naturally adapted to the local climate and soil properties further supports their activity and stability in contaminated environments.

Although numerous studies, including those summarized in Table 1, have demonstrated that many microorganisms are capable of surviving in petroleum-contaminated environments, their efficiency in hydrocarbon biodegradation is often constrained by unfavorable environmental conditions. Factors such as low hydrocarbon bioavailability, along with deficiencies in nutrients, oxygen, or moisture, can significantly limit their metabolic activity and overall remediation potential. Recognizing these limitations is important both for selecting the most suitable microbial communities for bioaugmentation and for designing effective bioremediation strategies that optimize microbial efficiency in biodegrading PHs in contaminated soils.

### *Biosurfactant-producing bacteria*

The bioavailability of hydrocarbons is one of the main problems in bioremediation, as these compounds dissolve poorly in water and often adsorb strongly onto soil particles, limiting microbial access and slowing degradation.

Bacterial strain	Isolation source	Reference
<i>Acinetobacter junii</i>	Soil samples (oil exploration areas of Ahvaz, Iran)	(Ohadi et al., 2017)
	Oil-contaminated soil sample (Sichuan Province, China)	(Jiang et al., 2023)
<i>Acinetobacter lwoffii</i>	Crude oil-contaminated soil (Zhejiang Province, China)	(Bidja Abena et al., 2019b)
<i>Acinetobacter pittii</i>	Crude oil-contaminated soil (Zhejiang Province, China)	(Bidja Abena et al., 2019b)
<i>Bacillus atrophaeus</i>	Aged crude oil-contaminated soil samples (oil fields, Iran)	(Kiamarsi et al., 2019)
<i>Bacillus cereus</i>	Crude oil-contaminated soil (Yanchang County, China)	(Deng et al., 2020)
	Soil and water samples from hydrocarbon-contaminated environments (Gauteng, South Africa)	(Mandree et al., 2021)
	Crude oil-contaminated soil (Croatia)	(Bertović et al., 2024)
	Soil samples contaminated with PAHs (abandoned chemical plant, Beijing, China)	(Zhao et al., 2018)
<i>Bacillus halotolerans</i>	Oil-contaminated soil samples (Khurais oil field, Saudi Arabia)	(Al-Dhabaan, 2019)
	Crude oil-contaminated soil (Yanchang County, China)	(Deng et al., 2020)
<i>Bacillus licheniformis</i>	Crude oil-contaminated soil (Croatia)	(Bertović et al., 2024)
<i>Bacillus megaterium</i>	Crude oil-contaminated soil (Croatia)	(Bertović et al., 2024)
<i>Bacillus subtilis</i>	Soil and water samples from hydrocarbon-contaminated environments (Gauteng, South Africa)	(Mandree et al., 2021)
	Crude oil-contaminated soil (Zhejiang Province, China)	(Bidja Abena et al., 2019b)
	Aged crude oil-contaminated soil samples (oil fields, Iran)	(Kiamarsi et al., 2019)
	Oil-contaminated soil samples (Khurais oil field, Saudi Arabia)	(Al-Dhabaan, 2019)
<i>Bacillus thuringiensis</i>	Soil samples contaminated with PAHs (abandoned chemical plant, Beijing, China)	(Zhao et al., 2018)
<i>Bacillus velezensis</i>	Soil and water samples from hydrocarbon-contaminated environments (Gauteng South Africa)	(Mandree et al., 2021)
<i>Citrobacter sedlakii</i>	Soil contaminated crude oil (Shiraz Oil Refinery, Iran)	(Ghoreishi et al., 2017)

