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Enhanced In-Situ Bioremediation of Petroleum-Contaminated Soil: A Review

Rizka Amelia Situmorang[1*], Qomarudin Helmy[2]

[1]Master Program of Environmental Engineering, Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, Indonesia
[2]Environmental Engineering Study Program, Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, Indonesia
***Corresponding author**: rizkaasitumorang@gmail.com

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Abstract
Petroleum is still one of the main sources of energy even though many energy sources have been discovered today. The growing petroleum industry is directly proportional to the contamination that can be caused by this industry. Bioremediation is an environmentally friendly and cost-effective technology that has recently become a favorite technology in dealing with petroleum contaminated soil waste. This technology can be carried out directly in the polluted area, to minimize dredging and transportation costs. However, natural attenuation has limited potential and needs a longer time to degrade hydrocarbon. The article aims to outline the application and factors to consider in in-situ bioremediation of petroleum-contaminated soil.
Keywords: *biodegradation, column test, in situ bioremediation, petroleum, soil*

Abstrak
Minyak bumi masih menjadi salah satu sumber energi utama meskipun sudah banyak sumber energi lain yang ditemukan saat ini. Industri minyak bumi yang terus berkembang berbanding lurus dengan kontaminasi yang dapat ditimbulkan oleh industri ini. Bioremediasi merupakan teknologi ramah lingkungan dan hemat biaya yang akhir-akhir ini menjadi teknologi favorit dalam menangani limbah tanah yang terkontaminasi minyak bumi. Teknologi ini dapat dilakukan secara langsung di area yang tercemar, untuk meminimalisir biaya pengerukan dan transportasi. Namun, atenuasi alami memiliki potensi yang terbatas dan membutuhkan waktu yang lebih lama untuk mendegradasi hidrokarbon. Artikel ini bertujuan untuk menguraikan aplikasi dan faktor-faktor yang perlu dipertimbangkan dalam bioremediasi in-situ pada tanah yang terkontaminasi minyak bumi.
Kata Kunci: *biodegradasi, uji kolom, bioremediasi in-situ, minyak bumi, tanah*

1. Introduction

Petroleum is the primary energy with the highest consumption among other energy sources. Petroleum is processed in various stages before it is finally distributed to consumers. Every process of handling, processing, distribution, and management has the potential to contaminate the environment. Contamination can come from the E&P process, storage tank leaks, spills from pipelines, drilling sites, spills in the loading and unloading process, used oil spills, improper waste disposal practices, as well as landfill leaching, and the use of paving materials from hazardous waste [1], [2], [3], [4], [5], [6].

Petroleum-derived products exhibit diverse physical, chemical, and toxic properties, characterized by various refinement additives that result in different environmental impacts. The danger posed to nature using petroleum and its derivatives is indisputable. Hence, identifying the toxicity of specific petroleum components is crucial, as it allows for the recognition of potential ecological threats associated with the exploitation of these products [7]. Petroleum consists of various hazardous chemicals such as Benzene, Toluene, Ethylbenzene, and Xylene (BTEX), as well as PAH (Polycyclic Aromatic Hydrocarbon) which have been recognized as carcinogens and mutagenic substances [8].

Bioremediation has become one of the favorite treatments to remediate petroleum-contaminated soil. In the process, bioremediation uses bacteria that can utilize petroleum as a carbon source and break it down into simpler compounds [9], [10]. The products of bacterial mineralization are less toxic when compared to the products of chemical and physical remediation processes, hence the process of bioremediation is a safe option [11]. Bioremediation can be carried out in situ to prevent excavation and transportation to reduce costs and disruption to the site [12]. Naturally, in situ bioremediation takes a long time and has low degradation efficiency, so various efforts and engineering are carried out to improve the in-situ bioremediation process. This article aims to review various studies that discuss in situ bioremediation of oil-polluted soil.

Table 1. In Situ Bioremediation research for 10 years

Contaminants	Bioremediation Scale	Methods	Initial Concentrations	Final Concentrations	Literatures
Diesel oil and engine oil	Lab Scale	BS	20,000 mg/kg	10,912 mg/kg	[13]
Petroleum Hydrocarbon Leachate	Pilot Scale	BA	300 µg benzene/L	200 µg benzene/L	[14]
TCE Manufacturing Waste	Field Scale	BABS	24,500 µg TCE/L	µg TCE/L	[15]
Diesel oil	Lab Scale	BS	70,000 mg/kg	38,500 mg/kg	[16]
Gasoline	Pilot scale	BA	29,000 mg/kg	7,018 mg/kg	[17]
Diesel oil	Lab Scale	BS	75,000 mg/kg	22,500 mg/kg	[18]
Crude Oil	Pilot Scale	BS	28,000 mg/kg	2,240 mg/kg	[19]
Crude Oil	Microfield Scale	BABS	33,500 mg/kg	7,604.5 mg/kg	[20]
Crude Oil	Lab Scale	BS			[21]
Gasoline	Field Scale	BS	89 mg MBTE/L	44.856 mg MBTE/L	[22]
Crude Oil	Lab Scale	BA	5000 mg/kg	1,750 mg/Kg	[23]
Crude Oil from Mud Pit	Lab Scale	BS	32,200 mg/kg	1,500 mg/kg	[24]
Crude Oil	Micro	BABS	35,000 mg/kg	1,750 mg/kg	[25]
Refining Oil	Scale	BS	-	6% degraded	[26]
Diesel and Gasoline	Lab Scale	BS	1,133 mg/kg	504 mg/kg	[27]
Crude Oil	Pilot Scale	BS			[13]

