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Microbial action to remediate petroleum-hydrocarbon contaminated soil

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Abstract
The primary energy source for many companies and daily living is petroleum. Priority pollutants include hydrocarbons, which are a major component of petroleum pollution. Oil spills and other accidental releases of petroleum-derived chemical wastes into the environment that poses the contamination of soil, sediments, and marine environments, frequently have detrimental effects on human health. It is reported that petroleum hydrocarbons alter the genetic sequences that leads to mutation and more importantly it exhibits carcinogenic effects also. They also have detrimental socio-economic effects in the host communities that produce the oil. In comparison to the conventional physico-chemical procedures for the restoration and reclamation of polluted areas, bioremediation strategies have been recognised as an economical and environmentally benign alternative. Depending on the contaminants in the polluted soil, different organisms are used with various bioremediation techniques. Microbial bioremediation is the technique of using microorganisms or microbial processes to remove or degrade pollutants from soil. Extracellular enzymes are produced by microbes to break down complex hydrocarbons into smaller, more accessible molecules. These compounds are subsequently transferred into microbial cells to provide energy and growth. Microbes frequently build biofilms on soil particles, resulting in localised microenvironments that improve degradation efficiency and shield cells from harmful circumstances. This paper contrasts the degradative properties of remediation techniques depending on microbial action.

Keywords: Petroleum pollution, contamination of soil, mutation, carcinogenic effects, microbial bioremediation

Introduction

Petroleum (Or crude oil) is a complex heterogeneous mixture that occurs in nature and is mostly composed of hydrocarbons. However, it also occasionally includes substantial amounts of nitrogen, oxygen, and sulphur as well as trace amounts of metals including copper, iron, nickel, vanadium, and other elements. Petroleum hydrocarbons are known to exist naturally in large quantities and in a range of forms, including solid (For example, asphalt), liquid (For example, crude oil), and/or gaseous (For example, natural gas) (Ite *et al*. 2019) [15]. Petroleum enters the soil environment through extraction, processing, and transportation (Pipe rupture). The most dangerous and poisonous aliphatic, cycloaliphatic, and aromatic hydrocarbons are the main pollutants in petroleum-contaminated soil (Suganthi *et al*. 2018) [23]. They harm soil fertility, reduce plant and microbial variety, upset the biological balance of the soil, and potentially endanger human health (Ansari *et al*. 2018) [16]. When cultivated in highly petroleum-contaminated soil, crop germination is delayed, the chlorophyll content is low, and certain crops fail to thrive. High-molecular-weight hydrocarbon pollutants will persist in the soil for a very long time due to their hydrophobicity, generating secondary pollution throughout the ecosystem, but certain low-molecular-weight hydrocarbon pollutants can weather and degrade over time Pollutants can also enter into human body by inhaling, skin contact, or eating food contaminated with petroleum, leading to gastrointestinal problems, contact dermatitis, auditory and visual hallucinations, and a significantly increased risk of leukaemia in children (Kumari *et al*. 2018) [18] (Ozigis *et al*. 2019) [35]. The health of people and the natural ecosystem is greatly endangered when several various petroleum products, are present in large numbers in soil or water. These pollutants build up in the tissues of animals and plants and have the potential to accumulate in our food chain, having major toxic, carcinogenic, and mutagenic impacts (Varjani *et al*. 2017) [27].

The actions of living organisms can cause enzymatic assault on the majority of organic compounds as well as many inorganic ones. These compounds make up the majority of environmental pollutants produced by contemporary society, and the effects of enzymes on them are typically referred to as "biodegradation." Bioremediation is the beneficial application of biodegradative processes to remove or detoxify chemicals that have gotten into the environment and endanger public health, often as contaminants of soil, water, or sediments. The ability of some bacteria to break down petroleum hydrocarbons and use them as their only source of carbon and energy for growth has been understood for 80 years. There are several techniques for cleaning up polluted soil, including both physico-chemical and biological ones. Compared to chemical and physical approaches, biological ones are more cost-effective and effective (Thapa *et al*. 2012) [26]. A novel technique for cleaning up petroleum hydrocarbon contamination has been made possible by the ongoing research and advancement of microbial remediation technology, which has garnered a lot of interest (Dombrowski *et al*., 2016) [10] (Dvoˇrák *et al*., 2017) [11].

Materials and Methods

The appropriate material for this review paper was found by searching PubMed, PubMed Central, Google, and published research work and the review articles from around the world on the environmental pollutants produced by the leather industries and the microbial bioremediation strategy to remove the pollutants for a cleaner world. Only published data were considered, and vague statements of exposure were excluded. Information acquired from reputable sources of publications on the subject is part of these inclusion criteria. The study did not include any other languages than English.

