



## A Global Systematic Review of Factors Affecting the Biological Treatment of Wastewater Containing Petroleum Substances

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### ABSTRACT

**Introduction:** A variety of treatment methods, including biological remediation, have been employed to address oil-contaminated wastewater. Bioremediation, which involves using microorganisms to mitigate or eliminate pollutants, is recognized as an environmentally friendly, cost-effective, and evolving technique for removing and breaking down various environmental contaminants, including those from oil industry.

**Materials and Methods:** This systematic review not only introduces biological treatment but also explores factors contributing to its success. In this study, a search was performed with keywords including petroleum substances, bioremediation, and biological treatment on Scopus, ScienceDirect, Web of Science, and PubMed, and 1349 studies were obtained, and 61 articles were finally chosen according to exclusion and inclusion criteria.

**Results:** A significant increase was observed in research articles over the past five years, likely reflecting the growing awareness of the need to remediate petroleum pollution in recent years. The nature of petroleum wastewater varies depending on the specific crude oil refining process, and factors that have the greatest effect on biological treatment include temperature, pH, inhibitors, time, oxygen, nutrients, nature, concentration of pollutants, and microorganism type. No single species of microorganism can break down all petroleum compounds.

**Conclusion:** This study allows decision-makers to evaluate these factors before implementing and investing in this method, ensuring its effectiveness in reducing petroleum pollution concentrations.

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### Introduction

Historically, industrialized nations have been the largest consumers of oil. However, oil consumption in developing countries is projected to increase and eventually match that of industrialized countries, with a significant portion of this growth likely occurring in the transportation sector. As a result, crude oil continues to play a vital role in the fossil fuel energy mix<sup>1</sup>. The

production of excess water is an inherent aspect of crude oil production and initial processing operations<sup>2</sup>. The volume of saline water produced alongside crude oil in desalting units is significant, and this wastewater poses a major environmental challenge for areas surrounding oil facilities due to its distinctive characteristics, such as high dissolved salt content, the presence of oil, volatile and non-volatile organic compounds, and other

environmentally hazardous pollutants<sup>3</sup>. Moreover, the global increase in the volume of this wastewater over the last decade has raised additional concerns. Oil-contaminated water makes up about 77 percent of the total wastewater generated by oil and gas industries, containing a variety of complex and toxic organic and inorganic substances, as well as heavy metals, and falls short of the standards required for safe environmental discharge. It is estimated that, on average, each barrel of crude oil production requires between 0.8 and 8 barrels of water.

Refineries are among the most water-intensive industrial facilities, and their produced wastewater contains some of the highest levels of pollution; many refineries are located near major urban centers. In 2008, there were 700 refineries operating in the world, including 47 located in Asia and the Pacific with a capacity of 371 MMt/y. Now, in 2024, there are 825 crude oil refineries operating worldwide, and this number is expected to grow by 181 units between 2024 and 2030. Through various processes such as desalting, distillation, thermal cracking, and catalytic cracking, refineries generate significant volumes of wastewater while producing valuable products from crude oil. These processes require substantial amounts of water, typically consuming between 0.4 and 1.6 times the amount of crude oil processed. This inevitably leads to an increase in wastewater output<sup>4</sup>. Treating oily wastewater from refineries is a critical step in the oil extraction process, with significant implications for environmental health and water usage. Therefore, it is essential that wastewater from the oil industry is treated effectively to prevent any harm to living organisms<sup>5</sup>.

A significant cause for concern in recent years is the presence of polycyclic aromatic hydrocarbons (PAHs) like anthracene, phenanthrene, benzo[a]pyrene, and benzo[a]anthracene in oily wastewater. Some of these compounds are persistent in the environment and have potential carcinogenic properties<sup>6</sup>. Oil pollution and its derivatives are among the most prevalent and widespread environmental contaminants, posing

serious risks to human health<sup>7</sup>. The existence of these pollutants in the environment not only adversely affects the local ecosystem but also infiltrates the food chain over time, eventually posing a threat to human health<sup>8</sup>.