2. Material and Methods

This review workflow is summarized in Figure 1. The journals used in this review were obtained from an online database in the form of Scopus, which is considered to have the largest journal database. Journals were searched using the following keywords "in situ bioremediation" OR "column test" AND "soil" AND "petroleum" OR "oil" OR "gasoline" OR "diesel". The results of the search obtained 304 journals which were then given a filter as a limitation of journals that could be used. Journals were limited to publications from 2013 to 2023 (last 10 years), using English, and in the form of articles and books which reduced the number of journals to 98. From the various filtering processes, 15 journals were used as references for this journal which are shown in **Table 1**.

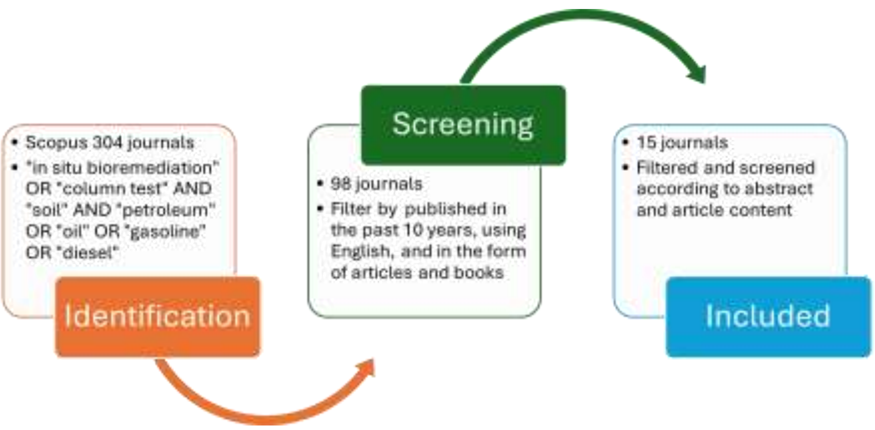

Figure 1. Literature Review Workflow

3. Source and Fate of Petroleum Contamination in Soil

Petroleum contamination of soil and the environment results from anthropogenic activities related to the petroleum industry. The process in this industry consists of various stages, each of which can potentially spread pollutants to the environment. The E&P process is one of a series of upstream stages in the petroleum industry. The initial product of the E&P process is crude oil which is naturally formed from fossils resulting from carbon and organic material deposits buried in the ground. From the refining process, crude oil can then produce various products such as petrol, diesel, and various petroleum products derived from distillation results so that the compound characteristics of each product are different [28]. This also causes

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each petroleum industry process to release different contaminant characteristics. Soils contaminated by crude oil have a larger density and low volatility, whereas petroleum product leachates tend to have high volatility due to their smaller density and carbon chains.

Petroleum can contaminate soil not only from the E&P process but also from upstream to downstream [26]. Figure 2 shows that PHs can also contaminate soil due to the distribution process. According to USEPA data, by 2023, 570,000 USTs were releasing hazardous materials with about 58,000 UST sites remaining to be cleaned up. The downstream process also plays a role where good disposal processing is required. In a case that occurred in Italy where bioremediation had to be carried out on a former landfill site that had high TPH values, this was thought to be due to accumulated leachate from hydrocarbon petroleum waste [14].

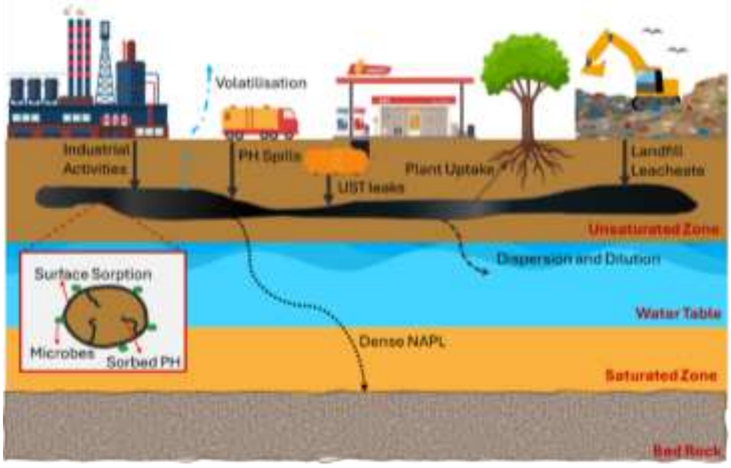

Figure 2 .Fate of Petroleum Hydrocarbon in Environment

Aforementioned, the use of fuel has the potential to cause environmental pollution. The greater the amount of its usage, the higher the potential for pollution. The negative impact is not only present at the site of contamination but will also spread and move. Petroleum that contaminates soil near the surface moves vertically upwards due to evaporation and downwards due to gravitational force and diffusion [29].