Bacterial strain	Isolation source	Reference
<i>Enterobacter cloacae</i>	Unpolluted soil (Shiraz Oil Refinery, Iran)	(Ghoreishi et al., 2017)
	Hydrocarbon-contaminated soils (Punjab province, Pakistan)	(Ejaz et al., 2021)
<i>Enterobacter hormaechei</i>	Crude oil-contaminated and clean soil (Shiraz Oil Refinery, Iran)	(Ghoreishi et al., 2017)
	Oil-based muds waste samples (drilling sites, Egypt)	(El-Liethy et al., 2022)
<i>Nocardioides</i> sp.	Soil samples contaminated with PAHs (abandoned chemical plant, Beijing, China)	(Zhao et al., 2018)
<i>Plantibacter auratus</i>	Aged crude oil-contaminated soil samples (oil fields, Iran)	(Kiamarsi et al., 2019)
<i>Pseudomonas aeruginosa</i>	Crude oil-contaminated soil (petroleum production site, Thailand)	(Mukjang et al., 2022)
	Oil-contaminated soil samples (Khurais oil field, Saudi Arabia)	(Al-Dhabaan, 2019)
	Motor oil-contaminated soil (Kairouan, Tunisia)	(Chebbi et al., 2017)
<i>Pseudomonas chlororaphis</i>	Crude oil-contaminated soil (Croatia)	(Bertović et al., 2024)
<i>Pseudomonas guineae</i>	Soil samples exposed to diesel fuel (Antarctic)	(Gran-Scheuch et al., 2017)
<i>Pseudomonas resinovorans</i>	Aged crude oil-contaminated soil samples (oil fields, Iran)	(Kiamarsi et al., 2019)
<i>Raoultella ornithinolytica</i>	Artificially contaminated soil with crude oil, diesel, and gasoline (Zhejiang Province, China)	(Bidja Abena et al., 2020)
<i>Rhodococcus erythropolis</i>	Soil samples exposed to diesel fuel (Antarctic)	(Gran-Scheuch et al., 2017)
<i>Serratia marcescens</i>	Artificially contaminated soil with crude oil, diesel, and gasoline (Zhejiang Province, China)	(Bidja Abena et al., 2020)
<i>Sphingobium xenophagum</i>	Soil samples exposed to diesel fuel (Antarctic)	(Gran-Scheuch et al., 2017)
<i>Staphylococcus pasteurii</i>	Two aged crude oil-contaminated soil samples (oil fields, Iran)	(Kiamarsi et al., 2019)

The application of surfactants can help overcome this limitation by increasing hydrocarbon solubility and making them more accessible to degrading microorganisms. Synthetic surfactants are commonly used for this purpose, but these chemicals are often recalcitrant and can accumulate easily in the soil, posing a risk to the environment. In contrast, biosurfactants are less toxic to the environment, can be easily biodegraded, and offer a more sustainable alternative (da Silva et al., 2021; Stepanova et al., 2022).

Research on the isolation and identification of biosurfactant-producing bacteria has revealed that strains from several genera, including *Pseudomonas* (Abbasi et al., 2013; Fardami et al., 2022), *Enterobacter* (Fardami et al., 2022), *Pantoea* (Fardami et al., 2022), *Agrococcus* (Soltanighias et al., 2019), *Arthrobacter* (Soltanighias et al., 2019), *Dietzia* (Soltanighias et al., 2019), *Kocuria* (Soltanighias et al., 2019), *Lysinibacillus* (Soltanighias et al., 2019), *Serratia* (Almansoori et al., 2019; Karlapudi et al., 2018) and *Bacillus* (Fardami et al., 2022; Feng et al., 2019; Soltanighias et al., 2019) are capable of producing biosurfactants. These findings highlight the dual role of many naturally occurring bacteria in hydrocarbon-contaminated environments - not only to degrade hydrocarbons but also to enhance the process by producing biosurfactants that increase the bioavailability of otherwise poorly soluble compounds. The use of biosurfactant-producing microorganisms, which are also capable of hydrocarbon degradation, has proven to be a highly effective bioremediation strategy (Figure 1) (Łebkowska et al., 2011; Soltanighias et al., 2019).

