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Enhancing Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soil Using *Brachiaria ramosa*: A Review of Plant Growth Promotion and Degradation Potential

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Abstract

This paper examines rhizoremediation as a sustainable and environmentally acceptable remediation technique in response to the growing problem of petroleum hydrocarbon pollution in soils. Brachiaria ramosa, a tropical grass species that may be useful in contaminated settings, is the main emphasis. A critical analysis of the literature on plant growth-promoting rhizobacteria (PGPR), microbial consortia involved in hydrocarbon breakdown, and rhizosphere-mediated degradation is conducted. There is a thorough discussion of important mechanisms such as root exudation, microbial stimulation, increased hydrocarbon bioavailability, and interactions between plants and microbes. The physicochemical characteristics of the soil, the surrounding environment, and the dynamics of the microbial population are all factors that affect the effectiveness of remediation. Despite the paucity of direct experimental evidence specific to B. ramosa, established rhizoremediation principles and insights from related species point to its potential use. This review identifies existing knowledge gaps and stresses the necessity of field-scale research and controlled experimental validation. All things considered, combining B. ramosa with efficient microbial systems is a viable approach to the long-term cleanup of hydrocarbon-contaminated soils.

Keyword: Rhizoremediation, *Brachiaria Ramosa*, petroleum hydrocarbons, plant growth-promoting rhizobacteria (PGPR), microbial consortia, soil remediation, sustainable agriculture, environmental restoration

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INTRODUCTION

Soil pollution by petroleum hydrocarbons has been recognized as a major environmental issue due to the accelerated pace of industrialization, urbanization, and the increased use of petroleum products [1–3]. Crude oil exploration, transportation, and leakage from storage tanks have resulted in large-scale soil contamination [4, 5]. Petroleum hydrocarbons are complex mixtures of aliphatic and aromatic compounds that persist in soil and cause adverse effects on soil structure, fertility, microbial diversity, and overall soil health [6–8]. These pollutants are of major concern due to the toxic, mutagenic, and carcinogenic properties of some petroleum hydrocarbons [9, 10]. Conventional remediation analytical frameworks, such as excavation, soil washing, and chemical treatments, are expensive, energy-intensive, and may cause secondary environmental impacts, thereby necessitating the development of eco-friendly remediation technologies [3, 11, 12]. Rhizoremediation, a plant-assisted bioremediation strategy, has been increasingly recognized as a promising and environmentally sustainable approach for the remediation of hydrocarbon-polluted soils [13–15]. This method exploits the symbiotic interactions between plant roots and microorganisms in the rhizosphere to enhance the biodegradation of organic pollutants [13, 16]. The rhizosphere is characterized by high concentrations of root exudates, including sugars, amino acids, organic acids, and phenolic compounds, which stimulate microbial growth and activity [16, 17]. These microorganisms can utilize petroleum hydrocarbons as carbon and energy sources, thereby accelerating their transformation into less toxic compounds [6, 7]. Unlike phytoextraction, rhizoremediation does not rely on direct uptake of hydrocarbons by plants but rather on microbially mediated degradation stimulated by plant–microbe interactions [13, 14]. Several studies have demonstrated that rhizoremediation can enhance hydrocarbon degradation compared to unplanted systems due to increased microbial activity in the rhizosphere [14, 15].

Grasses have been identified as suitable candidates for rhizoremediation because of their rapid growth, high biomass production, extensive fibrous root systems, and ability to tolerate poor and disturbed soil conditions [14, 18]. Among these, species belonging to the genus *Brachiaria* (Poaceae family) have shown considerable potential for the remediation of petroleum hydrocarbon-contaminated soils and for promoting plant growth under stressed soil conditions [18, 19].

Brachiaria ramosa, a tropical grass species, shares ecological and morphological characteristics with other *Brachiaria* species, including a dense root system, adaptability to poor soils, and tolerance to environmental stresses [19, 20]. Direct experimental evidence on the rhizoremediation potential of *Brachiaria ramosa* is currently limited. Its physical and ecological traits, however, suggest that it is very suitable for use in soils contaminated by petroleum hydrocarbons. The ability of this plant to sustain active rhizosphere microbial populations and improve soil physical properties indicates its potential to enhance petroleum hydrocarbon degradation [14, 16].

In addition, the application of plant growth-promoting rhizobacteria (PGPR) can further improve plant growth, stress tolerance, and microbial degradation efficiency in contaminated environments [21–23].

This review investigates the potential of *Brachiaria ramosa* to enhance rhizoremediation of oil-polluted soils by evaluating its plant growth-promoting effects and hydrocarbon degradation capacity. By integrating plant growth responses, rhizosphere microbial activity, and contaminant degradation, this review contributes to a better understanding of sustainable, plant-based remediation strategies [13–15]. The reviewed articles provide insight into the application of *Brachiaria ramosa* as an effective and environmentally friendly option for the remediation of petroleum hydrocarbon-contaminated soils.

This work is a narrative review that gathers and evaluates the body of research on rhizoremediation of soils contaminated by petroleum hydrocarbons, with a focus on *Brachiaria ramosa*. This study did not include any novel experiments, plant growth experiments, or microbiological investigations. Rather, conclusions are made using known theoretical frameworks and findings that have already been published.

METHODOLOGICAL STRUCTURE FOR SYNTHESIZING LITERATURE

A systematic strategy was used to perform this review, incorporating research on plant–microbe interactions, hydrocarbon degradation, and rhizoremediation.