The contamination process in the unsaturated zone is complex, involving multiple phases: soil, water, air, and contaminants. In this layer, hydrocarbons experience several processes such as being dissolved in soil moisture, sorbed in soil particles, presenting as nonaqueous phase liquids (NAPLs), and forming an envelope of organic vapours. Light nonaqueous phase liquids (LNAPLs), which have a lower density than water, will migrate to the water table, accumulate, and float on the surface. Some compounds that are denser than water will sink to the bottom of the water table [30]. Residual phase contaminants are adhered to by microorganisms and taken up by plant roots due to their organic nature, resulting in the bioremediation of petroleum compounds.

Contaminants in soil are always in a dynamic state, interacting with soil particles or undergoing transformations until they move according to their energy, in the direction where their energy is balanced. In addition, the soil solution or groundwater can be lower. With the movement of the water mass, the contaminants in the water will also move. Advection-driven transport is a physical mechanism where water carries contaminants as it moves. In contrast, hydrodynamic dispersion involves the movement or mass transfer of contaminants driven by diffusion due to concentration gradients, leading to Brownian motion and dispersion mechanisms. This occurs because of the irregular flow paths of water through pores. Retardation refers to the phenomenon where the amount of contaminants changes during transport due to interactions with the soil medium, which effectively slows down the contaminant movement. Changes in solute concentration in the liquid phase can result from sorption-desorption processes and transformation phenomena. Sorption involves changes in contaminant amounts due to interactions with soil particles, while transformation involves changes due to chemical reactions.

These reactions can be abiotic, involving reactions with other substances, or biotic, involving biodegradation processes. Both abiotic and biotic transformations play crucial roles in reducing contaminant concentrations in groundwater. [29]. The behavior and interaction of pollutants with soil comprise various physical, chemical, and biological processes that take place in all three main groups of processes such as retention on and within the soil body, infiltration, diffusion, and transport by soil solution, and alteration, transformation, and initiation of chemical changes within the soil [31].

4. Petroleum Contamination Effects on Environment

Oil phytotoxicity is caused by multiple processes, including (1) disruption of plant-plant relations; (2) modifications in plant metabolism; (3) toxicity of plant cells; and (4) decreased exchange of oxygen with the environment. Certain impacts, which happen as soon as the oil encounters the plants, are more serious and noticeable right away, while others take longer to manifest and give the plants time to adapt to the pollution. da Silva Correa et al., 2022 mention that the immediate effects of oil reach the plants via root and leaf stem. The presence of petroleum hydrocarbons can result in SEM analysis showing physical changes in soil contaminated by hydrocarbons in the form of crude oil with a weight ratio of 1:5. Hydrocarbons cause the previously agglomerated natural soil structure to become compacted due to van der Waals forces and hydrogen bonding [33]. Soil agglomeration inhibits the distribution of nutrients, water, and oxygen diffusion in the soil. Oil will stick to the root surface, erode the root structure, and have an impact on the water and nutrients absorbed by the root system, which leads to the plant dying from a shortage of both [34]. The content of petroleum hydrocarbons in the soil can reduce seed germination and nutrient translocation, induce oxidative stress, disrupt plant metabolic activities, and inhibit plant physiology and morphology which ultimately reduces crop yield [35].

5. Petroleum Contamination Effect on Human

Hydrocarbon compounds are hazardous compounds. The dominant compounds in petroleum hydrocarbons such as BTEX and PAHs can potentially be carcinogenic. PAHs are volatile compounds and at short periods and high doses can cause eye irritation, nausea, diarrhoea, and confusion. Chronic diseases can also be caused by PAH exposure such as decreased immune function, cataracts, lung function abnormalities, and skin inflammation. Specific to naphthalene, inhalation, and ingesting large amounts can cause red blood cell damage [36].

BTEX consists of benzene, toluene, ethylene, and xylene which are known to be hazardous compounds. Benzene is the most abundant compound in BTEX which is classified as a carcinogen and haematotoxic by the USEPA. Prolonged exposure to benzene can cause leukaemia and aplastic anaemia in humans [37].