When designing the bioremediation strategies, it is important to consider whether the selected microorganisms can produce biosurfactants. Limited pollutant bioavailability is often one of the main reasons why biodegradation fails, and biosurfactant production can help overcome this problem. If the chosen strains can't produce biosurfactants, synthetic surfactants may need to be applied. However, the use of chemicals can negatively affect the environment and substantially increase the overall cost of the bioremediation process.

### ***Enzymatic mechanisms of petroleum hydrocarbon biodegradation***

The biodegradation of PHs relies on a series of enzymatic reactions that enable the transformation of these complex contaminants (Figure 1). As bioremediation of PHs is predominantly an aerobic process, the first step typically involves the incorporation of oxygen into hydrocarbon molecules. This reaction is catalysed by enzymes such as monooxygenases and dioxygenases, depending on whether one or two oxygen atoms are incorporated into the substrate (Koshlaf and Ball, 2017).

During alkane degradation, terminal oxidation converts methyl groups into primary alcohols, which are further oxidized to aldehydes by alcohol dehydrogenases, and then to fatty acids by aldehyde dehydrogenases (Pandolfo et al., 2023). Fatty acids are further metabolized via  $\beta$ -oxidation to acetyl coenzyme A, which subsequently enters the Krebs cycle (Koshlaf and Ball, 2017). In the degradation of aromatic hydrocarbons, a key enzymatic step is ring activation and cleavage. Catechol dioxygenases play a crucial role in the cleavage of aromatic rings, enabling further mineralization of these compounds (Varjani, 2017).

### ***Rhizoremediation***

Phytoremediation is another important biological technique that can be used for the remediation of soil contaminated with PHs. Generally, phytoremediation is described as the use of plants to remediate contaminated environments, but it includes many different mechanisms like phytotransformation, phytoextraction, phytostabilization, phytovolatilization, rhizofiltration and rhizoremediation (Stepanova et al., 2022; Correa-García et al., 2018). Although all these mechanisms can be applied to soil contaminated with PHs, rhizoremediation is one of the most important due to plant-microbe interactions (Figure 1) (Stepanova et al., 2022). Rhizoremediation involves the degradation of contaminants in the rhizosphere zone through microbial activity, which is stimulated by the release of root exudates, such as organic acids, sugars, and amino acids, which serve as

carbon and energy sources for microorganisms. These compounds enhance microbial growth and enzymatic activity, thereby promoting the biodegradation of hydrocarbons (Stepanova et al., 2022; Ossai et al., 2020). Furthermore, root exudates can increase the bioavailability of contaminants by altering the geochemical characteristics of soil. Root systems also improve oxygen diffusion and increase moisture content, creating favourable conditions for aerobic degradation processes, but also create a habitat for autochthonous microorganisms (Ossai et al., 2020).

Generally, indigenous hydrocarbon-degrading microbes, adapted to contamination, can be efficiently stimulated by plants, so for rhizoremediation to be successful, the selection of plant species is crucial (Correa-García et al., 2018). Different plants vary in their ability to stimulate microbial degradation of petroleum hydrocarbons. For example, degradation of polycyclic aromatic hydrocarbons (PAHs) differs significantly among plant species such as *Echinacea purpurea*, *Festuca arundinacea*, and *Medicago sativa*, although degradation rates were higher in all planted systems compared to the control (Liu et al., 2015). Similarly, in field conditions, the impact of different tree species (*Albizia lebbek*, *Cassia siamea*, *Delonix regia*, and *Dalbergia sissoo*) was evaluated (Mukhopadhyay et al., 2017). The PAH removal efficiencies varied among species, ranging from approximately 50% to over 80%, highlighting the importance of plant selection in rhizoremediation strategies. Rhizoremediation using non-edible plant *Jatropha curcas* has been shown to enhance the removal of waste lubricating oil from soil, up to 89% and 96% (Mukhopadhyay et al., 2017). The study indicates that the presence of *J. curcas* significantly increases the abundance of hydrocarbon-degrading microorganisms in the rhizosphere, including species of *Pseudomonas*, *Bacillus megaterium*, *Micrococcus* and *Corynebacterium*, suggesting that contaminant removal is primarily driven by rhizodegradation rather than plant uptake. Another study showed that the roots of both wild desert plants (*Senecio glaucus*, *Cyperus conglomeratus*, *Launaea mucronata*, *Picris babylonica*, *Salsola imbricata*) and crop species (*Vicia faba* and *Lupinus albus*) grown in oil-contaminated