Results and Discussion

Petroleum is made up of a variety of hydrocarbons, composed of carbon (83–87%), hydrogen (11–14%), and sulfur (0.06–0.8%), nitrogen (0.02–1.7%), oxygen (0.08–1.82%), and trace metal components (nickel, vanadium, iron, antimony, etc.) (Aguelmous *et al*. 2020) [4]. About 95% to 99% of petroleum is made up of hydrocarbons, which are compounds created when carbon and hydrogen combine. Alkanes, cycloalkanes, and aromatic hydrocarbons are three categories of hydrocarbons that are separated based on their structural makeup (Sui *et al*. 2021) [24]. Although just a metre deep over the surface of the earth, soil is one of the natural resources that must be conserved with the highest care and attention. The everyday usage of petroleum is essential to our contemporary culture. It serves as a primary source of energy for heating, transportation, and manufacturing, as well as a raw material for synthetic rubber and plastic. In today's culture, there are so many petroleum-based products that are common environmental pollutants (Such petrol, kerosene, fuel oil, mineral oil, and asphalt) (Adipah *et al*. 2019) [3].

Effects of Petroleum Hydrocarbons (PHC) on ecosystem: The main source of hydrocarbon pollution in the soil is oil spills. There are 600,000 metric tonnes of natural petroleum leaks recorded annually on the planet (Abioye*et al*. 2011) [2]. Different nations and regions have various sampling and transit methods, as well as various petroleum pollution sources and intensities. In addition, rainfall-induced washing and leaching of pollutants into the surface and deep soil in both horizontal and vertical orientations as well as into the groundwater system. Oil significantly affects soil moisture, pH, total organic carbon, total nitrogen, exchangeable potassium, and enzyme activity (Urease, catalase, and dehydrogenase), impairing the ecological structure and function of soils. As pollutant concentrations increase, the amount of clay in contaminated soil increases, soil porosity decreases, impermeability and hydrophobicity increase, and plant root development and bacterial population reduce (Sui *et al*. 2021) [24]. When PHCs are released into the environment due to accidents, industrial releases, by-products of commercial or private uses, spills, or leaks, they move from the soil to the groundwater where some organisms may break down some of the contaminants into smaller fractions, while others may evaporate into the atmosphere, while still others will stay in the soil for a longer period of time and will be broken down by other organisms found in the soil, causing damage. The volatility of PHCs, if released into the earth, might firstly provide a risk of fire or even explosion, particularly when vapour enters tight places. Contaminants can obstruct the flow of nutrients and water, which degrades the soil. Petroleum residues can bind to soil particles and stay there for a long time. Last but not least, PHCs might endanger the ecosystem (Adipah *et al*. 2019) [3]. Low petroleum hydrocarbon concentrations (10 g/kg) were shown to promote plant root vitality, but medium concentrations (30 g/kg) and high concentrations (50 g/kg) were found to diminish it. In addition, the chlorophyll content of soil polluted with 50 g/kg of petroleum is over 60% lower than that of uncontaminated soil (Zhen *et al*. 2019) [34]. Through migration and dispersion, the petroleum in the soil also contaminates the groundwater environment, posing a threat to many aspects of human existence (Sui *et al*. 2021) [24].

Effect of Petroleum Hydrocarbons on human health: Numerous petroleum contaminants are hazardous, mutagenic, and carcinogenic, including benzene and polycyclic aromatic hydrocarbons. Some aromatics impair human liver and kidney function and potentially increase the risk of cancer (Haller *et al*. 2020) [14]. According to the Centres for Disease Control and Prevention (CDC), symptoms will go away when exposure is stopped, but if exposure continues for a longer period of time, permanent damage to the central nervous system may result. Additionally, benzene is known to be associated with a higher incidence of leukaemia at sufficient concentrations. The Environmental Protection Agency in Washington, D.C. (USEPA) states that exposure to soil toxins by direct touch, inhalation, or ingestion can be fatal (Adipah *et al*. 2019) [3]. Long-term exposure to polluted environments can result in fatigue, respiratory issues, eye irritation, headaches, and a higher risk of spontaneous abortion in women (O'Callaghan-Gordo *et al*. 2016) [21]. Long term exposure of PHC significantly increased risk of leukaemia in children (Ozigis *et al*. 2019) [35].