Relevance to the following criteria was used to choose peer-reviewed articles:

- Degradation of petroleum hydrocarbons in soil systems.
- Microbial processes mediated by the rhizosphere.
- The function of grasses, particularly *Brachiaria* species.
- Remedial techniques aided by PGPR.

Data from several studies were compared with an emphasis on:

- Reduction of total petroleum hydrocarbons (TPH).
- The dynamics of microbial populations.
- The abundance of functional genes such as *alkB*.
- Parameters of plant growth.

Instead of focusing on discrete observations, the synthesis highlights mechanistic relationships between plant characteristics, microbial activity, and degradation efficiency.

PETROLEUM HYDROCARBON CONTAMINATION OF SOILS

Petroleum hydrocarbon contamination of soil is a major environmental problem arising from human activities, such as crude oil exploration, transportation, refining, and accidental spills or leakage, during improper handling and storage [1–5]. Petroleum hydrocarbons are complex mixtures of aliphatic and aromatic hydrocarbons, resins, and asphaltenes and are, therefore, collectively referred to as total petroleum hydrocarbons (TPH) [6, 7].

While some aliphatic and aromatic components are relatively biodegradable and may decompose or volatilize within a short period, others – particularly polycyclic aromatic hydrocarbons (PAHs) – are more stable and can persist in soils for long periods due to their hydrophobic nature [10, 24, 25]. Once petroleum hydrocarbons enter the soil, they tend to be strongly adsorbed onto soil particles, leading to reduced soil porosity and alterations in the physical, chemical, and biological properties of the soil [8, 25]. As a consequence, contaminated soils often exhibit reduced fertility, aeration, and water-holding capacity, which negatively affects plant growth [2, 18].

Petroleum hydrocarbons may coat soil particles and plant roots, thereby restricting oxygen diffusion to the root zone and creating anaerobic conditions that inhibit normal root respiration [3, 18]. In addition, several petroleum-derived compounds possess toxic, mutagenic, or carcinogenic properties, posing risks to plants, animals, and humans either directly or indirectly through the food chain [6, 9, 26]. These toxic effects commonly result in stunted plant growth, inhibition of seedling development, chlorosis, and, in severe cases, complete failure of vegetation establishment in contaminated soils [2, 18]. The absence or poor establishment of vegetation further limits microbial activity and slows the biodegradation of petroleum hydrocarbons, thereby increasing their persistence in the environment [11, 12].

Microorganisms play a central role in the natural attenuation of petroleum hydrocarbons in soils, as indigenous bacteria and fungi possess the enzymatic systems required for hydrocarbon degradation [6, 7]. However, in heavily contaminated soils, the rate of degradation is frequently constrained by nutrient deficiency, limited oxygen availability, low bioavailability of hydrocarbons, and phytotoxic effects [2, 12, 25]. Consequently, natural attenuation alone is often insufficient to restore petroleum hydrocarbon-contaminated soils within an acceptable time frame [3, 11].

The presence of vegetation at contaminated sites has been shown to significantly enhance the degradation of petroleum hydrocarbons, leading to the development of the concept of rhizoremediation [13–15]. In petroleum hydrocarbon-contaminated soil, plant roots create a zone of intense biological activity known as the rhizosphere, where microbial populations and activities are considerably higher than in bulk soil (Figure 1) [13, 16]. Root exudates, including sugars, organic acids, amino acids, and phenolic compounds, serve as substrates and signaling molecules for hydrocarbon-degrading microorganisms, thereby stimulating their growth and activity [16, 17].

As a result, petroleum hydrocarbons are preferentially degraded in the root zone [14, 15]. Grasses are particularly suitable for use in petroleum hydrocarbon-contaminated soils due to their rapid growth, extensive fibrous root systems, and tolerance to contaminated conditions [14, 18].

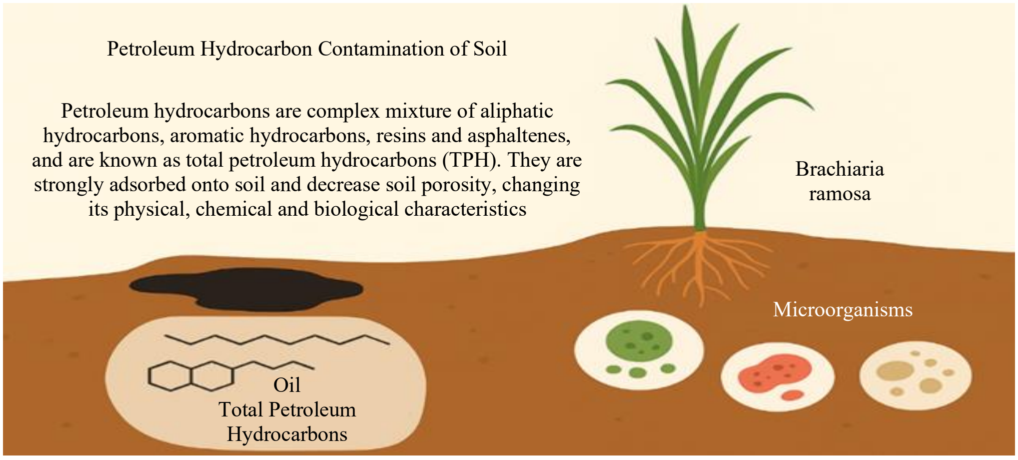


Figure 1. Petroleum hydrocarbon contamination of soils.