6. Contamination on Soil

Various technologies are used to reduce the toxicity of petroleum hydrocarbon compounds. Physical, chemical, and biological processes have been used to treat PHs-containing waste, but biological processes are considered to have various advantages such as simple process, lower cost, and safer product. Soil naturally contains bacteria that can break down hydrocarbon compounds into simpler and less toxic compounds for the environment. The natural bioremediation process that occurs is referred to as natural attenuation, which often takes a long time due to several problems that can be experienced in natural attenuation, namely the low number and ability of hydrocarbon-degrading bacteria, lack of nutrients and electron acceptors, and low bioacceptability. A supportive environment increases the removal of PHs compounds in the soil, this can be done by providing a setting in the form of an environment that supports microorganisms in degrading PHs. Several enhanced bioremediation processes are carried out in removing PHs in soil in situ.

6.1 Biostimulation

Biostimulation is the most successful and efficient bioremediation method compared to other in situ remediation in simulated soils contaminated with petroleum hydrocarbons. Biostimulation can be carried out on land that has microorganisms that are appropriate for bioremediation but have inadequate land characteristics so that stimulation is carried out for the growth of microorganisms. One of the most common stimulations is by adding nutrients to contaminated soil.

In-situ bioremediation conducted on a lab scale using a column test and with the addition of nutrients at a ratio of 100:10:1 can degrade 7% (w/w) diesel-contaminated soil by 45.4% in 60 days [16]. In-situ bioremediation was also conducted at a gas station in Israel on gasoline-contaminated soil by adding nutrients and aeration using CaO_2 [22]. Using bio barriers, Casiraghi et al., 2022 have successfully remediated hydrocarbon leachate-contaminated land with high PHs (Petroleum Hydrocarbons) and CAHs (Chlorinated Aromatic Hydrocarbons) in Italy. The bioremediation process takes place aerobically and anaerobically with the addition of nitrogen and phosphate. The aerobic process is achieved by sparging water, and the anaerobic process is done by adding an electron acceptor in the form of sulfate. The biostimulation process is also carried out by adding substrates such as molasses, cheese whey, and soy oil.

6.2 Bioaugmentation

This process involves the addition of exogenous microbial cultures, native microbes, communities or genetically engineered microbes with specific catabolic activities that have adapted and proven to degrade contaminants to enhance degradation or increase degradation rates [1], [38]. Significant progress has been made in identifying bacterial strains capable of degrading petroleum hydrocarbons in contaminated soil. These bacteria employ various mechanisms to reduce Total Petroleum Hydrocarbon levels, including the production of biosurfactants. Biosurfactants enhance the bioavailability of contaminants, a topic further explored in the biosurfactant subsection. For instance, *Pseudomonas aeruginosa* ZS1, a bacterial isolate, produces rhamnolipids, a type of biosurfactant. *Rhamnolipids* effectively emulsify crude oil, increasing its accessibility for microbial uptake and degradation. This characteristic makes P. *aeruginosa* ZS1 a promising candidate for bioremediation of petroleum pollutants, particularly in environments like the Zhoushan coastal areas [39].

60.6-86.6% of petroleum contamination can be removed within two months in a gas station In China, the isolate used was an indigenous bacterium identified as Rhodococcus sp. OBD-3 [40]. A field-scale in situ bioremediation has been carried out on the former PHC storage terminal using the bioaugmentation method, which on a pilot scale shows that using biostimulation does not get maximum results, so a commercial organohalide bacterial consortium is added which in 3 months has reduced to more than 85% [15]. Not only bacteria, but fungi are also commonly used in enhancing petroleum bioremediation. Covino et al., 2015 also demonstrated bioaugmentation using fungi native to petroleum hydrocarbon-contaminated soil to degrade clay contaminated with petroleum hydrocarbons and achieved a removal efficiency of 79.7% after 60 days.

6.3 Factors Affecting Petroleum Bioremediation

Numerous studies have investigated the bioremediation of hydrocarbon-contaminated soil. Researchers have demonstrated that using single strains or native bacterial consortia in laboratory settings effectively degrades hydrocarbons. However, this efficacy often does not translate to field conditions due to various factors influencing biodegradation efficiency and rate. Key factors include microbial characteristics (such as microbial consortium composition, metabolic potential, population density, biosurfactant production, and competition), the physicochemical properties of contaminants (including chemical structure, concentration, toxicity, and bioavailability), and environmental conditions (such as soil type, temperature, pH, oxygen levels, salinity, nutrients, and water availability). These factors impact microbial activity, enzyme activity involved in degradation, and overall hydrocarbon breakdown. This suggests that bacterial bioremediation can be more effective if these influencing factors are carefully manipulated, optimized, and controlled.