Green strategy to reduce risks: A variety of physical-chemical and biological treatments have been studied as ways to control oil pollution (Yuniati *et al*. 2018) [33]. The most economical and environmentally beneficial methods for cleaning up petroleum-contaminated soil and water to restore it to its original habitat are provided by bioremediation (Borah *et al*. 2017) [6]. By optimising the conditions for degradation, bioremediation uses the potential biological organisms to clean up polluted matrices. Additionally, it provides the opportunity to eliminate or significantly reduce other inert pollutants through their inherent biological activity, which quickens biological destruction and precipitation of pollutants (Ma *et al*. 2016). A single microbe or a microbial community is regarded as a fundamentally important component in the elimination of hydrocarbons without compromising soil stability (Varjani *et al*. 2017) [27].

Bacterial action to remediate PHC contaminated Soil: The biological degradation of petroleum hydrocarbons in the environment is ultimately one of the most significant natural mechanisms by which these organic pollutants could be mineralized by native soil microbes and/or transformed into harmless by-products. Petroleum hydrocarbon degrading microbes are widely distributed in the environment (Ite *et al*. 2019) [15]. The right microbial selection that can break down pollutants without losing microbial viability or competing with other autochthonous microorganisms is essential for an efficient bioremediation procedure. Bacterial communities are the most dynamic notable microbes in the degradation of petroleum oil and are regarded as the most powerful environmental oil spill (Could feed exclusively on hydrocarbons) degraders. Their particular genes in relation to hydrocarbon disintegration assume a crucial role in the degradation process of petroleum hazards (Varjani *et al*. 2017) [27]. Although there are numerous microorganisms that may break down the crude oil in the soil, it has been observed that using mix cultures rather than pure cultures in bioremediation is advantageous since it demonstrates the synergistic interactions. Commercially available freeze dried bacteria for hydrocarbon breakdown can be employed for bioremediation once they have multiplied to a minimum of 2108 CFU/ml. Pseudomonas, Aeromonas, Moraxella, Beijerinckia, F lavobacteria, Chromobacteria, *Nocardia*, Corynebacteria, Acinetobacter, Mycobacteria, Modococci, Streptomyces, Bacilli, Arthrobacter, Aeromonas, Cyanobacteria, etc. are among the bacteria that may break down petroleum compounds (Thapa *et al*. 2012) [26]. Alcanivorax borkumensis, Geobacillus thermodenitrificans, Desulfatibacillum alkenivorans, Polymorphumgilvum SL003B-26A1T, and Pseudomonas aeruginosa N002 are only a few examples of bacteria having hydrocarbon removal abilities (Varjani *et al*. 2017) [27]. 99 different petroleum-degrading bacterial strains were identified, and it was found that 85% of them belonged to the families Actinomycetes, Enterobacteriaceae, coryneforms, Klebsiella aerogenes, *Micrococcus* spp., *Nocardia* spp., and *Sphaerotilus natans*. Six different strains of bacteria from the families Bacillus, Klebsiella, and Serratia were part of a microbial consortium that improved the biodegradation of petroleum oil in soils containing bagasse waste (Varjani *et al*. 2017). According to estimates, the microbes *Gordonia* sp., *Brevibacterium* sp., *Aeromicrobium* sp., *Dietzia* sp., Burkholderia sp., and Mycobacterium sp. found in petroleum-contaminated soil areas may be capable of degrading hydrocarbons (Jain *et al*. 2010) [16]. A powerful and effective hydrocarbon-degrading bacteria called Bacillus licheniformis was discovered in China in soil that had been contaminated by crude oil. It has a remarkable capacity to break down both short- and long-chain alkanes (Liu *et al*. 2016) [19].

In a study on the biodegradation of petroleum hydrocarbons at a ship-breaking yard, it was discovered that *Fusarium moniliforme* caused the maximum degradation of octane (58%) and diesel (56%), while *Penicillium corylophilum* caused the same for kerosene (40%). *Bacillus* spp. demonstrated a degradation of hydrocarbon content of 92.5% during the spillage of lubricating oil in the water. Microorganisms are also used to treat soil pollution. Bacillus subtilis strains are discovered in Kuwaiti soil that has been polluted with oil. Environmental contaminants can be broken down by soil bacteria by adapting to them; some soil types may already have native bacteria that are well-suited for this purpose. Bacterial degradation is achievable when the pollutant concentration is below the threshold of toxicity. However, high concentrations of diesel can be toxic to microorganisms and hinder decomposition (Ahmed *et al*. 2018) [5]. They discovered six bacterial genera that can break down and use diesel oil as a source of carbon, including Staphylococcus, Enterobacter, Yersinia, Proteus, and Alcaligenes. The study conclusively showed that Ghanaian soils contain a variety of bacterial genera that can do this. A consortium of bacteria that can degrade diesel oil was used to biodegrade the fuel both in a lab setting and on a small scale. In both the laboratory (135 to 19.32 g of diesel per kg of soil) and the pilot scale (118 to 17.5 g of diesel per kg of soil), the concentration of diesel in soil treated with the bacterial consortium was decreased to around 15% of the original concentration over the course of five weeks (Ahmed *et al*. 2018) [5].