In this context, tropical grasses belonging to the genus *Brachiaria* have attracted attention because of their ability to thrive in petroleum hydrocarbon-contaminated soils and to stimulate microbial degradation processes [18, 20]. Although direct experimental evidence for *Brachiaria ramosa* is still limited, studies on related *Brachiaria* species have reported significant reductions in TPH concentrations in planted soils compared with unplanted controls, indicating the positive role of these grasses in enhancing biodegradation [18, 19].

This effect is mainly attributed to improvements in soil structure and aeration, which increase the bioavailability of hydrocarbons following their desorption from soil particles and facilitate microbial degradation [14, 25]. Therefore, based on the known mechanisms of rhizoremediation and evidence from related species, *Brachiaria ramosa* is a promising candidate for enhancing the biodegradation of petroleum hydrocarbons in contaminated soils.

MECHANISM OF RHIZOREMEDIATION

Rhizoremediation is an *in situ* bioremediation technique that increases the breakdown of petroleum hydrocarbons in contaminated soils by utilizing the synergistic interactions between soil microorganisms and plant roots (Figure 2) [13, 14]. Instead of eliminating pollutants directly, plants promote microbes that break down hydrocarbons in the rhizosphere, where microbial activity is much higher than in bulk soil [6, 16]. Sugars, amino acids, organic acids, phenolics, and enzymes make up root exudates, which act as carbon sources and signaling molecules that encourage microbial growth and trigger degradation pathways, resulting in the selective enrichment of communities that break down hydrocarbons [14, 15].

Furthermore, rhizoremediation increases the bioavailability of hydrocarbons because exudates and microbial biosurfactants make hydrophobic compounds more soluble and desorbable, which helps break down complex hydrocarbons like polycyclic aromatic hydrocarbons [6, 7].

According to several studies, plant roots enhance soil conditions by increasing porosity, oxygen availability, and controlling moisture and pH. Key hydrocarbon-degrading genera with enzymes like monooxygenases and dioxygenases for hydrocarbon breakdown, such as *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Bacillus*, and *Mycobacterium* [7, 27], are enriched by rhizosphere interactions [15, 16] (Table 1).

Additionally, plant growth-promoting rhizobacteria (PGPR) indirectly increase root biomass, microbial colonization, and degradation efficiency by producing phytohormones, solubilizing nutrients, and increasing ACC deaminase activity [24, 28]. According to recent studies, rhizoremediation is an environmentally friendly and economical remediation strategy [13–15]. It is a multi-mechanistic process driven by plant–microbe interactions that enhances microbial degradation through improved bioavailability, favorable soil conditions, and enriched microbial communities (Table 2).

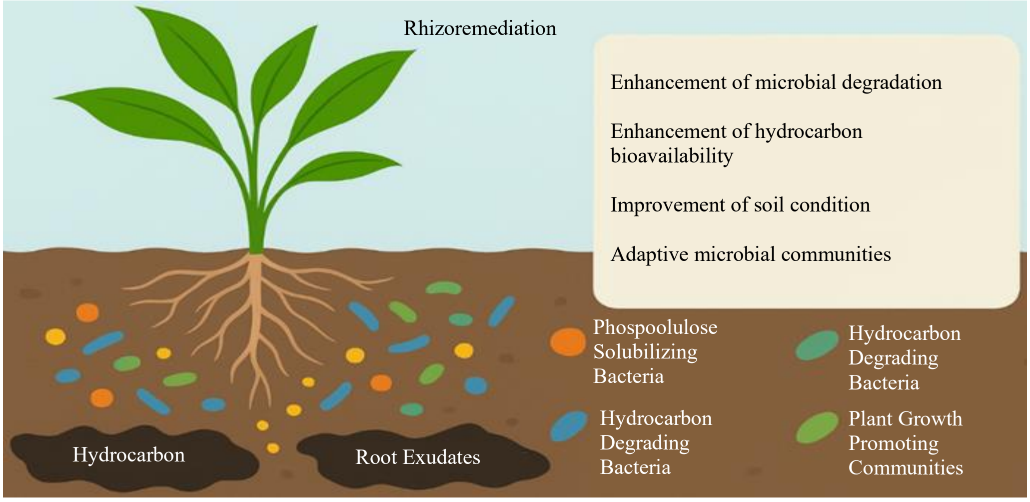


Figure 2. Mechanism of rhizoremediation.

Table 1. Mechanisms of hydrocarbon degradation and rhizosphere processes.

Component	Mechanism	Functional role in rhizoremediation	Key references
Soil Modification	Increased porosity, oxygen, moisture regulation	Supports aerobic degradation and microbial activity.	[8, 18, 27]
Root Exudation	Release of sugars, amino acids, organic acids, phenolics	Provides carbon source, stimulates microbial growth, induces degradation genes.	[9, 16, 17, 23]
Rhizosphere Interaction	Plant–microbe synergy	Enhances microbial population and biodegradation activity in root zone.	[13, 14, 16]
Bioavailability Enhancement	Biosurfactant production, desorption	Increases solubility and mobility of hydrophobic hydrocarbons.	[2, 6, 25]

Table 2. Microbial dynamics and contributions to plant growth.