Contaminant

This journal discusses one type of contaminant commonly found in soil in the form of petroleum, specifically. petroleum consists of various types of complex hydrocarbons. so that each type of compound has its unique characteristics that also affect the degradation process. Gasoline, a ubiquitous fuel, is a complex mixture composed of petroleum hydrocarbons and highly hazardous additive compounds. Jet fuel consists of over 300 hydrocarbon compounds. Creosote is made up of 200 chemicals, of which 85% are polycyclic aromatic hydrocarbons (PAHs), 12% are phenolic compounds, and the remaining are heterocyclic compounds [42]. Petroleum essentially consists of several groups of hydrocarbon compounds. The hydrocarbon group includes saturated hydrocarbons, which are further divided into n-alkanes (straight chain), isoparaffins (branched chain), and cycloparaffins. The unsaturated hydrocarbon group is divided into monocyclic, bicyclic, polycyclic aromatic hydrocarbons, and naphthenoaromatic hydrocarbons. Each group of compounds has its level of degradability, ranging from the easiest to degrade to the most difficult: straight chain paraffins, branched paraffins, cycloalkanes, naphthene aromatics, hydrocarbons, and aromatic hydrocarbons [43].

As a source of energy for microorganisms or plants used in bioremediation, the factor or influence of contaminants on the bioremediation process is very important. The influence can be in the form of type, concentration, co-contaminant, and age of contamination. Guirado et al., 2021 showed that soils freshly contaminated by diesel fuel have greater degradation efficiency when compared to soils that have long been polluted by spilt tanks. Similarly, soils from oil production fields as historical-polluted soil had lower TPH reduction when compared to newly oil-added soils at 24.67% and 51.84% respectively [45].

Salinity

It has been previously discussed that petroleum exploration and production (E&P) processes contribute to environmental pollution. The soil contamination around production sites contains high concentrations of oil and salt. Previous research has shown that although some microbes, known as halophiles, require salt for their growth, the general relationship between microbial growth and salinity is inversely proportional [46], [47]. Salinity can alter the microbial community in the soil through the effects of increased osmotic pressure and the accumulation of harmful ions. In addition, salt content can alter the physicochemical properties of contaminants, limiting bioavailability and the ability of microorganisms to utilize substrates, in this case, hydrocarbons. However, various studies have shown that salt content in soil can inhibit hydrocarbon biodegradation. Bioremediation of 1% NaCl addition to crude oil-contaminated soil compared to non-saline conditions resulted in TPH degradation of 19% and 67% [48]. An increase in salt content inversely correlates with biological activity, leading to a decrease in the biodegradability of petroleum contaminants. A study demonstrated that the addition of a bacterial consortium can enhance the biodegradation of petroleum hydrocarbons in saline-alkaline soil [49].

Overcoming saline soil conditions can be done with bioadmendment in the form of biochar and residual mushroom compost. Biochar is effective in reducing PAHs in soil, while residual mushroom compost is more effective in reducing alkene compounds [48]. Bioadmendments can reduce soil salinization while increasing water holding capacity and high salt adsorption ability [50], [51].

Biosurfactant

Surfactants are molecules with dual characteristics, featuring a hydrophobic tail and a hydrophilic head. They gather at the interface of different liquid phases, like oil/water or air/water, and at increased concentrations, they organize into micelles. This critical concentration threshold for micelle formation is known as the critical micelle concentration (CMC). Micelles play a crucial role in reducing surface tension, which is the resistance of a liquid to external forces across phases. Consequently, surfactants are employed to cleanse oil from water or soil. While they can be synthesized chemically or biologically, excessive use of chemical surfactants poses environmental risks [52]. Biosurfactants, produced by various microorganisms including bacteria, fungi, and yeast, emerge as secondary metabolites either externally secreted or adhered to cell surfaces. Offering a more environmentally friendly alternative, biosurfactants boast characteristics such as lower toxicity, biodegradability, enhanced foaming ability, and lower CMC compared to chemical counterparts. These attributes render them valuable across numerous sectors like bioremediation, healthcare, cosmetics, food, and oil industries. Beyond their surfactant role, biosurfactants also contribute to cellular communication [53].

Biosurfactants are metabolites produced by microbes, for this reason, microbial sources can have an important effect on their characteristics. Biosurfactants are not only influenced by the origin of microorganisms but also by various factors such as their chemical composition, molecular weight, physicochemical traits, and mode of operation. Regarding molecular weight, they are classified into two groups: low-molecular-weight biosurfactants, which include glycolipids, phospholipids, and lipopeptides, and high-molecular-weight biosurfactants, comprising amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins, or complex combinations of these biopolymers. Low-molecular-weight biosurfactants excel in reducing surface and interfacial tensions, while high-molecular-weight ones are more adept at stabilizing oil-in-water emulsions [54]. In the previous study, there was an increase in TPH degradation after biosurfactant production by *Burkholderia sp*. and assisted by the addition of 32.6 C/N nutrients resulting in 186.48 mg/kg per day of macro alkene with an increase of 10.59-46.71% [55].