Because they require energy and carbon for growth and reproduction as well as the need to relieve physiological stress brought on by the presence of petroleum hydrocarbons in the microbial bulk environment, native bacteria eventually degrade or metabolise the majority of petroleum hydrocarbons encountered in the environment (Kleindienst *et al*. 2015) [17]. In fact, numerous studies have shown that there are many hydrocarbon-degrading bacteria in oil-rich environments, such as oil spill sites and oil reservoirs (Yang *et al*. 2015) [32], and that their quantity and abundance are strongly correlated with the types of petroleum hydrocarbons and the local environmental factors (Fuentes *et al*. 2015) [12] (Varjani *et al*. 2017) [27]. It has proven possible to identify and use several common and unusual bacterial species as biodegraders to cope with petroleum hydrocarbons (Xu *et al*. 2018) [31]. Interestingly, it has been observed that "conditionally rare taxa" in soil, including *Alkanindiges* sp., show rare-to-dominant bacterial changes that are significantly impacted by environmental constraints like diesel pollution (Fuentes *et al*. 2015) [12]. Similar to this, several obligate hydrocarbonoclastic bacteria (OHCB), such as Alcanivorax, Marinobacter, Thallassolituus, Cycloclasticus, Oleispira, and a few others, displayed low abundance or undetectable status before to pollution but were discovered to be dominant following petroleum oil contamination (Xu *et al*. 2018) [31]. These observations imply that these microbes play a key part in the breakdown of petroleum hydrocarbons and have a big impact on how they change and end up in the environment (Wang *et al*. 2011) [30]. This is due to the fact that various native bacteria have unique catalytic enzymes, which causes a broad range in their functions in oil-contaminated areas.

This also suggests that in order to obtain the optimum environmental purification impact, the remediation of petroleum hydrocarbon pollution necessitates the cooperative activity of several functional bacteria (Dombrowski *et al*. 2016) [10]. Based on this hypothesis, the halotolerant Hydrocarbon Utilising Bacterial Consortium (HUBC) was developed, which included the bacterial isolates *Ochrobactrum* sp., Stenotrophomonas maltophilia, and Pseudomonas aeruginosa. It was discovered that this consortium was effective at degrading crude oil (3% v/v), with a degradation percentage as high as 83.49% (Varjani *et al*. 2015) [27]. To efficiently speed up the breakdown of crude oil, a specified co-culture between a local bacterial consortium and exogenous Bacillus subtilis was used (Tao *et al*. 2017) [25].

Table 1: Potential Petroleum Hydrocarbon degrading bacterial species (Thapa *et al*. 2012) [26] (Jain *et al*. 2010) [16] (Varjani *et al*. 2017) [27] (Fuentes *et al*. 2015) [12] (Liu *et al*. 2016) [19] (Ahmed *et al*. 2018) [5] (Tao *et al*. 2017) [25] (Varjani *et al*. 2015) [29].

PHC degrading bacterial species	Acinetobacter, *Aeromicrobium* sp., Aeromonas, *Alcanivorax borkumensis*, *Alkanindiges* sp., *Arthrobacter*, *Bacillus licheniformis*, *Bacillus* sp., *Bacillus subtilis*, Beijerinckia, *Brevibacterium* sp., *Burkholderia* sp., *Desulfatibacillum alkenivorans*, *Dietzia* sp., *Geobacillus thermodenitrificans*, *Gordonia* sp., *Micrococcus* spp., *Mycobacterium* sp., *Nocardia* sp., *Ochrobactrum* sp., *Pseudomonas*, *Pseudomonas aeruginosa*, *Sphaerotilus natans*, *Stenotrophomonas maltophilia*

Influence of environmental factors on bioremediation of PHC: Different environmental factors, including oxygen accessibility, temperature, pH, water availability, nutrients, and the presence or concentration of contaminants, have an impact on the biodegradation rates, biomass production, and fate of petroleum hydrocarbons in the natural environment (Brune *et al*. 2012) [8] (Guarino *et al*. 2016) [13].