Component	Mechanism	Functional role in rhizoremediation	Key references
PGPR Activity	Phytohormones, ACC deaminase, nutrient solubilization	Enhances plant growth, stress tolerance, and indirectly microbial degradation.	[21, 22, 24, 29]
Functional Enzymes	Monooxygenases, dioxygenases	Catalyze oxidation and breakdown of hydrocarbons.	[9, 10]
Microbial Enrichment	Selection of degraders (*Pseudomonas*, *Rhodococcus*, etc.)	Promotes efficient degradation of aliphatic and aromatic hydrocarbons.	[6, 9, 15]
Overall Outcome	Integrated plant–microbe processes	Improved hydrocarbon degradation and sustainable remediation.	[2, 3, 13]

ROLE OF *BRACHIARIA RAMOSA* IN HYDROCARBON DEGRADATION

Brachiaria ramosa is a tropical grass belonging to the family Poaceae and is increasingly considered a promising candidate for the rhizoremediation of petroleum hydrocarbon-contaminated soils because of its good adaptability and extensive root system [18–20]. There is presently little direct experimental data on *Brachiaria ramosa*'s ability to degrade hydrocarbons. To determine its possible role, this section summarizes research from similar *Brachiaria* species and generic rhizoremediation studies that directly evaluates the potential of *B. Ramosa* for hydrocarbon degradation. Therefore, studies on related *Brachiaria* species demonstrate that general principles of rhizoremediation [2, 13, 14]. Despite this knowledge gap, the documented characteristics of the *Brachiaria* rhizosphere and its effects on microbial communities provide a strong rationale for considering *B. Ramosa* as a suitable plant for enhancing the biodegradation of petroleum hydrocarbons [15, 16]. The most commonly proposed mechanism by which *B. Ramosa* enhances hydrocarbon degradation is through stimulation of microbial activity in the rhizosphere (Figure 3) [13, 14].

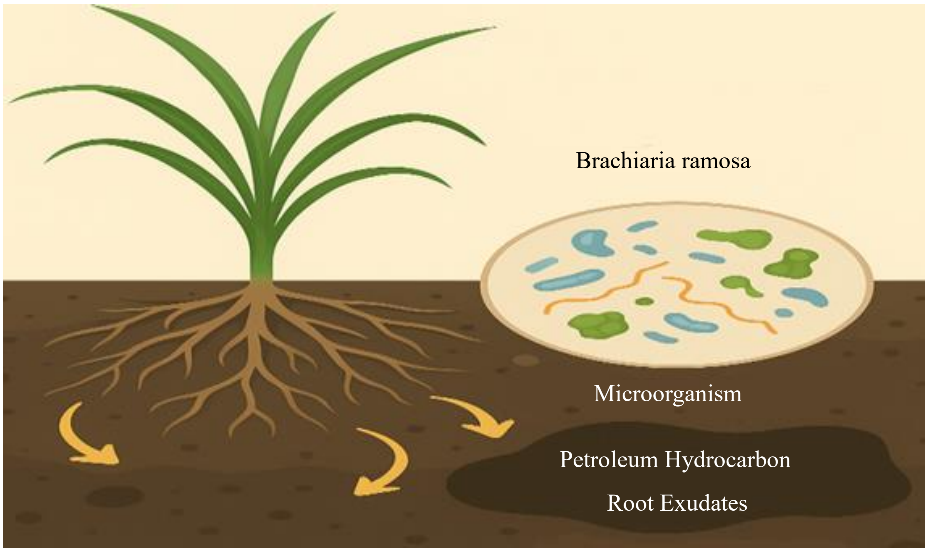


Figure 3. Role of *Brachiaria ramosa* in hydrocarbon degradation.

Plant roots exude a variety of organic compounds, including sugars, amino acids, organic acids, and phenolic substances, which serve as carbon and energy sources for soil microorganisms [16, 23]. These exudates stimulate microbial growth, thereby increasing microbial biomass and the activity of enzymes involved in hydrocarbon degradation [2, 6]. Studies on other *Brachiaria* species have shown that planted soils generally exhibit higher microbial counts and greater reductions in total petroleum hydrocarbons compared to unplanted controls, indicating that root-driven microbial stimulation plays a critical role in biodegradation [14, 18].

In addition to nutrient supply, the *Brachiaria* rhizosphere provides a favorable microenvironment for bacteria and fungi capable of degrading hydrocarbons [13, 16]. Many hydrocarbon-degrading microorganisms possess functional genes, such as alkane monooxygenases (*alkB*) and dioxygenases, which are essential for initiating the degradation of aliphatic and aromatic hydrocarbons [9, 10]. It has been reported that the abundance of such functional genes is often higher in the rhizosphere of grasses, supporting the hypothesis that *Brachiaria* species may enhance hydrocarbon degradation through the induction and enrichment of relevant microbial genes [13, 14].

Although specific data on gene-level effects in *B. Ramosa* are not yet available, its similarity in root architecture and growth habit to other *Brachiaria* species suggests a positive influence on the proliferation of hydrocarbon-degrading microorganisms [18, 20]. Another important contribution of *B. Ramosa* to hydrocarbon degradation is its role in improving the physical properties of the surrounding soil. The dense and fibrous root system enhances soil aeration and increases oxygen penetration into contaminated soil layers [14, 18]. This is particularly important because oxygen acts as a key electron acceptor in the aerobic degradation of many petroleum hydrocarbons [9, 10]. Moreover, plant roots can facilitate the desorption of hydrocarbons from soil particles, thereby increasing their bioavailability for microbial degradation [6, 25].

Evidence for the potential of *B. Ramosa* in hydrocarbon degradation is further supported by studies on other *Brachiaria* species. For instance, *B. Decumbens* and *B. Brizantha* have been reported to significantly reduce total petroleum hydrocarbon concentrations in contaminated soils compared to unplanted controls, indicating stimulation of biodegradation through rhizosphere effects and improved soil conditions [14, 18]. These findings suggest that *B. Ramosa*, which shares similar ecological and morphological characteristics, may also contribute to hydrocarbon removal in contaminated sites [19, 20]. However, direct experimental studies on *B. Ramosa* are still required to confirm its effectiveness and to elucidate the specific microbial and biochemical interactions involved.

