

Microbiological Perspectives of Hydrocarbon Degradation: A Review

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ABSTRACT: Hydrocarbons are found in abundant quantities, naturally. A complex mixture of hydrocarbons is present in a majority of crude oil. The organic matter is decomposed into hydrogen and carbon, which are then bonded to form chains. Microorganisms like bacteria can degrade these, fungi and archaea etc., by different metabolic pathways. Hydrocarbons are considered toxic to the human race and environment. So, there is a need to remove these from the environment. Various methods are available, but some methods like the chemical method are not cost-effective. Therefore, microbial biodegradation is an effective alternative method. Employing microbial population for this purpose does not require high cost as compared to other technologies.

Microbial degradation occurs either in aerobic or anaerobic conditions. The degradation processes are classified as aerobic or anaerobic microbial degradation of hydrocarbons. Many biochemical challenges are faced during the anaerobic degradation because of the inertness of hydrocarbons. However, even in the abundance of oxygen, the entire breakdown of hydrocarbons is a complex mechanism. In simple words, microbial biodegradation of hydrocarbon is a way to detoxify the environment by employing microorganisms. However, still, there is a requirement for efficient microorganisms and enzymes for hydrocarbon degradation. Also, there is a need for process optimization of the existing microbiological degradation methods. This review focuses on studying microbe-assisted degradation of hydrocarbons, the factors affecting degradation in anoxic (anaerobic) and aerobic conditions, and the enzymatic mechanisms involved. It describes the elimination of undesired hydrocarbons using different strategies such as bio-augmentation, bio-stimulation, and the use of bio-surfactants.

Keywords: Biodegradation, Hydrocarbons, Microbes, Sustainable

INTRODUCTION

Hydrocarbons can be defined as compounds consisting of carbon and hydrogen. They are widely non-polar. They have very low chemical reactivity at room temperature. Hydrocarbons represent the major components of natural oil, gas, coal etc. (Choudhary and Yadav, 2013; Rabus *et al.*, 2016). The biodegradation process depends on various factors such as the number of hydrocarbons present and their nature, environmental conditions such as presence or absence of oxygen, optimum temperature (which is 25-30°C), and the microorganism.

Anthropogenic activities dealing with hydrocarbons like transport of oil or gasoline, leakage etc., have led to contamination of groundwater and oil spills in the ocean. This has led to environmental pollution (Christova *et al.*, 2019). Therefore, there is a need to degrade them. Various techniques are employed for this purpose. As far as aromatic hydrocarbons are considered, it is essential to understand their degradation pathways and their factors due to their higher water solubility and toxicity than alkanes. The susceptibility of different hydrocarbons to microbial attack differs. In general, the response of hydrocarbons to degradation (via microorganisms) is ranked as cyclic

alkanes < small aromatics < branched alkanes < linear alkanes (Das and Chandran, 2011). Not all hydrocarbons are easily degradable or even degradable. Some like petroleum, benzene, xylenes, alkanes, toluene, ethylbenzene and polycyclic aromatic hydrocarbons (PAH) are biodegradable under low salinity marine habitats and proper environmental conditions.

On the other hand, compounds like methyl tertiary butyl ether (MTBE) and higher molecular PAHs are not easy to degrade (Olajire and Essien, 2014). As a result of such diversity in the environmental conditions for hydrocarbon breakdown, in-situ or in-vitro degradation requires a lot of research and optimization. This review provides insights into different methods for hydrocarbon degradation.

A. Factors affecting the microbial degradation of hydrocarbons

Temperature. Temperature is one factor that highly affects hydrocarbon biodegradation by affecting their physical and chemical composition. It influences the rate of hydrocarbon metabolism done by microorganisms. At lower temperatures, rates of degradation of hydrocarbons is slow as there is a reduction in enzymatic activity rates (Bisht *et al.*, 2015).

Due to an increase in the viscosity of the hydrocarbons, a reduction in the volatilization of toxic short-chain alkanes is observed. In contrast, their water solubility increases, making the hydrocarbons highly toxic and resistant to microorganisms, which causes a delay in the onset of biodegradation. However, at high temperatures, an increase in the rate of hydrocarbons metabolism by microbes is observed. In different habitats, such as soil, marine and freshwater, the temperature ranges for degradation are different, i.e., 30-40°C, 20-30°C and 15-20°C, respectively (Al-Hawash *et al.*, 2018). This degradation rate might be observed due to a decrease in the enzymatic activity, which results from decreasing temperature.

