

# POTENTIAL APPROACHES TO ENHANCE BIODEGRADATION OF PETROLEUM HYDROCARBONS

## Abstract

Petroleum hydrocarbons, derived from crude oil, are essential energy sources but pose a significant environmental concern due to their widespread use and potential for accidental releases. Biodegradation, a natural process mediated by microorganisms, has come out as a successful approach for the treatment of environments that are polluted by petroleum hydrocarbons. This abstract provides information on the biodegradation mechanisms and factors influencing the breakdown of petroleum hydrocarbons by microorganisms. The book chapter encompasses various aspects of petroleum hydrocarbon biodegradation, including the microbial communities involved, metabolic pathways employed by microorganisms, and environmental factors affecting the degradation process. Microorganisms such as fungi have a key role in petroleum hydrocarbon biotransformation, using enzymes to disintegrate compound hydrocarbon compounds into simpler, more manageable forms. Several factors influence the rate and efficiency of biodegradation, such as hydrocarbon composition, pH, temperature, availability of nutrients, oxygen levels, and the existence of co-contaminants. Understanding these factors is vital for optimizing biodegradation processes and developing effective bioremediation strategies. Overall, this review emphasizes the significance of biodegradation as a legitimate and environmentally safe approach to remediate petroleum sites spoiled by hydrocarbons. It underscores the need for further research for surveying the untapped possibilities of groups of microorganisms and their metabolic capabilities for enhanced

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biodegradation. By harnessing the power of nature, biodegradation offers a promising solution for mitigating the environmental impact of petroleum hydrocarbons and restoring contaminated ecosystems.

**Keywords:** Biodegradatio, Petroleum Hydrocarbons.

## I. INTRODUCTION

Complex organic molecules known as petroleum hydrocarbons are produced from crude oil and are widely employed as energy sources and in a variety of other sectors. However, they can cause substantial ecological harm if they are accidentally released into the environment, such as through oil spills or leaks from storage facilities. Pollution from petroleum hydrocarbons is a widespread issue on a global scale. According to Margesin and Schinner (1999), the primary origin of pollution by petroleum at cold climate terrestrial sites are either above and below ground fuel deposits, petroleum products transported through pipelines, and oil and gas production-related accidents and leaks of natural gas condensate. (in Alaska, the United States, Canada, and Russia) [1]. Soil and groundwater have been contaminated at numerous locations as a result of the usage of petroleum fuels on a large scale and additional items in regions with chilly climates. The harsh weather and isolated settings of these areas make rehabilitation more difficult. In general, groundwater has not explicitly addressed sites in cold climates. The cold-adapted bacteria aerobic biodegradation processes in soils have typically been the review of bioremediation strategies' main concern for pollution by petroleum hydrocarbons in locations in cold climates [2]. Sites in cold climates have often not been specifically addressed by groundwater. Reviews of bioremediation methods for pollution by petroleum hydrocarbons in cold climate locations usually concentrated on the aerobic biodegradation activities carried out in soils by cold-climate adapted bacteria [3]. By synthesizing the material currently available regarding the bioremediation of petroleum-contaminated underground water in cool locations, this review aims to close the science gap mentioned above.

1. Details on the locations' methods of aerobic and anaerobic biodegradation;
2. Possible use of such techniques to clean up groundwater is assessed;
3. Identifying the most important areas of research needed to advance such groundwater bioremediation applications in cold climate settings [4].

"A managed or spontaneous process in which biological, especially microbial, catalysis acts on pollutant compounds, thereby remedying or eliminating environmental contamination" (Madsen, 1991) is the meaning of bioremediation. This paper covers data pertinent to the application of bioremediation methods for groundwater polluted with petroleum in cold climates [5]. These hydrocarbons are known to be harmful to a variety of organisms and can linger in the environment for a long time, endangering ecosystems. Growing interest has been seen in recent years in creating environmentally acceptable and sustainable methods to clean up petroleum hydrocarbon-contaminated places. Fungi are one promising strategy for biodegradation. Through enzymatic activity, the broad group of microorganisms known as fungi may break down complex organic substances, including petroleum hydrocarbons [6]. In situ bioremediation techniques for groundwater applications include bio-sparging with oxygen or air, specifically formulated compounds releasing oxygen's addition, nutrients and/or electron acceptors delivered via liquid like nitrate or Hydrogen peroxide, in addition to intrinsic("naturally occurring") bioremediation (also known according to observed natural attenuation) [7]. Certain systems for remediation integrate tested methods (physical, chemical, or biological). For instance, bio-slurping (several phases of extraction), seen in the 1990s, integrates sedentary venting and vacuum elimination of free products close to the water level [8].