Future work should focus on measuring total petroleum hydrocarbon reduction in soils planted with *B. Ramosa*, analyzing microbial community shifts using molecular tools, and correlating functional gene abundance with actual degradation rates [2, 14, 15]. Such studies will provide a solid scientific basis for the application and optimization of *B. Ramosa* in rhizoremediation under different contamination levels and environmental conditions.

Overall, based on the available information on *B. Ramosa*, evidence from related *Brachiaria* species, and established mechanisms of rhizoremediation, it can be reasonably assumed that *B. Ramosa* has the potential to stimulate hydrocarbon degradation in contaminated soils [2, 13, 14]. Through root exudation, improvement of soil aeration, and enhancement of hydrocarbon bioavailability, *B. Ramosa* enhances promotion of microbial activity and contributes to the effective remediation of petroleum-polluted soils [6, 15, 25].

PGPR AND RHIZOSPHERE MICROBIAL ACTIVITY

In petroleum hydrocarbon-contaminated soils planted with grasses, like *Brachiaria ramosa*, plant growth-promoting rhizobacteria (PGPR) are essential for boosting rhizosphere microbial populations and increasing rhizoremediation efficiency [13–15]. The rhizosphere, which is rich in root exudates that include sugars, amino acids, organic acids, and phenolics, offers vital nutrients and signaling compounds that promote the growth of microorganisms and the breakdown of hydrocarbons [16, 23]. The rhizosphere of *B. Ramosa* is thought to work similarly to other *Brachiaria* species recognized for successful rhizoremediation, despite the paucity of species-specific evidence (Table 3) [18, 20].

Table 3. Mechanisms of direct plant growth-promoting PGPR.

Component	Mechanism	Functional role	Key references
Nutrient Acquisition	Nitrogen fixation, phosphorus solubilization	Improves nutrient availability and plant growth under stress.	[22, 29]
Phytohormone Production	Indole-3-acetic acid (IAA) synthesis	Enhances root growth and rhizosphere interaction.	[23, 24]
Stress Alleviation	ACC deaminase activity	Reduces ethylene levels and improves stress tolerance.	[17, 21]
Rhizosphere Enrichment	Root exudates (sugars, amino acids, organic acids, phenolics)	Stimulates microbial growth and hydrocarbon degradation.	[6, 16, 23]

Through nitrogen fixation, phosphorus solubilization, phytohormone production (such as indole-3-acetic acid), and stress ethylene reduction via ACC deaminase, PGPR directly promote plant growth, resulting in improved root development and increased rhizosphere interactions [22, 24]. While important genera, like *Pseudomonas* and *Bacillus*, have hydrocarbon-degrading enzymes like monooxygenases and dioxygenases, PGPR indirectly promote hydrocarbon degradation by generating siderophores, enzymes, and biofilms that enhance microbial activity and root colonization (Table 4) [23, 28].

Table 4. Microbial dynamics and hydrocarbon degradation: PGPR's indirect functions.

Component	Mechanism	Functional role	Key references
Microbial Stimulation	Siderophores, antibiotics, enzymes, biofilm formation	Enhances microbial activity, colonization, and pathogen suppression.	[23, 28]
Community Structuring	Increased microbial diversity and functional genes	Enriches degraders and enhances ecosystem stability.	[8, 14]
Hydrocarbon Degradation	Monooxygenases, dioxygenases (PGPR-associated genes)	Catalyze breakdown of aliphatic and aromatic hydrocarbons.	[6, 9, 10]
System Outcome	Plant–PGPR synergy	Improved plant growth and hydrocarbon remediation efficiency.	[2, 13, 15]

Additionally, PGPR influence the organization of the rhizosphere microbial population by boosting diversity and enriching functional genes linked to stress tolerance, nutrient cycling, and degrading processes [14, 17]. Root exudation and PGPR activity work together to improve soil aeration, nutrient availability, and microbial collaboration in *B. Ramosa* systems, which in turn promotes plant development and the breakdown of petroleum hydrocarbons [15, 16, 18]. Current research indicates that PGPR-assisted rhizoremediation is a successful method for hydrocarbon-contaminated soils, despite the paucity of species-specific investigations (Figure 4) [15, 20].