Nutrients. Marine water bodies are not very rich in nitrogen or phosphorus, and the absence of N and P limits microbial degradation. Therefore, there is a need to add these nutrients to increase the biodegradation process (Hesnawi and Adbeib, 2013). However, excess C/P or C/N ratios are unfavourable for the growth of microbes. These are formed due to discharging the hydrocarbons into water bodies with low concentrations of inorganic nutrients (Zafra *et al.*, 2015). Adjustment of C/N/P ratios by adding the required nutrients, i.e., nitrogen and phosphorus, can help stimulate hydrocarbon biodegradation.

The physical state of Hydrocarbons. Oil spill in water tends to spread and eventually form thick films on the surface of water bodies. Because of the actions of wind and wave, oil-in-water or water-in-oil emulsion is formed. When hydrocarbons get dispersed inside the water column within the 'oil-in-water emulsions, it leads to an increase in the oil area and its availability for attack by microorganisms for degradation purposes. The emulsions formation via the microbial production and the discharge of bio-surfactants is a crucial procedure within the intake of hydrocarbons by microbes (bacteria and fungi). The petroleum biodegradation in terrestrial and aquatic conditions is very different. Mainly it is related to the movement of the oil along with the particulate presence. These two factors significantly affect the physical as well as chemical characteristics of hydrocarbons. As a result, its susceptibility to microbial degradation is also affected. Terrestrial oil spills are generally characterized by vertical movement of the oil penetrating the soil instead of the horizontally spreading related to slick formation. Infiltration of oil into the soil prevents evaporative losses of volatile hydrocarbons, which are considered toxic to microorganisms. Particulate can reduce the effective toxicity of the components, but absorption or adsorption of hydrocarbons to humic substances contributes to persistent residues.

Oxygen. Oxygen is one of the crucial and significant factors in the biodegradation of hydrocarbons by microbial population. Biodegradation pathways significantly utilize dissolved molecular oxygen. Usually, 3 to 4 ml of dissolved oxygen is utilized to convert 1 ml of petroleum hydrocarbons into CO₂ and H₂O. The availability of oxygen for the terrestrial environment is determined mainly by the soil structure.

It depends on various factors such as: whether the soil is water-logged, utilizable substrates are present or not, which leads to depletion of oxygen, moisture quantity and the rate of biodegradation (Haritash and Kaushik, 2009). Due to the air and water interface and wind activity, surface water has an abundant oxygen supply, but the dissolved oxygen decreases with depth. When the oxygen is thoroughly utilized, hydrocarbon substances' biodegradation occurs under anaerobic conditions (without oxygen). This is why petroleum hydrocarbon substances reaching the deepest in the oceans and seas take a long time for microbial degradation.

Salinity. Microorganisms are well suited to the wide range of salinity of the oceans around the world. Estuaries provide numerous situations because of the variation in their salts amounts according to different scales of comparison to seas and oceans' values. For biodegradation of hydrocarbon, microorganisms are incorporated into the aqueous environment. It should be taken care that if these microbes are compatible with the marine environment's salinity level or not (Ebadi *et al.*, 2017). Various studies have been conducted which deal with the effects of salinity on the microbial degradation of hydrocarbons. It is observed that the rate of hydrocarbon metabolism decreases as salinity increases.

Pressure. When we talk about deep-sea environment pressure as a variable in hydrocarbons' biodegradation, it is considered an essential factor. When hydrocarbon pollutants reach the deep ocean environment, hydrocarbons pollute the deep benthic zones of the oceans. These pollutants are biodegraded for a long time (Varjani and Upasani, 2017). Microbial populations very slowly degrade it, and therefore, some fraction of oil could remain for many years.

Water activity. The water activity in the aquatic environment is stable at a value near 0.9, and the water activity of soil ranges mainly from 0.0-0.99. Therefore, hydrocarbon degradation in the soil ecosystem is restrained by water availability for microbial growth and metabolism. A study done by Dibble and Bartha on oil sludge biodegradation in soil claimed that the most optimum rate for microbial biodegradation is at 30-90% water saturation. The inhibition of the degradation process at the lower values is not observed because of hydrocarbon assisted reduction in the soil's water holding capacity. Atlas recommended that tar balls settle on the beach because available water limits hydrocarbon biodegradation.

pH. Most of the heterotrophic bacteria, as well as fungi, grow at a neutral pH. Fungi are supposed to be more tolerant to acidic pH. Therefore, extreme pH conditions have a negative influence on the ability of microorganisms to degrade hydrocarbons. It was reported by Verstraete *et al.* that a doubling rate of degradation of gasoline is observed in acidic soil (pH 4.5) when the pH is adjusted to 7.4. However, on further raising the pH to 8.5, the doubling rate drops significantly.