Active bioremediation techniques like bio-stimulation and bio-augmentation may be used in ex-situ as well as in-situ settings (see Table 1). These entail adding crucial nutrients to boost the already-existing bacterial population naturally (bio-stimulation) or adding [9].

There has been an increase in interest in recent years in developing ways for cleaning up petroleum hydrocarbon-contaminated areas that are both environmentally acceptable and sustainable [10]. Fungi are also adaptable and can endure many environmental circumstances, like high or low alkalinity levels and salinity. They can survive in petroleum-contaminated areas where other creatures might struggle, thanks to their flexibility [11]. Numerous fungi have proven to have amazing capacities to break down petroleum hydrocarbons. For instance, the biodegradation capability of organisms from the genera *Aspergillus*, *Penicillium*, *Trichoderma*, and *Cladosporium* has received substantial research [12]. These fungi can use a variety of hydrocarbon substances, including polycyclic aromatic hydrocarbons (PAHs), aromatic chemicals, and alkanes, as carbon sources for growth [13].

## II. ROLE OF DIFFERENT MICROBES IN HYDROCARBON DEGRADATION

Hydrocarbons are organic molecules consisting of atoms of carbon and hydrogen, and microbes are essential to their breakdown. These hydrocarbons can be found in a variety of environmental contexts, including oil spills, contaminated soil, and industrial waste [14]. Their breakdown is crucial for carbon sequestration and the cleaning up of polluted areas. Through several methods, numerous microbes, like fungi and bacteria, take part in the breakdown of hydrocarbons. Here is a list of their responsibilities [15].

**1. Mechanism of Petroleum Hydrocarbon Degradation:** In an aerobic atmosphere, the majority of organic pollutants break down fast and completely. Oxidation and activation are the main enzymatic catalysts for oxygen incorporation via peroxidases and oxygenates, which is the primary intracellular organic pollutant assault [16]. Using the tricarboxylic acid cycle as an example, Chandran and Das (2011) described how the peripheral degradation pathways change biological contaminants progressively in the metabolic intermediates of the central intermediary pathway. Primary precursors' metabolites, such as acetyl CoA, pyruvate, and succinate, are used in the production of cell biomass [17]. Through gluconeogenesis, the saccharides needed for many types of growth and biosynthesis are created. According to Rahman et al. (2003), there are three potential ways to degrade petroleum hydrocarbons: (a) using a particular system of enzymes; (b) adhesion of the microbial cell to substrates; or (c) producing biosurfactants [18]. A specific microbe strain/ a group of microbial strains related to the different groups can preferentially metabolize petroleum hydrocarbons. According to Al-Hawash and colleagues (2018), the consortium has demonstrated that it's more capable than individual cultures in metabolizing or degrading petroleum hydrocarbons [19]. Recently, it was discovered that the HDMP2 breakdown of diesel might be a three-stage process:

- Adsorption to the surface.
- Absorption of cell.
- Biodegradation.

The components of diesel were initially readily absorbed upon the HDMP2's surface [20].

- 2. Enzymes Involved in the Decomposition Of Hydrocarbons:** Cytochrome P450's alkane hydroxylases. One super group of ubiquitous monooxygenases of Heme-thiolates which have been isolated from several *Candida* species, such as *Candida tropicalis*, *Candida apicola*, and *Candida maltose* play an important role in the degradation of microbes in oil, various mixtures, gasoline additives, and chlorinated hydrocarbons. According to the length of the chain, systems of enzymes are necessary to add oxygen to the substrate to start bio-degradation (Table 2). Superior eukaryotes often have many P450 families, each with a distinct P450 isoform that might serve as a group to metabolically convert a specific substrate. The cytochrome P450 enzyme systems were active during the biodegradation of petroleum hydrocarbons [22]

There must be many microsomal Cytochrome P450 variants for several yeast species to use n-alkanes and other aliphatic hydrocarbons as their only source of carbon and energy. In eukaryotes and prokaryotes that actively participate in the debasement of alkanes under aerobic conditions, alkane oxygenase systems include cytochrome P450 enzyme, soluble di-iron methane monooxygenases, integral membrane di-iron alkane hydroxylases (such as alkB), membrane-bound copper containing methane monooxygenases, lipases and esterase [23]. Another significant point to note is that none oil-degrading microorganisms which are shown as brown can be influenced by metabolites and other compounds released by oil-degrading bacteria and vice versa (Head et al.,2006). Primary oil degraders must compete with other microorganisms for scarce nutrients like phosphorus [24].