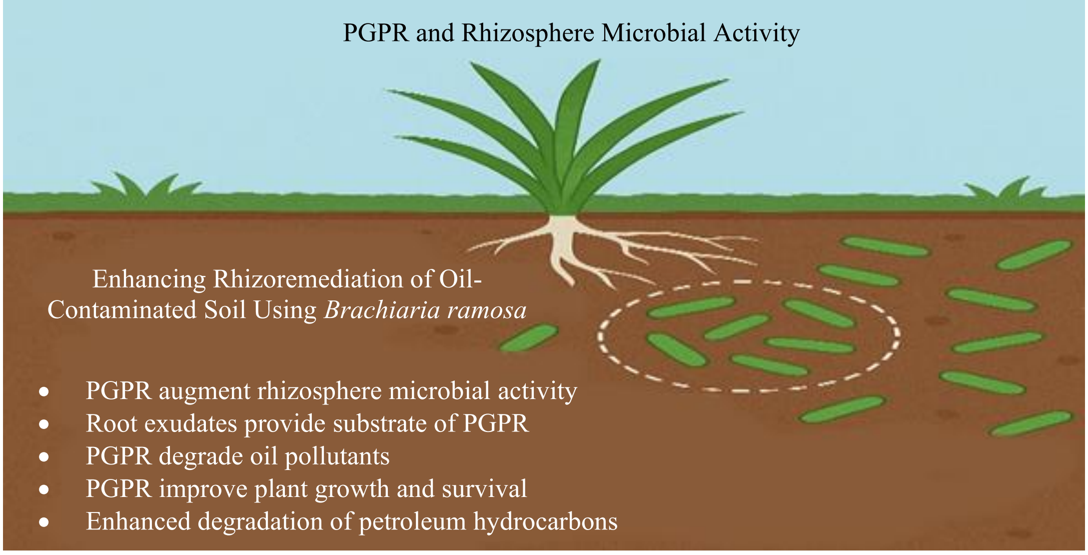


Figure 4. PGPR and rhizosphere microbial activity.

BRACHIARIA RAMOSA: BIOLOGICAL AND ECOLOGICAL CHARACTERISTICS

Brachiaria ramosa, a tropical grass belonging to the Poaceae family, is a good option for rhizoremediation of petroleum hydrocarbon-polluted environments due to its quick growth, high biomass production, fibrous root system, and adaptability to poor and contaminated soils [19, 20]. Through the release of root exudates, like sugars, amino acids, organic acids, and phenolics, which act as energy sources for microorganisms that break down hydrocarbons, its vast root system improves rhizosphere development, boosting soil–root interactions and stimulating microbial populations [17, 23]. According to current studies, these microbial communities are essential to the enzymatic oxidation and mineralization processes that break down hydrocarbons [14, 15]. In terms of ecology, *B. Ramosa* enhances microbial activity and supports aerobic degradation processes by increasing porosity, aeration, water infiltration, and nutrient availability (Table 5) [16, 18].

Table 5. Features of rhizoremediation.

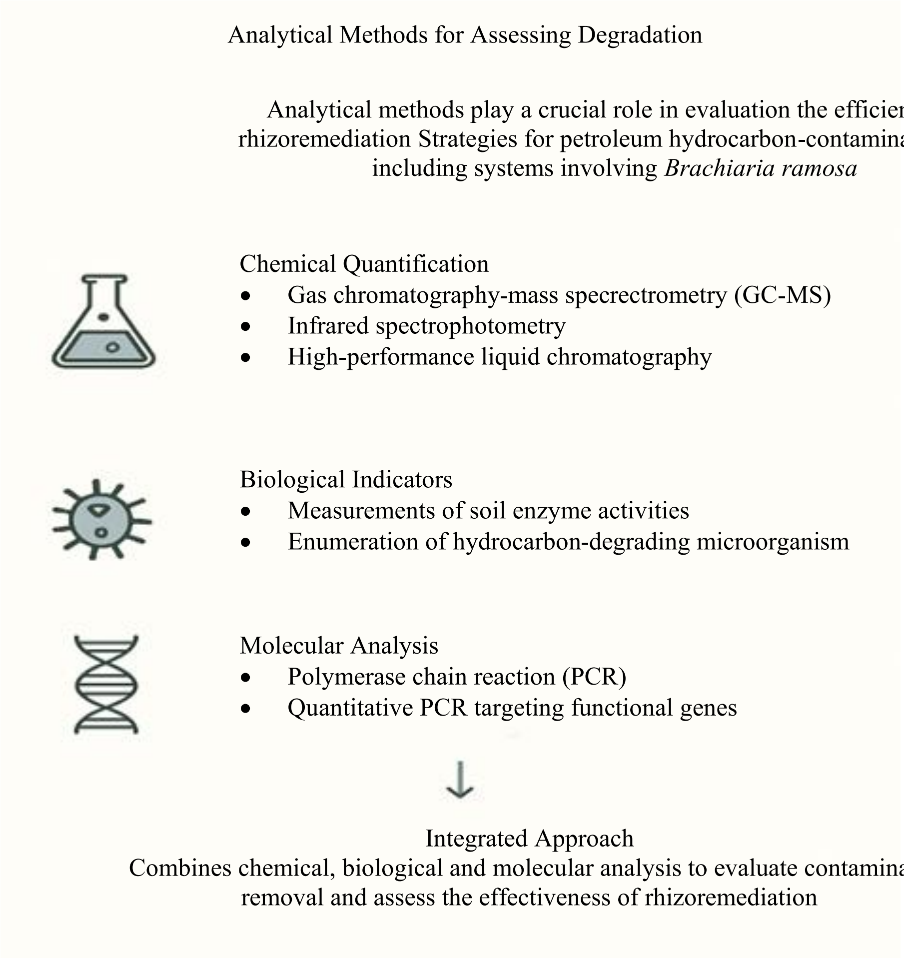
Features	Rhizoremediation observation	Supporting reference
Fast growth and high biomass	Enables quick establishment and root expansion in contaminated soil, enhancing microbial interactions	Sharyn E. Gaskin et al. – Rhizoremediation of hydrocarbon contaminated soil using Australian native grasses.
Fibrous root systems	Expands rhizosphere volume and promotes soil–root–microbe interactions essential for degradation	Sharyn Gaskin et al. – Screening of Australian native grasses for rhizoremediation.
Root exudation	Stimulates hydrocarbon-degrading microorganisms by releasing organic compounds	Journal of Chemistry & Analytical Biochemistry – root exudates increase microbial populations and hydrocarbon availability in rhizosphere.
Soil aeration improvement	Root growth increases soil porosity, aiding oxygen diffusion and aerobic biodegradation	[14]
Stress tolerance	Grasses, like *Brachiaria*, show tolerance to petroleum hydrocarbon stress with sustained growth	[18]

Through desorption processes, root activity may also raise the bioavailability of hydrocarbons [14, 25]. Studies on allied *Brachiaria* species show considerable reductions in total petroleum hydrocarbons, indicating similar potential for *B. Ramosa* despite the lack of direct experimental data [15, 18]. Although more species-specific research is needed to confirm its degradation efficiency, its resilience, root architecture, and ecological functions generally support its use in sustainable rhizoremediation [15, 20].