Lipoptide is a type of biosurfactant which is a secondary metabolite of bacteria produced by bacteria of the genus *Bacillus*. Up to 59% of TPH was reduced from soil under conditions of salinity (0-8%), pH (6-9), and temperature (15-45^{o} C) using *Bacillus sp*. Z-13 bacteria, which were isolated from petroleum-contaminated soil [56]. As a simulation of the use of lipopeptide biosurfactants in in situ bioremediation, a lab-scale study was conducted in a leaching column test using the Bacillus genus that has been improvised into mutant 1-24 which degraded 45.44% of TPH [13].

Nitrogen Intake

Nitrogen and phosphorus are the most important inorganic nutrients required for microbial growth. Some soil environments may be deficient in nitrogen and/or phosphorus, and several studies have shown an increase in hydrocarbon biodegradation due to macronutrient addition, whereas others have shown little change. The success of nutrient addition will depend on soil composition and the presence of nitrogen-fixing bacteria [57]. Various studies have been conducted to support deeper information about nutritional factors on bacterial activity in the hydrocarbon remediation process. The results of the flourency intensity analysis indicated that the ratio of C:N:P can be useful to determine the right level of nutrients for bacteria. The ratio of 100:5:2 can effectively reduce TPH higher when compared to the ratio of 100:5:1 [58].

In general, ammonium chloride is added to achieve a more optimum C:N ratio for bioremediation but according to the results of research conducted by Liu et al., 2023 that the addition of KNO3 can improve petroleum biomineralization compared to NH_4Cl. W. Wang et al., 2023 have specifically examined the compounds used to provide nutrients to soil polluted by diesel. Peptone, with a carbon-to-nitrogen (C:N) ratio of 20:1, offers more effective remediation results compared to potassium nitrate (KNO_3). This is because peptone aids in the conversion of nitrogen into amino acids, whereas KNO_3 is prone to rapid denitrification.

Humidity

Moisture content is a crucial variable in the field of bioremediation, a process that utilizes microorganisms to degrade or remove contaminants from the environment. The moisture content of soil affects various aspects of bioremediation, including the availability of contaminants, the transfer of gases, the effective toxicity level of contaminants, the movement and growth of microorganisms, and the distribution of microbial species. Low moisture levels can restrict bacterial activity by limiting cellular movement and metabolism reactions [42]. This can significantly impact the competitive advantage between bacteria and fungi in the soil, as fungi are generally more drought-tolerant than bacteria due to their ability to transfer moisture from water-filled micropores to drained pores, whereas bacteria require water films for motility and substrate diffusion [29].

High water content can increase the removal of oil content in the soil and increase the growth of oil-degrading microorganisms. The addition of 15% water to contaminated soil removed 18% of the TPH in the soil whereas the sample without water addition was only 3% [60]. However, too high water content can also reduce the oxygen diffusion coefficient which reduces oil degradation in soil where soil that has 30% moisture content results in a 42% reduction in TPH while excess moisture content results in 28% only [61]. in contaminated soil, especially for the in-situ bioremediation process has a slight difference from the ex-situ process. In the ex situ process, moisture content can be homogenized but in the in situ process there are differences in water content at each depth [16], [62].

Aeration/Oxygen Availability

Hydrocarbon-degrading (oleophilic) bacteria can breathe in the presence of oxygen (aerobic) and in the absence of oxygen (aerobic or facultative). The availability of molecular oxygen in soil is the limiting factor for aerobic bioremediation and acts as the final electron acceptor and chemical reactant (oxygenase reaction enzyme) for terminal, subterminal, and biterminal oxidation and aromatic ring cleavage of hydrocarbon contaminants. Studies show that without considering the total potential biomass of hydrocarbon-degrading bacteria, 3.1 mg/ml of oxygen is required for the degradation of 1 mg/ml of hydrocarbon contaminants, and 10-40% oxygen levels are required for hydrocarbon biodegradation. Therefore, aerobic catabolism results in higher biodegradation rates than anaerobic metabolism.

Reports show that the use of isolated bacterial strains degraded 20-25% of the total amount of oil under aerobic conditions within 10 days, but the same strain took 50 days under anaerobic conditions to degrade 15-18% of the total petroleum present and there was no or little degradation of hydrocarbon pollutants in anoxic soil areas. However, the low level of oxygen in hydrocarbon-contaminated sites inhibits the aerobic degradation route and results in low removal efficacy. Factors resulting in slow biodegradation and depletion of free oxygen in soil include oxygen concentration, bacterial oxygen consumption rate, physical and chemical properties of the contaminated soil (type and porosity), and the presence of usable substrate or accumulated organic matter. Therefore, the availability of oxygen in the soil is important to have effective biodegradation [63].