soils are closely associated with hydrocarbon-utilising bacteria such as *Cellulomonas flavigena*, *Rhodococcus erythropolis*, and *Arthrobacter* sp. (Radwan et al., 1998). The rhizosphere soils contained a higher number of these microorganisms compared to the bulk soil. This highlights the important role of plant-microbe interactions in enhancing hydrocarbon degradation.

Despite its potential, rhizoremediation has several limitations. The efficiency of the process depends strongly on plant species, soil conditions, and contaminant properties, which can vary significantly between sites. In addition, the process is relatively slow and often limited to the root zone, making it less effective for deeply contaminated soils (Stepanova et al., 2022).

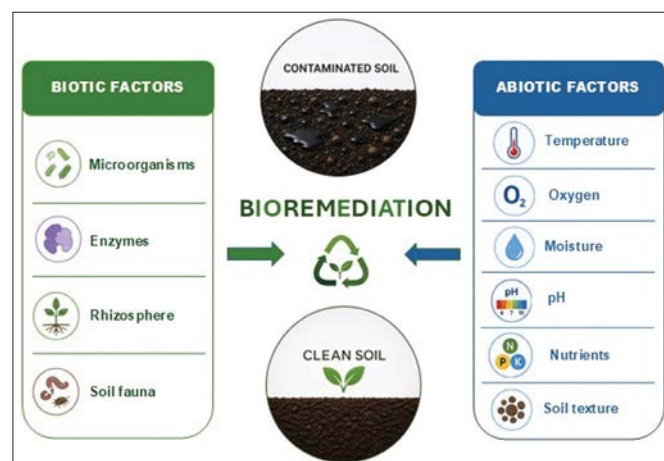


Figure 1. Biotic and abiotic factors important for effective bioremediation of soil contaminated with PHs

### Soil fauna

In addition to remediation with microorganisms and plants, soil fauna such as nematodes, collembola, protists and earthworms can also be used as a tool for improving the efficiency of bioremediation processes (Figure 1) (Correa-García et al., 2018). The most commonly used and studied organisms are earthworms (Ossai et al., 2020). This technique is known as vermiremediation, and its main principle is the use of earthworms to extract, transform, accumulate and degrade the contaminants in soil (Javed and Hashmi, 2021). Earthworms also interact with biotic and abiotic factors in soil, improving the soil properties, increasing aeration and bioavailability of contaminants, and enhancing microbial activity by creating

the optimal conditions (Ossai et al., 2020; Correa-García et al., 2018).

One of the most promising earthworm species for soil remediation is *Eisenia fetida* (Javed and Hashmi, 2021). The study shows that *E. fetida* can survive and enhance the bioremediation of soil contaminated with crude oil, even at high contamination levels (e.g. 30,000 mg/kg) (Martinkosky et al., 2017). The degradation rates in the experiment with *E. fetida* were higher (~90 mg/day) compared to treatments without earthworms (~40 mg/day). Another study reported reductions of some aliphatic hydrocarbons of up to 100%, along with a decrease of approximately 67% and 50% of octadecane and tetracosane after 120 hours (Njoku et al., 2016). Earthworms such as *Eudrilus eugeniae* have also shown a high capacity to adapt to petroleum-contaminated soils, maintaining survival and activity over experimental periods of up to 20 days under controlled moisture conditions (70–80%) (Veena et al., 2023). The study indicates that their presence can lead to changes in the chemical composition of contaminants, including transformations of functional groups, suggesting active bioconversion processes.