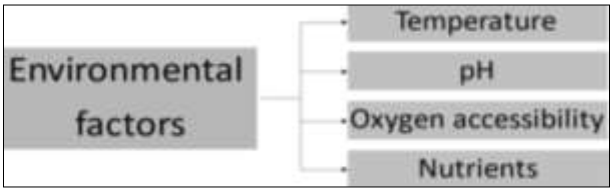

Fig 1: Environmental factors that influence the bioremediation of PHC (Brune *et al*. 2012) [8] (Guarino *et al*. 2016) [13].

Temperature: The chemical makeup of the pollutants, physiology, and variety in the microbial flora community are all impacted by temperature, which is crucial for hydrocarbon biodegradation. Although biodegradation occurs at varying temperatures, hydrocarbon degradation occurs throughout a wide temperature range (Das *et al*. 2010) [9]. Low temperatures have an impact on the characteristics of an oil spill, raise the viscosity of the oil and the physiological characteristics of microorganisms, and reduce the volatility of low molecular weight hazardous hydrocarbons, which slows down the biodegradation process (Varjani *et al*. 2017) [27]. The soil exhibits the highest levels of biodegradation at temperatures between 30°C and 40 °C (Das *et al*. 2010) [9].

Oxygen accessibility: The rates at which microbiological oxygen is utilised, the kind of soil, independent of whether the soil is wet, and the proximity to utilisable substrates that can cause oxygen exhaustion all affect the availability of oxygen in soils. It has been established that the rate-restricting factor in the biodegradation of crude oil in soil and groundwater is the centralization of oxygen (Varjani *et al*. 2017) [27]. Utilising oxygen as an electron acceptor is crucial for aerobic degradation processes; however in settings where petroleum oil is present, the air permeability is frequently insufficient (Xu *et al*. 2018) [31].

Nutrients: Biodegradation occurring in soil, more specifically in freshwater and marine environments, nutrients like phosphorus, nitrogen, and iron may be a limiting factor. When an oil spill occurred in a marine or freshwater habitat, the addition of carbon was noticeably increased, and the availability of nitrogen and phosphorus became a crucial factor in the biodegradation of the oil (Varjani *et al*. 2017) [27]. Low bioremediation efficiency are caused by issues with soluble and insoluble nutrients during the remediation process (Xu *et al*. 2018) [31].

pH

The impact of soil pH on the biodegradation of petroleum hydrocarbons may be significant for terrestrial ecosystems since soil pH varies greatly, ranging from 2.5 to 11, and most microbial species prefer neutral pH. Additionally, excessive pH has a negative impact on the capacity of hydrocarbon-degrading microbial communities (Varjani *et al*. 2017) [27].

Conclusions

The majority of hydrocarbons that make up petroleum (Also known as crude oil) in natures complicated heterogeneous combination. Through extraction, processing, and transportation (Pipe rupture), petroleum reaches the soil environment. They impair soil fertility, lessen plant and microbial diversity, disturb the soil's biological equilibrium, and may even be dangerous to human health. There are a variety of methods, including physico-chemical and biological ones, for cleaning up contaminated soil. Biological methods are more economical and successful than chemical and physical ones. The development of microbial remediation technology, which has attracted considerable interest, has enabled a revolutionary method for removing petroleum hydrocarbon pollution. Bacteria have been chosen and utilised to digest waste materials produced by the food, agricultural, chemical, and pharmaceutical industries. Using bacteria to remove pollutants from the environment has lately acquired favour as a promising method since it is affordable and advantageous to the environment. It has been known for 80 years that certain bacteria can degrade petroleum hydrocarbons and use them as the only source of carbon and energy for growth.

Future Scope

It's conceivable that ongoing research will produce microbial strains and bioremediation methods that are more effective. This could result in polluted site remediation that is quicker and more affordable. Microbial bioremediation offers an environmentally beneficial method of restoring soil, coinciding with expanding environmental rules and business responsibilities, as sustainability gains prominence. These factors imply a promising future for microbial remediation in the treatment of soil polluted with petroleum hydrocarbons. So, it is necessary to look for other efficient microbes for the detoxification and degradation of petroleum hydrocarbons. In order to design effective bioremediation solutions, it is necessary to understand their genetic make-up and biochemistry. This will help to ensure the long-term viability of natural habitats on land and in the marine. More eco-friendly and cost-effective waste treatment technologies must be used in future.

Conflict of Interest

There is no conflict of interest related to the study.

Author contributions

Acquisition and interpretation of data is done by Sohani Das. Conception, design and revising of the article are done by Rupesh Dutta Bani

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