### III.MICROORGANISMS THAT DEGRADE HYDROCARBONS

Bacteria play a significant role in the degradation of hydrocarbons, which are organic molecules consisting of hydrogen and carbon atoms. They have created several processes to break down hydrocarbons and utilize them as a source of carbon and energy [25]. Here is a summary of how bacteria break hydrocarbons:

- 1. Uptake of Hydrocarbons:** Bacteria can absorb hydrocarbon molecules from their surroundings thanks to transport systems on their cell membranes. These transporters are only capable of moving certain hydrocarbons like polycyclic aromatic hydrocarbons (PAHs), aromatic hydrocarbons, and alkanes [26].
- 2. Production of Extracellular Enzymes:** Once the bacteria have absorbed the hydrocarbons, they release extracellular enzymes like hydroxylases or oxygenases that start the degradation process. The type of hydrocarbons present determines the specific enzymes that are produced [27].
- 3. Aliphatic Hydrocarbon Degradation:** Aliphatic hydrocarbon oxygenases are produced by bacteria for aliphatic hydrocarbons (such as alkanes). These enzymes add oxygen atoms to the hydrocarbon chains, creating hydroxylated intermediates as a result. Through beta-oxidation pathways, the hydroxylated compounds are then further metabolized [28].
- 4. Aromatic Hydrocarbon Degradation:** By producing aromatic hydrocarbon oxygenases, bacteria break down aromatic hydrocarbons like benzene, toluene, and xylene. The formation of dihydroxy compounds is facilitated by these enzymes, which enables oxygen

to be added to the aromatic ring which is broken down in subsequent ring-cleavage reactions, resulting in smaller aromatic compounds and other metabolites [29].

5. **Co-Metabolism and Co-Utilization:** Bacteria can co-metabolize, where they use another carbon source as their main source of energy and degrade hydrocarbons as a by-product. By using hydrocarbons that they cannot be able to use as their only source of energy and carbon, bacteria can degrade them [30].
6. **Formation of Microbial Consortia:** In some circumstances, the coordinated efforts of numerous bacterial species working in a microbial consortium may be necessary for hydrocarbon degradation. The specific enzymes or abilities that each species contributes to the overall degradation process complement it, increasing its efficiency [31].
7. **Environmental Factors:** Environmental elements like oxygen availability, pH, temperature, nutrient levels, and the existence of other pollutants can affect how effectively bacteria degrade hydrocarbons. These elements may affect how hydrocarbon-degrading bacteria develop and function in their natural environments [32].

Bioremediation, the process of using microorganisms to restore damaged areas, such as those caused by oil spills and locations for industrial waste, depends critically on bacteria's capacity to degrade hydrocarbons. It is crucial to comprehend the mechanisms underlying bacterial hydrocarbon degradation to create efficient bioremediation plans and reduce the environmental effects of hydrocarbon pollution [33].

**Table 1: List of Bacteria that Break down Petroleum Hydrocarbons and their Preferred Degradation Substrates**

Hydrocarbon Types	Bacteria Species	Hydrocarbon Compounds	References
Aliphatics	Pseudomonas aeruginosa	Long chain alkanes (C12-C38)	[34]
	Dietzia sp.	n-alkanes (C13-C17)	[35]
	Rhodococcus ruber	n-alkanes (C6-C40)	[36]
	Gordonia sihwensis	Branched-chain and n-alkanes	[37]
Aromatics	Marinobacter sp.	n-alkanes	[38]
	Mycobacterium cosmeticum	Mono aromatic compounds (toluene, o-xylene, ethylbenzene, and benzene)	[39]
	Pseudomonas aeruginosa	Monoaromatic compounds	[34]
	Achromobacter xylosoxidans	Mono/polyaromatic compounds	[40]
	Bacillus licheniformis	compounds	[41,42]
	Bacillus mojavensis	Polyaromatic compounds	[42]
Resins	Pseudomonas sp.	Resins	[43]

Asphaltenes	Staphylococcus sp.	Asphaltenes	[43]
	Bacillus sp.		[44]
	Pseudomonas sp.		[45]
	Citrobacter sp.		[46]
	Enterobacter sp.		[47]
	Lysibacillus sp.		[48]

#### IV. HYDROCARBON-DEGRADING FUNGI

Through a process known as biodegradation, fungi assist in the breakdown of hydrocarbons. They have specific enzymes that help break down complicated hydrocarbon molecules into simpler ones so that fungi and other bacteria can utilize them as the origin of carbon and energy. The following steps make up the mechanism by which fungi degrade hydrocarbons [49].