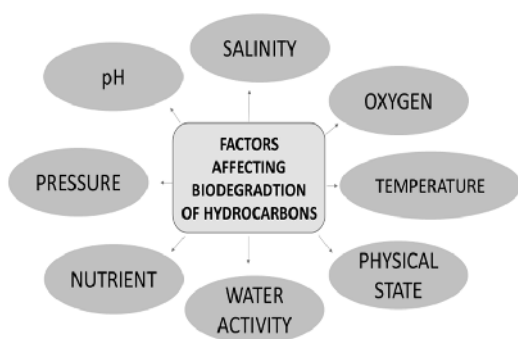


Fig. 1. Factors affecting microbial degradation of hydrocarbons.

Aerobic Biodegradation of Hydrocarbons. The major dominating factor in the aerobic biodegradation process is oxygen. Availability of oxygen depends on its ability to diffuse towards the environmental location and being available readily to microorganisms. Oxygen in the environment can affect the rate of degradation by many times compared to the degradation occurring in anoxic conditions. Hydrocarbons with double bonds (alkenes and alkanes) and hydrocarbons having single bonds only (short carbon chain alkanes) can be easily degraded, followed by aromatic hydrocarbons. Under aerobic conditions, hydrocarbon substances are easy to degrade. Aerobic hydrocarbon biodegradation can be done by bacteria, fungi and algae as well.

Aromatic hydrocarbons are present in different chemical structures and are widely distributed. Microorganisms, especially bacteria, degrade these structures. The main principle of biodegradation of aromatic hydrocarbons is their ability to transform through peripheral reactions. The narrow range of central intermediates is also formed, subjected to the ring cleavage and funnelling into the TCA cycle (Pérez-Pantoja *et al.*, 2010).

The biodegradation rate varies depending on various ecological parameters. This decreases with increasing complexity in hydrocarbon. Biodegradation efficiency is observed on nitrogen source addition as the nitrogen is known to fulfil a few microorganisms' nutrient requirements. Generally, the commonly found molecules in petroleum hydrocarbons are alkanes, cyclo-alkanes, and hydrocarbon mono-aromatics called BTEX, which stands for benzene, toluene, ethylbenzene, and xylene isomers. Apart from the environmental contamination, the toxicity of BTEX compounds is a potential threat to human health (Claro *et al.*, 2018). A metabolic pathway for aerobic degradation of the BTEX compounds is provided by two enzymatic systems, i.e., dioxygenases and monooxygenases (Jindrová *et al.*, 2002). The monooxygenase enzyme attacks the ethyl or methyl substituents of the aromatic ring structure; these are, as a result, changed by quite a few several oxidation reactions to corresponding substituted phenylglyoxal or pyrocatechols, respectively.

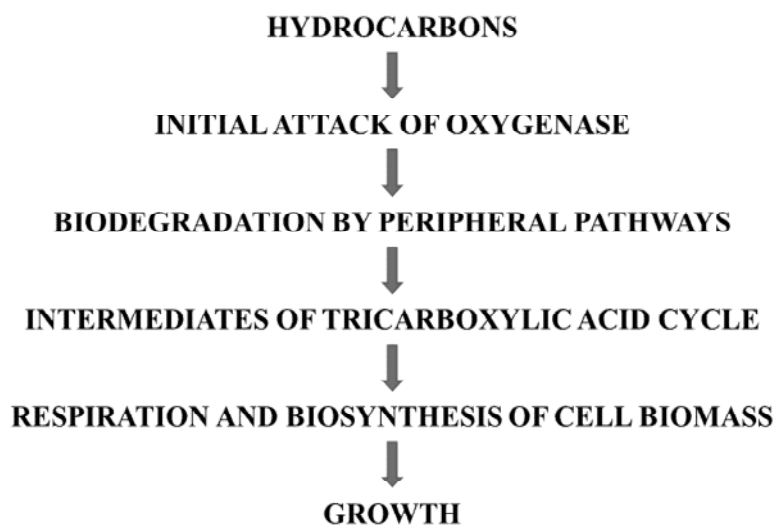


Fig. 2. Method of Aerobic Microbial Degradation of Hydrocarbons.