## ABIOTIC SOIL PROPERTIES IMPORTANT FOR BIOREMEDIATION

### Soil texture

The relative amount of sand, silt and clay in soil is a very important abiotic factor influencing the fate and transport of PHs but also affecting microbial activity in contaminated soil (Figure 1). Soil texture is not only important for the distribution and bioavailability of contaminants but also for the bioavailability of essential nutrients and oxygen (Atlas and Bartha, 1992; Haghollahi et al., 2016). Sandy soils, which are mostly made of large particles, exhibit high porosity and permeability, allowing fast and efficient air and water movement through the soil profile. These soil properties have a positive influence on bioremediation efficiency by enhancing oxygen and water diffusion (Abdel-Moghny et al., 2012). On the other hand, clayey soils contain fine particles with

high specific surface area and cation exchange capacity (CEC). Because of these properties, these soils strongly adsorb HCs and nutrients, making them less bioavailable to microbes (Haghollahi et al., 2016). The low permeability and compact structure of clay-rich soils also limit oxygen transfer and potentially lead to anaerobic conditions. This can significantly slow the bioremediation process, as anaerobic degradation of hydrocarbons is generally less efficient and slower than aerobic pathways (Varjani, 2017). An advantage of this soil type is the minimal risk of contaminant migration into deeper soil layers and groundwater, because the contaminants are strongly bound to soil particles. Loamy soils, which are a mixture of sand, silt and clay, have the optimal nutrient availability, moisture retention and oxygen flow. These properties promote microbial growth and activity; consequently, the microbial community in loamy soils is more abundant, active and capable of biodegrading a wide range of PHs (Afzal et al., 2011).

### Temperature

Temperature is a crucial factor that influences not only bacterial growth, abundance and diversity of microbial communities, but also affects the physicochemical properties of PHs. These effects can significantly influence the overall efficiency of biodegradation processes (Figure 1) (Aziz et al., 2024; Mekonnen et al., 2024). Although many microorganisms are capable of surviving under extreme temperature conditions, most species exhibit optimal growth and metabolic activity within a relatively narrow temperature range. Different studies have reported temperatures ranging from 20 °C to 40 °C as optimal for bioremediation of soil contaminated with crude oil (Ullah, 2022; Vidali, 2001). However, most of the research evaluating the effectiveness of selected bacterial strains in the biodegradation of PHs has been conducted at temperatures between 20 °C and 25 °C (Annie et al., 2020; Bidja Abena et al., 2019b; Varjani and Upasani, 2019). At lower temperatures, microbial activity and abundance are reduced, which negatively affects the rate of hydrocarbon degradation (Margesin and Schinner, 2001). Nonetheless, some specific microorganisms,

adapted to cold environments, have demonstrated the ability to biodegrade hydrocarbons but at slower rates in comparison to biodegradation in an optimal environment (Gran-Scheuch et al., 2017). Elevated temperatures may increase the bioavailability of hydrophobic hydrocarbons by reducing their viscosity and surface tension, but also inhibit microbial growth and denature enzymes (Atlas and Bartha, 1992). Therefore, maintaining soil temperatures within the optimal range is necessary for successful bioremediation.

### **Soil pH**

Soil pH is a critical environmental factor in the biodegradation of PHs, as it directly affects the growth, abundance, and metabolic activity of soil bacteria (Figure 1) (Aziz et al., 2024). Most bacteria, including those responsible for hydrocarbon biodegradation, thrive in neutral to slightly alkaline conditions. The optimal pH for the biodegradation of crude oil is between 6.5 and 8.0 (measured in H<sub>2</sub>O) (Vidali, 2001). Because of that, for successful bioremediation of soil, it is essential to monitor and optimise pH.