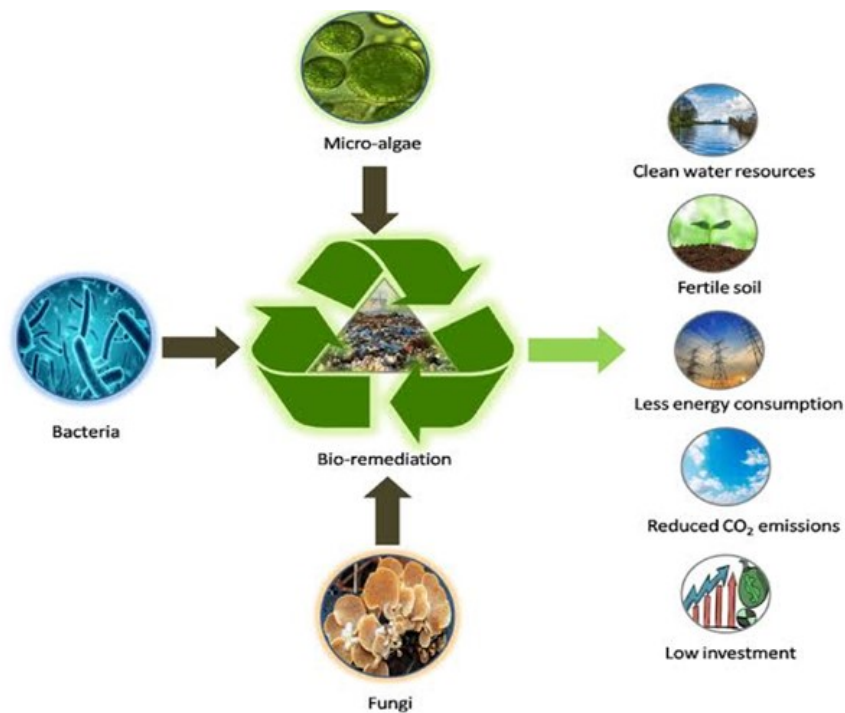
- 1. Production of Extracellular Enzymes:** Fungi release extracellular enzymes into their environment, including oxidases, peroxidases, and lipases. These enzymes work as catalysts in particular chemical reactions to break down the hydrocarbons [50].
- 2. Adsorption to the Fungal Cell Surface:** On the surface of fungus cells, hydrocarbon molecules that are present in the environment are adsorbed. The hydrocarbons are concentrated around the fungal cells as a result of this process, making it easier for the enzymes to reach [51].
- 3. Enzymatic Oxidation:** The extracellular enzymes begin to work on the hydrocarbons once they have been adsorbed to the surface of the fungus cells. The oxidation of hydrocarbons is the most typical method of hydrocarbon breakdown by fungi. For instance, aliphatic hydrocarbons' oxidation (such as alkanes) can result in the formation of fatty acids and alcohols. Similar ring-cleavage reactions occur with aromatic hydrocarbons, such as xylene, benzene, and toluene which produce smaller aromatic molecules [52].
- 4. Metabolic Assimilation:** The smaller molecules produced by the early hydrocarbon breakdown by extracellular enzymes are then transferred inside the fungal cells. These substances are further digested within the fungal cells via a variety of mechanisms to provide energy and biomass for the fungus' growth and maintenance [53].
- 5. Synergistic Interactions:** In some circumstances, fungi and other microbes, including bacteria, may develop mutualistic interactions. Some bacteria make biosurfactants, which aid in the solubilization of hydrocarbons and increase their accessibility to fungi for microbial breakdown. The total effectiveness of hydrocarbon breakdown in the environment can be improved by these synergistic interactions [54].