Technologies to accelerate naturally occurring in situ bioremediation include biosparging, bioventing, and the use of oxygen-releasing agents such as pure oxygen, hydrogen peroxide, ozone, and commercial oxygen-releasing compounds. These technologies work by providing an additional supply of oxygen to the subsurface, which is then available to aerobic bacteria. Most enhanced aerobic bioremediation technologies primarily address contaminants dissolved in groundwater or adsorbed to soil particles in the saturated zone. Enhanced aerobic bioremediation technologies have typically been used outside the source area [64].

The bio-barrier technology used by Casiraghi et al., 2022 aerobically proved to be capable of remediating soil contaminated with hydrocarbon leaching from landfills at microcosms and pilot scale.

Oxygen provision was carried out by air sparging method with the rate of 1600 L/h. The aerobic condition was also carried out in Moshkovich et al., 2017 by using O_2 slow-release compounds (CaO_2).

Soil Characteristics

Different soil types can have different effects on hydrocarbon degradation processes. An et al., 2023 examined the behaviour of oil contaminants in vadose soils, where in their journal it was found that the finer the soil particles, the less contaminant transport but the more efficient the natural degradation. Sandy loam soils have a higher petroleum degradation rate when compared to sandy and loamy sand types. This is supported by research conducted by Sun et al., 2023 which shows that the biodegradation of petroleum volatile compounds is in line with the silt content of the soil.

Permeability or soil conductivity is the ability of soil to drain water, which will affect the transportation of contaminants, nutrients, and bacteria. Permeability can decrease during the contamination process of the soil, and this affects the activity of bacteria to degrade hydrocarbons. In aerobic processes, permeability also plays a role in diffusing oxygen. Low permeability hinders bacterial activity and stimulates anaerobic zones in the soil that slow down contaminant degradation [67]. Unlike ex-situ bioremediation, in situ bioremediation relies on specific site characteristics to achieve effective remediation. According to Anderson, 1995 one of the key factors for successful in situ bioremediation of contaminated soils is the hydraulic conductivity or soil permeability. In saturated zones, matrices with hydraulic conductivity greater than 10^{-4} cm/sec are preferred for liquid delivery methods. For unsaturated layers, matrices with hydraulic conductivity ranging from 10^{-5} to 10^{-6} cm/sec are effectively treated with bioventing, which delivers more air per unit volume than liquid. However, these fluid-flow values for treatability are not fixed; higher levels of contamination impose greater limitations on fluid-flow properties.

Microorganisms

Microorganisms play an important role in the remediation of soil contaminated with petroleum hydrocarbons. Heterotrophic bacteria use hydrocarbons as a carbon source so that they can break the complex carbon chain of petroleum into simpler and safer compounds. Factors considered in selecting hydrocarbon-degrading bacteria are type, single strain or consortium, number, and interaction between bacteria.

Many types of microorganisms can be used in oily soil bioremediation. several bacterial genera can successfully reduce petroleum hydrocarbon compounds under extreme conditions. four bacterial strains from the genera *Pseudomonas*, *Rhodococcus*, *Arthrobacter*, and *Sphingomonas* were shown to degrade petroleum n-alkanes at temperatures of -1.5°C to 35°C in the presence of 0-8% NaCl (w/v). That research conducted by Semenova et al., 2023 has the potential to be used for bioaugmentation of polar regions contaminated by petroleum. the incident of collapsed fuel containers at a power plant located in the Siberian city of Norilsk in June 2020 which accidentally spilled 15000 tonnes into the river and 600 tonnes into the ground in the Siberian city of Russia is one of the causes of the Arctic Circle area being polluted with petroleum [70].

The microorganisms used can be bacteria or fungi. Spent Mushroom Compost (SMC) is a waste compost residue obtained from the mushroom industry which is a consortium of fungi and bacteria. SMC *Agaricomycetes* has been studied to decontaminate soil containing petroleum pollutants. The results showed that SMC *Agaricomycetes* can reduce 64.7% of TPH in three months [71]. A mixture of microorganisms between fungi and bacteria was also studied by Nwankwegu and Onwosi, 2017 The bacteria used were a single strain of *Micrococcus luteus* (Mb) and the fungus *Rhizopus arrhizus* (Rb) The waste used was soil from mangrove forests which was added with 150 mL of gasoline with a result of 66%, but by using bacteria or fungi alone a greater reduction result was obtained, namely 75.7% and 71.1% respectively. These results can be influenced by bacterial interactions, where in Kebede et al., 2021, hydrocarbon-degrading bacterial communities exist in cooperation (with synergistic effects) and/or competition (with antagonistic effects) for their survival in hydrocarbon-contaminated environments.

However, bacterial composition either interspecific (between bacteria and fungi) or intraspecific (between bacterial species themselves) is a limiting factor for biodegradation efficacy. For example, for interspecific competition: hydrocarbon-degrading fungi and hydrocarbon-metabolizing bacteria may compete to utilize hydrocarbon contaminants as a source of carbon and other limited available nutrients (nitrogen and phosphorus) for their growth and metabolism. In addition, some hydrocarbon-degrading microbial species also release metabolites that inhibit the growth and development of other hydrocarbon-degrading bacterial species.