Anaerobic Biodegradation of Hydrocarbons. Under anoxic conditions, hydrocarbon biodegradation is slow in comparison to aerobic biodegradation. Facultative and anaerobic archaea and bacteria are primarily known for their proficiency to degrade hydrocarbons despite the presence of oxygen (Wartell *et al.*, 2021). These anaerobic microorganisms quickly emerge at oil-spill locations and are formed due to immediate oxygen consumption. The degradation of polycyclic aromatic hydrocarbons (PAHs) in anoxic conditions is further

improved by treating the contaminated zone with nitrates. It is thermo-dynamically favourable, highly soluble and cost-effective (Zhang *et al.*, 2021). During anaerobic petro-hydrocarbon degradation, the first few steps involving the trigger of the hydrocarbon molecules by adding oxidized functional groups are limited. Generally, it takes a few days to several months to anaerobically degrade the hydrocarbon. The efficient degradation of different hydrocarbons happens in the absence of oxygen despite the slow growth rate. Under

anaerobic metabolism, aromatic hydrocarbons are first oxidized to phenols or organic acids converted to long-chain volatile fatty acids and finally degraded to CH₄ and CO₂ (Wilkes *et al.*, 2016).

The function of Enzymes in Hydrocarbon biodegradation. Various enzymes are present in eukaryotes and prokaryotes, which are actively involved in hydrocarbon biodegradation. Fungi have many advantages over bacteria. They can act upon a broader range of substrates and can also produce many extra-cellular enzymes. These enzymes penetrate through the contaminated soil and remove the pollutants. The efficiency of biodegradation by fungal enzymes is affected by various factors, like nutrients availability, oxygen, pH, temperature, and media's chemical composition. Fungi can degrade organic pollutants and mineralize them too. The extra-cellular peroxidases of fungi are responsible for biodegradation. Fungal lignin peroxidases directly oxidize several Hydrocarbons (Kadri *et al.*, 2017). Cytochrome P450 hydroxylases engage in microbes assisted biodegradation of hydrocarbons and other compounds. Scheller *et al.* isolated cytochrome P450 enzymes from *Candida* species (*Candida apicola*, *C. maltose* and *C. tropicalis*). P450 monooxygenase, epoxide hydrolases, dioxygenases, proteases, cytochrome, and lipases, have been studied for their ability to degrade hydrocarbons (Balaji *et al.*, 2014).

Bio-surfactants for Biodegradation of Petroleum Hydrocarbons. Biosurfactants are heterogeneous series of substances with a chemically active surface. The hydrocarbon-degrading microorganisms can create biosurfactants of varied chemical nature. These surface-active compounds on the surface increase the surface area for hydrophobic water-insoluble substrates, resulting in an increment in their availability (Hazra *et al.*, 2012). Hence enhancing the bioremediation rate as the microorganism's population is increased. These surfactant substances promote solubility, mobility, and degradation of pollutants.

Hydrocarbon utilizing as well as biosurfactant producing bacteria enhances the effectiveness of bioremediation technology. This happens as the bio surfactant-assisted microorganism method makes the hydrocarbons bioavailable for degradation (Lee *et al.*, 2018). In an experimental study, *Pseudozyma*, a biosurfactant, was supplemented at varied concentrations to *Pseudomonas putida* culture medium with crude oil its carbon source. It was observed that the supplementation of biosurfactants to the medium improved the biodegradation of the crude oil by *P. putida*. The utmost biodegradation of hydrocarbons was seen at the supplementation of 2.5 mg L⁻¹ of biosurfactants (Sajna *et al.*, 2015).

Table 1: List of microbial strains and known hydrocarbon substrates.

Microorganisms	Substrate (pollutant)
<i>Pseudomonas</i> sp.	Aliphatic and aromatic hydrocarbons- Naphthalene, xylene, toluene, parathion, malathion, organophosphates
<i>Arthrobacter</i> sp.	Benzene, polycyclic aromatics, pentachlorophenol
<i>Alcaligenes</i> sp.	Halogenated hydrocarbons, polychlorinated biphenyls
<i>Mycobacterium</i> sp.	Branched hydrocarbons, benzene, cycloparaffins
<i>Nocardia</i> sp.	Naphthalene, alkylbenzenes
<i>Corynebacterium</i> sp.	Halogenated hydrocarbons, phenoxy acetate
<i>Candida</i> sp.	Polychlorinated biphenyls
<i>Xanthomonas</i> sp.	Polycyclic hydrocarbon
<i>Streptomyces</i> sp.	Phenoxy acetate, Halogenated hydrocarbons

APPLICATIONS

For environment clean up. The capability of microorganisms to treat hydrocarbons and degrade them has been studied. This is utilized for the benefit of the environment.