It's important to keep in mind that fungi's capacity to break down hydrocarbons can vary depending on the species and the particular enzymatic abilities they possess. In addition, environmental elements like temperature, pH, moisture, and the presence of other substances may also have an impact on how effectively fungi degrade hydrocarbons. In some environments, fungi may be more prominent in the hydrocarbons'

degradation, especially in conditions with low oxygen levels where aerobic bacteria may not be as successful [55].

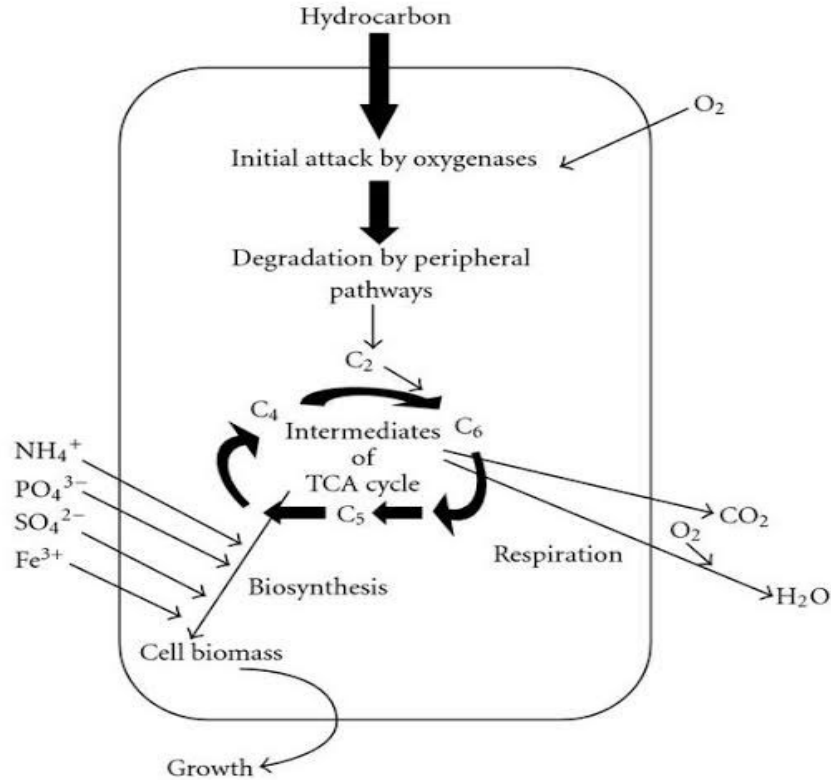
**Table 2: Fungus that Break down Petroleum Hydrocarbons and their Preferred Substrates for Degradation**

Chemical Class	Fungus	Compounds	References
Polycyclic aromatic Hydrocarbons	<i>Cunninghamella elegans</i>	Acenaphthene	[56]
	<i>Cunninghamella elegans</i> , <i>Pleurotus ostreatus</i> , <i>Phanerochaete laevis</i> , <i>Rhizoctonia solani</i> , <i>Naematoloma frowardii</i> , <i>Phanerochaete chrysosporium</i> , <i>Pleurotus sajor-caju</i> , <i>Trametes versicolor</i> , <i>Ramaria</i> sp., <i>Bjerkandera</i> sp.,	Anthracene	[57,58,59]  [60,61]
	<i>Candida maxima</i> , <i>Pleurotus ostreatus</i> , <i>Aspergillus niger</i> , AGHP-1 <i>Agrocybe aegerita</i> ,	Pyrene	[62]  [63]
	<i>Laetiporus sulphureus</i> , AGHP-1, <i>Pleurotus ostreatus</i> <i>Penicillium</i> sp.	Fluoranthene	[64] [63]



**Figure 1:** Overview of Bioremediation of Organic Pollutants using Bacteria, Fungi, and Micro-Algae [65].





**Figure 2:** Aerobic Biodegradation of Hydrocarbons' Principle [66]

## V. CONCLUSION

Petroleum hydrocarbons are among the contaminants that should worry us the most because of their severe toxicity to human and environmental health [67]. It is generally acknowledged that bioremediation, which uses microorganisms to break down petroleum hydrocarbons, is an efficient and environmentally benign method. Bioremediation has made use of a variety of bacterial species that can degrade petroleum hydrocarbons [68]. However, some problems that slow down the effects of biodegradation have been identified throughout actual use [69]. Before summarising the various solutions to these problems, the review first highlighted key hindrance factors, such as the hazards of petroleum hydrocarbons, the bioavailability of pollutants, environmental limits, metabolic limitations, and time requirements [70].

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