Studies show that exogenous bacteria (introduced inocula) are used to degrade hydrocarbon contaminants but are usually ineffective. This is because they cannot avoid competition with native bacteria, predators, and various abiotic factors. Some single strains are also proven to degrade hydrocarbon-contaminated soil such as *Proteus mirabilis* SB [72], a study conducted by Xu et al., 2023 reported that *Burkholderia* sp. can activate the performance of indigenous bacteria in degrading macro alkenes. The synergy of the bacterial community is the reason for accelerating the remediation of hydrocarbon-contaminated soil.

Co-Contaminant

There have been numerous successful cases of bioremediation of petroleum-contaminated soil, focusing on its hydrocarbon content. However, an important fact that is often overlooked is that petroleum hydrocarbon-contaminated soil frequently contains associated contaminants. Lead (Pb) is commonly present with total petroleum hydrocarbons (TPH) as a co-contaminant in numerous historical oil spills, owing to its extensive use as a fuel additive [73]. A study was conducted to assess the effectiveness of bioremediating TPH in soils co-contaminated with TPH and Pb, as well as to evaluate the subsequent impact on soil ecotoxicity. The research of Leadin S. Khudur et al., 2019 demonstrated that Pb significantly affects the remediation process, with biostimulation of Pb-contaminated soil resulting in only an 8% reduction in soil toxicity, compared to a 64% reduction in soils contaminated solely with TPH. In another study also found that petroleum contamination elevated the levels of cadmium, lead, nickel, and copper in the soil [75].

Co-contaminants are one of the factors that affect the nature of pollutants. Heavy metals in areas polluted by fuel oil can alter the local microorganism community without changing its composition in the soil. Each metal has a different effect on the microorganism community but in general, heavy metal stress has an effect on soil enzyme activity and weakens degrading bacteria. Mn, Se, Cd, and Sn have more effect on microbial functional genes, compared with Zn, Pb, and As [76].

7. Considerations for In Situ Bioremediation

Excavation and removal of contaminated soil can be disruptive and costly, leading to increased interest in cost-effective and non-invasive methods like in-situ bioremediation for treating oil-contaminated sites. The selection of designs and processes for in-situ bioremediation is influenced by factors such as regulations, site-specific geology, hydrogeology, geochemistry, and biogeochemistry. Early consideration of regulatory concerns is crucial, as ignoring them can result in resistance or rejection of treatment plans. While some countries have clear guidelines for permissible levels of petroleum hydrocarbons—which facilitates treatment approval—others lack specific regulations, necessitating extensive negotiation and collaboration with various stakeholders [68].

Key technical considerations in designing an in-situ bioremediation system include hydraulic control to isolate and direct contaminated groundwater, and clogging control to address issues like biofouling and chemical clogging. Selecting an appropriate oxygen source is vital; options range from air sparging and hydrogen peroxide injection to using oxygen-releasing compounds like magnesium peroxide. Off-gas treatment methods such as activated carbon adsorption, catalytic oxidation, and biofilters are employed to manage exhaust gases from the bioremediation process. Clean-up time is affected by factors like treatment area, chemical delivery rate, and site heterogeneity. Effective site monitoring—using strategically placed wells and automated systems—is essential for assessing environmental impact, tracking performance, and documenting progress during the remediation process [42].

8. Conclusion

There are a lot of factors that enhance in situ bioremediation of petroleum-contaminated soil. In a decade, researchers have used biostimulation or combined it with bioaugmentation to enhance the bioremediation of petroleum-contaminated soil. Better biodegradation is stimulated by setting the nutrients, and humidity, in addition to promoting the aerated process and biosurfactant amendment. But in some cases, the number of microorganisms is not enough to remediate contamination in soil, therefore, exogenous bacteria addition is operated in soil bioremediation. Process bioaugmentation-biostimulation can be applied to sites with insufficient conditions for bacterial growth. Providing in situ bioremediation needs a lot of considerations such as regulations, hydraulic control, clogging control, selection of the oxygen source, off-gas treatment, clean-up time, and site monitoring.

9. Abbreviations

Abbreviation	Definition
BA	Bioaugmentation
BABS	Bioaugmentation-Biostimulation
BS	Biostimulation
BTEX	Benzene, Toluene, Ethylbenzene, and xylene
CAHs	Chlorinated Aromatic Hydrocarbons
CMC	Critical Micelle Concentration
E&P	Exploration and Production
PAHs	Polycyclic Aromatic Hydrocarbons
pH	Potential of Hydrogen
PHs	Petroleum Hydrocarbons
SMC	Spent Mushroom Compost
TPH	Total Petroleum Hydrocarbon

10. References

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