Bioremediation is an important method used in the restoration of an environment that is polluted by hydrocarbons. This method uses the natural mechanism of microbial biodegradation. These hydrocarbons consuming microorganisms are present ubiquitously in the environment (Varjani, 2017). Using different strategies, the microbial population is employed to treat environmental pollution. Some of these are as in Fig. 3.

Bioaugmentation. Bioaugmentation is a technique in which microorganisms are added to the target location to reduce contaminant load effectively. This is done by transforming it into less dangerous compounds. Typically, microorganisms having specific degradation

capabilities are directly added to the target. The added microorganisms may be attached to the carrier or be in a free state (Ławniczak *et al.*, 2020).

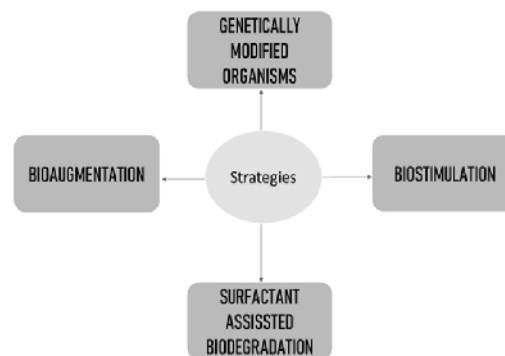


Fig. 3. Strategies to increase the efficiency of microbial biodegradation of hydrocarbons for environment clean up.

Biostimulation. Biostimulation is a technique that is environmentally friendly and cost-effective. It is achieved by adding nutrients to target or polluted sites to stimulate the existing bacteria or microbial population to degrade the toxic contaminants like hydrocarbons (Goswami *et al.*, 2018).

Genetically modified organisms. Recombinant DNA technology explores an organism's ability to metabolize a xenobiotic by detecting degradative genes and transforming them into appropriate hosts using a suitable vector. This technique explores polymerase chain reaction (PCR), site-directed mutagenesis, electroporation etc., for enhancing the ability of GMOs to degrade the contaminants (Adams *et al.*, 2015). In simple words, it works on a targeted mechanism. These organisms are constructed by rDNA technology and are specific.

Surfactant assisted methods. This method works by lowering the interfacial tension. Surface-active compounds can alter the properties of fluid interfaces and partitions at the interface between phases of fluid. This leads to the formation of micro-emulsion, and this imparts spreading and other detergent traits. When reducing the surface tensions and the interfacial tensions, bio-surfactants tend to increase hydrocarbons' mobility and bioavailability (Pacwa-Płociniczak *et al.*, 2011). This makes them practical to be utilized in the surfactant-enhanced process.

The impact of bio-surfactants on the remediation of salt contaminated soil and crude oil was studied by Zhang *et al.* The results showed a decline was observed in the concentration of total petroleum hydrocarbon (TPH value) within the soil while using rhamnolipid (a bio-surfactant) for 30 days, with a maximum TPH reduction value of 86.97% (Zhang *et al.*, 2011).

CONCLUSION AND FUTURE PERSPECTIVES

The comprehensive study of microbial biodegradation of hydrocarbons has focused on a variety of microbial populations and their degrading capacities. It deepens our knowledge of microbial procedures in hydrocarbon containing domains. Another significant conclusion of this study is the acknowledgement of microbial or biological preference over chemical methods. Chemically, hydrocarbon degradation methods are generally not suitable for the efficient synthesis of pure substances after degradation. For designing such experiments, some critical points have to be kept in mind, like the various factors which affect the degradation process, such as temperature, pH, salinity, concentration etc. Degradation of hydrocarbons can be performed in aerobic or anaerobic conditions. Depending on this, we classify the process of biodegradation as aerobic microbial degradation or anaerobic microbial degradation. To enhance this, biosurfactants are employed. Also, the role of enzymes in the entire process is very crucial. The use of biosurfactants and enzymes increases the overall efficiency of Hydrocarbon degradation. Microbial bioremediation is the term used to refer to the treatment of environmental pollutants through microorganisms. It

is considered to be a sustainable approach to cope up with environmental pollution. Different strategies employed are biostimulation, bioaugmentation, biosurfactant assisted and use of genetically modified organisms. These strategies can be conjugated with treatment methods such as absorption using immobilized microorganisms on absorbent surfaces like a loofah. The immobilized microorganisms can also provide continuous bioreactor capabilities for applications in the field of bioremediation.

Therefore, we can conclude that microorganisms are efficient for the degradation of hydrocarbons. Microbial metabolism can be utilized to degrade hydrocarbon that might result in products that can be used for power generation.

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Conflict of Interest. The authors declare no conflict of interest.

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