ANALYTICAL FRAMEWORK FOR ASSESSING DEGRADATION

Analytical framework plays a crucial role in evaluating the efficiency of rhizoremediation strategies for petroleum hydrocarbon-contaminated soils, including systems involving *Brachiaria ramosa*. The most common approach to assess degradation is the quantitative determination of total petroleum hydrocarbons (TPH) and specific hydrocarbon fractions in soil samples collected before and after treatment. Gas chromatography coupled with mass spectrometry (GC–MS) is widely regarded as a reliable and sensitive technique for identifying and quantifying individual aliphatic and aromatic hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), and has been extensively used in bioremediation and rhizoremediation studies [7].

In addition to GC–MS, infrared spectrophotometric method and gravimetric analysis after solvent extraction are often employed for routine estimation of TPH, especially in large-scale or long-term experiments, as they provide a practical measure of overall hydrocarbon removal [30]. High-performance liquid chromatography (HPLC) and LC–MS techniques are particularly useful for analyzing high-molecular-weight and more recalcitrant compounds, such as PAHs, which are known to persist in contaminated soils (Figure 5).



Analytical Methods for Assessing Degradation

Analytical methods play a crucial role in evaluation the efficiency of rhizoremediation Strategies for petroleum hydrocarbon-contaminated soil, including systems involving *Brachiaria ramosa*

Chemical Quantification
- Gas chromatography-mass specrectrometry (GC-MS)
- Infrared spectrophotometry
- High-performance liquid chromatography

Biological Indicators
- Measurements of soil enzyme activities
- Enumeration of hydrocarbon-degrading microorganism

Molecular Analysis
- Polymerase chain reaction (PCR)
- Quantitative PCR targeting functional genes

Integrated Approach
Combines chemical, biological and molecular analysis to evaluate contaminant removal and assess the effectiveness of rhizoremediation

Figure 5. Analytical framework for assessing degradation.

Besides chemical quantification, biological indicators are frequently used to support evidence of biodegradation. Measurements of soil enzyme activities, such as dehydrogenase, catalase, and urease, are commonly applied as indicators of overall microbial metabolic activity and have been shown to correlate with enhanced biodegradation in rhizosphere soils. Enumeration of hydrocarbon-degrading microorganisms using culture-dependent framework, such as the most probable number (MPN) technique, is also widely reported to assess changes in microbial populations during remediation [26]. Furthermore, molecular techniques, particularly PCR and quantitative PCR targeting functional genes, such as alkane monooxygenase (alkB) and dioxygenase genes, provide valuable information on the presence and abundance of microbial communities directly involved in hydrocarbon degradation.

These molecular approaches help to link observed decreases in hydrocarbon concentrations with the underlying biological mechanisms operating in the rhizosphere. In rhizoremediation systems, including those based on grasses, such as *Brachiaria* species, the integration of chemical, microbiological, and molecular analyses is considered essential to obtain a comprehensive assessment of degradation performance. Such an integrated analytical framework allows not only the quantification of contaminant removal but also the evaluation of plant–microbe interactions, microbial functional potential, and overall soil recovery, thereby providing a robust basis for assessing the effectiveness of *Brachiaria ramosa* in enhancing the biodegradation of petroleum hydrocarbons in contaminated soils [14, 18].

CHALLENGES, LIMITATIONS, AND KNOWLEDGE GAPS

One of the major challenges in enhancing the rhizoremediation potential of petroleum hydrocarbon-contaminated soils using *Brachiaria ramosa* is the lack of species-specific experimental evidence for its remediation capacity. Although several studies have demonstrated the effectiveness of other *Brachiaria* species and different plant systems in the degradation of petroleum hydrocarbons, direct evidence for *B. ramosa* remains limited, and most assumptions are, therefore, extrapolated from related species and general rhizoremediation studies [2, 13].

Another important limitation is the heterogeneity of petroleum hydrocarbon contamination in soils. The composition, concentration, toxicity, and weathering stage of hydrocarbons strongly influence plant growth and microbial activity. High concentrations of petroleum hydrocarbons are phytotoxic and can inhibit seed germination, root development, and biomass production, which in turn reduces rhizosphere-driven biodegradation efficiency [3, 6, 7]. Since rhizoremediation depends heavily on healthy root development to stimulate microbial degradation, any constraint on plant growth can directly impair remediation performance [2, 14].

Physicochemical properties of soil also represent major constraints to effective rhizoremediation. Factors, such as soil texture, pH, organic matter content, nutrient availability, and moisture status, strongly regulate both plant establishment and microbial activity [8, 16]. Although *B. ramosa* possesses a fibrous root system, its growth and rhizosphere development can be restricted in compacted, nutrient-poor, or heavily polluted soils, thereby limiting microbial proliferation and hydrocarbon degradation [2, 18].