### **Salinity**

Increased salt concentration in soil increases osmotic pressure, which has a negative impact on enzyme secretion and other cellular processes (Aziz et al., 2024; He et al., 2017). Salinity also reduces oxygen availability for microorganisms, further limiting their metabolic activity. Moreover, high concentrations of salt additionally decrease the solubility of hydrocarbon compounds, making them even less bioavailable (Aziz et al., 2024). High salinity can also cause physical damage to microbial cells, including the disruption of cell membranes, ultimately leading to cell lysis and mortality. All this inhibits microbial activity in soil and reduces the overall effectiveness of bioremediation in saline environments (Mekonnen et al., 2024).

### **Oxygen content**

While it is well established that bioremediation of organic contaminants can occur under both aerobic and

anaerobic conditions, research efforts have predominantly emphasized the mechanisms and dynamics of aerobic metabolism in the degradation of PHs (Aziz et al., 2024; Ullah, 2022). Under aerobic conditions, the biodegradation process occurs at a significantly faster rate compared to anaerobic environments (Figure 1) (Aziz et al., 2024). The initial phase of petroleum degradation occurs in the presence of oxygen, which serves as a crucial element for the complete degradation process. Typically, for the degradation of 1 mL of hydrocarbons into water and carbon dioxide, 3–4 mL of oxygen is needed (Ullah, 2022).

### **Moisture content**

Moisture content in soil is an important factor for successful bioremediation (Figure 1). Low moisture levels can reduce microbial activity, while excessive water can hinder oxygen transfer and contribute to the spread of contamination into previously uncontaminated soil and groundwater (Schjønning et al., 2011). The moisture content in soil, optimal for hydrocarbon degradation, varies across studies, due to different soil types, pore size distribution and soil texture (Haghollahi et al., 2016). Most studies suggest that moisture content ranging from 15% to 20% is optimal for the bioremediation of petroleum hydrocarbon-contaminated soils, as it provides a balance that supports microbial activity without negative impact on oxygen availability (Szulc et al., 2014; Varjani, 2017).

### **Nutrients**

The biostimulation strategy typically involves adding essential nutrients, which are often limited in contaminated environments, to support the growth and development of microorganisms. These nutrients enable the synthesis of vital enzymes required for the biodegradation of organic pollutants (Vidali, 2001). The most important nutrients for microbial growth and function are nitrogen, phosphorus, and carbon (Figure 1) (Aziz et al., 2024; Ullah, 2022). Adequate supplementation of these nutrients can significantly accelerate the bioremediation process. In particular, the optimal carbon-to-nitrogen (C:N) and carbon-to-phosphorus (C:P) ratios are critical

for supporting microbial growth and metabolic activity. A C:N:P ratio of approximately 100:10:1 is commonly considered ideal for promoting microbial degradation processes and optimization of the overall bioremediation process (Vidali, 2001).

## CONCLUSION

Microbial remediation of crude oil-contaminated soil is a complex process that occurs under specific environmental conditions, and the efficiency of the process is influenced by numerous factors. Because crude oil is a mixture of over a thousand different compounds, the most important thing for effective bioremediation is the presence of a diverse and abundant microbial community capable of biodegrading a wide range of hydrocarbon compounds. Research on contaminated environments provides valuable information about microbial communities adapted to high contaminant concentrations and stressful environmental conditions. These adapted microorganisms possess significant biotechnological potential for the further optimization of soil bioremediation. In addition to microorganisms, other biotic components such as plants and soil fauna can further enhance bioremediation processes by stimulating microbial activity and improving soil conditions. Plant-microbe interactions in the rhizosphere, as well as the activity of soil fauna, can increase contaminant bioavailability, improve aeration, and support microbial degradation processes. Soil texture is another important factor because it directly affects the physical and chemical characteristics of the soil, influencing oxygen diffusion, moisture retention, and contaminant mobility. Although certain microorganisms can tolerate extreme temperatures and other extreme conditions, the majority require a narrow range of conditions for optimal growth and activity. Therefore, for safe, efficient and cost-effective bioremediation, it is important to ensure the presence of specific microorganisms in soil, but also to have knowledge of soil properties and maintain optimal environmental conditions necessary for microbial activity.

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