A further limitation is the insufficient understanding of plant–microbe interactions in the *B. ramosa* rhizosphere under hydrocarbon stress. Root exudates are known to play a key role in stimulating microbial growth and shaping rhizosphere microbial communities, yet the composition and quantity of exudates released by *B. ramosa* in petroleum hydrocarbon-contaminated soils remain poorly characterized [17, 23]. Moreover, information regarding the selective enrichment of hydrocarbon-degrading microorganisms in the *B. ramosa* rhizosphere is still scarce [13, 15].

There is also limited knowledge about the enzymatic and molecular mechanisms involved in hydrocarbon degradation in the *B. ramosa* rhizosphere. Although enzymes, such as alkane monooxygenases, dioxygenases, and dehydrogenases, are known to play central roles in petroleum hydrocarbon degradation, their expression, regulation, and activity in association with *B. ramosa* roots have not yet been well documented [6, 7, 9].

Another shortcoming of many previous studies is the weak linkage between plant growth promotion and hydrocarbon degradation outcomes. While plant growth parameters and contaminant removal are often assessed separately, there is still limited direct evidence demonstrating how enhanced plant growth translates into increased microbial degradation efficiency [14, 21, 22]. In addition, most available studies have been conducted under controlled laboratory or greenhouse conditions, which may not accurately reflect the complexity and variability of field environments [2, 3, 11].

FUTURE PERSPECTIVES

Future directions in the evaluation and optimization of degradation processes in bioremediation and rhizoremediation are increasingly focused on improving efficiency, predictability, and long-term sustainability in contaminated environments [1–3]. A major emerging direction is the application of advanced analytical and molecular techniques to achieve a more comprehensive understanding of degradation pathways. High-resolution chromatographic and mass spectrometric approaches [3], such as multidimensional GC and high-resolution LC–MS, are expected to play a central role in characterizing complex contaminant mixtures and in identifying transformation products and potentially toxic intermediates, rather than relying solely on the apparent disappearance of parent compounds [4, 5].

Another important perspective is the direct linkage of microbial community structure and functional potential with observed degradation rates through the application of omics-based approaches, including metagenomics, metatranscriptomics, and metaproteomics [8, 27]. These approaches provide insights into active metabolic pathways, key degradative enzymes, and microbial interactions in complex environments such as the rhizosphere [10, 13, 16]. Integration of omics data with conventional chemical measurements is expected to strengthen mechanistic understanding of degradation processes and support the development of predictive models for field-scale remediation [2, 12].

Plant-based remediation strategies are also likely to advance through improved screening of plant species and genotypes, as well as through the deliberate design of beneficial plant–microbe interactions. Plants with root exudate profiles that selectively stimulate hydrocarbon-degrading microorganisms indicate rhizosphere-driven degradation [23, 29]. In addition, the application of plant growth-promoting rhizobacteria and tailored microbial consortia may help overcome the limitations associated with single-strain inoculation strategies [24, 28]. Such approaches are particularly relevant for complex contaminants and heterogeneous soils, where synergistic microbial interactions are critical for effective degradation [12, 30].

From an environmental management perspective, future research is expected to place greater emphasis on long-term, field-based studies rather than short-term laboratory or greenhouse experiments, as field conditions better reflect the influence of soil heterogeneity, contaminant aging, and environmental variability on remediation performance [3, 14]. Greater attention will also be given to contaminant fate, ecotoxicological risks, and soil recovery, which are essential considerations for the regulatory acceptance of remediation technologies [4, 15, 26].

Finally, future research should increasingly integrate degradation studies with broader issues, such as climate change and sustainable land management, since changes in temperature and precipitation patterns can strongly influence microbial activity, plant growth, and contaminant mobility in soils [25, 27]. Overall, progress in this field will require a shift from discipline-specific approaches toward system-oriented, multidisciplinary strategies that combine advanced analytical chemistry, molecular biology, plant science, and environmental risk assessment to develop more reliable and sustainable remediation solutions [3, 13].

CONCLUSIONS

In bioremediation and rhizoremediation, degradation process evaluation is crucial for determining remediation effectiveness, environmental safety, and sustainability. Both compound-specific quantification of petroleum hydrocarbons and indirect evaluation of microbial activity through biomass, respiration, enzyme activities, and functional genes are made possible by sophisticated analytical techniques like chromatographic methods (GC, GC–MS, HPLC), spectroscopic tools (FTIR), and biological and molecular indicators. However, soil heterogeneity, contaminant aging, texture, organic matter, and nutrient availability all affect degradation efficiency, which causes differences between laboratory and field results.

The relationship between gene abundance and actual degradation is yet unknown, and relying just on bulk metrics, like total petroleum hydrocarbons, may miss changes of particular hazardous chemicals. To better understand contaminant dynamics, future developments will need to integrate multi-omics methodologies, stable isotope techniques, and high-resolution analytical tools with long-term field research. Therefore, creating dependable and long-lasting remediation techniques requires a multidisciplinary framework that combines chemical, biological, and molecular investigations.

The lack of species-specific experimental validation for *Brachiaria ramosa* underscores the need for further research involving controlled plant growth experiments, hydrocarbon degradation assays, and rhizosphere microbial profiling, even though this review offers a thorough synthesis of current knowledge.

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Authors' Contributions

- N. K. Madhumita and Dr. P. Suganya – Designed the review framework.
- Shellsiya M. – Conducted the literature search, compiled relevant data.
- Dr. R. Nirmal Kumar and Dr. Ranjith Santhosh Kumar Devanesan Sanjeevi – Provided critical revisions, refined the manuscript.

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