Oil pollution can be addressed through various physical, chemical, and biological remediation methods, each with its own set of pros and cons. Physical methods simply shift the pollution from one phase to another, making them effective only during the initial stages of treatment. Biological methods, while low in efficiency, are cost-effective, whereas chemical methods, though highly efficient and fast-acting, tend to be expensive<sup>9</sup>. Some chemical methods are known to alter the environment's natural state, and they are sometimes criticized for their harmful side effects on ecosystems, which can be even more severe than oil pollution itself. Given the issues linked to physical and chemical methods, there is a growing need for safer and more affordable approaches to degrading environmental pollutants. Effective bioremediation strategies continue to stand out as a viable solution<sup>7, 10</sup>. Additionally, biological methods are generally more cost-effective and efficient compared to physical and chemical alternatives<sup>11</sup>. Biological treatment of waste from the oil, gas, and petrochemical industries is considered the safest and most environmentally friendly method for waste elimination. In contrast to chemical methods, which often produce additional waste, biological methods utilize microbes that naturally decompose once their task is complete. Moreover, these methods require much less energy<sup>12</sup>.

The oil industry is a key and vital sector, playing an essential role in the national economy of any country while supporting various other sectors such as agriculture, energy, and transportation. Currently, there is a heightened focus on techniques for treating oily wastewater, making its treatment an urgent issue that needs to be addressed and resolved at every oil field and by companies operating in oil-producing regions. Due to the high volume of production of wastewater containing petroleum substances and their

dangerous environmental effects, the need for its treatment becomes essential. In the meantime, biological treatment can be very useful based on its advantages; some of them were mentioned above. Therefore, this study discusses factors affecting the biodegradability of wastewater containing petroleum substances based on previously published research. It is necessary to consider all the effective factors when using this type of treatment so as not to lead to failure and to achieve the highest efficiency.

**Materials and Method**

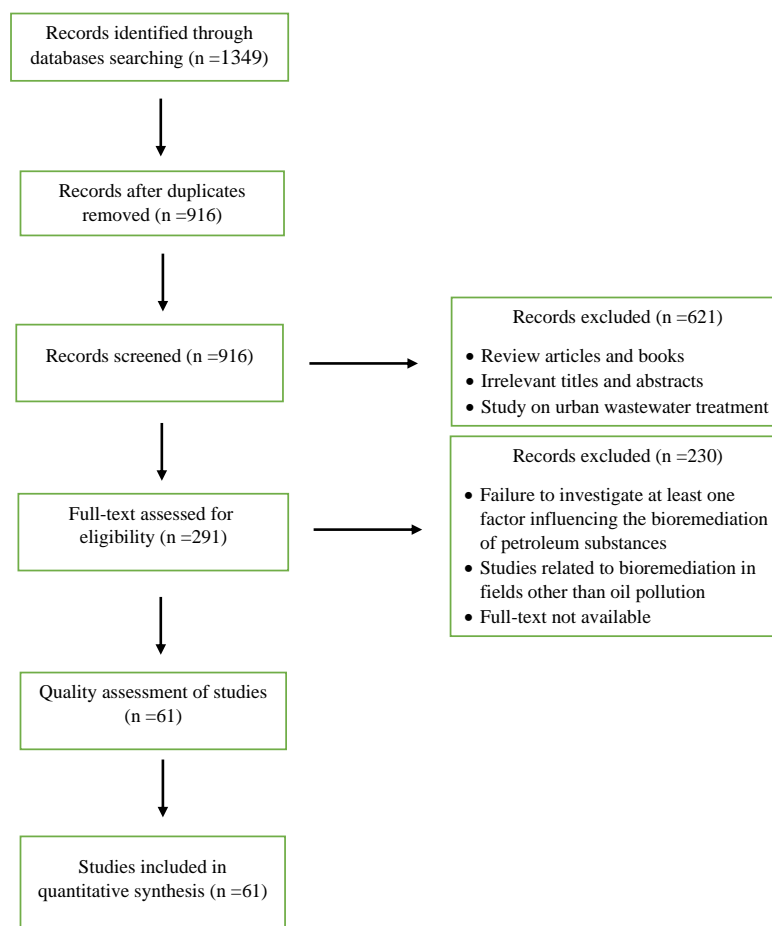
**Search strategy**

To conduct this systematic review, keywords including petroleum substances, bioremediation, and biological treatment were selected. Documents from Scopus, ScienceDirect, Web of Science, and PubMed were reviewed without any restriction on publication date. The search for documents was

conducted in July 2024 using the selected keywords. This step was carried out by two authors.

**Inclusion and exclusion criteria**

Many search results were duplicates or not relevant for the review and were removed. The inclusion criteria for this study required that the research address at least one effective factor in the bioremediation of petroleum substances. Only research studies, whether field or laboratory, were considered, and review studies were excluded. Studies that did not focus on biological processes as well as those related to bioremediation in contexts other than petroleum pollution were also excluded. Furthermore, studies that only examined the biological treatment of municipal or hospital wastewater were omitted from this systematic review. The study path can be seen in Figure 1.



**Figure 1:** The study diagram.

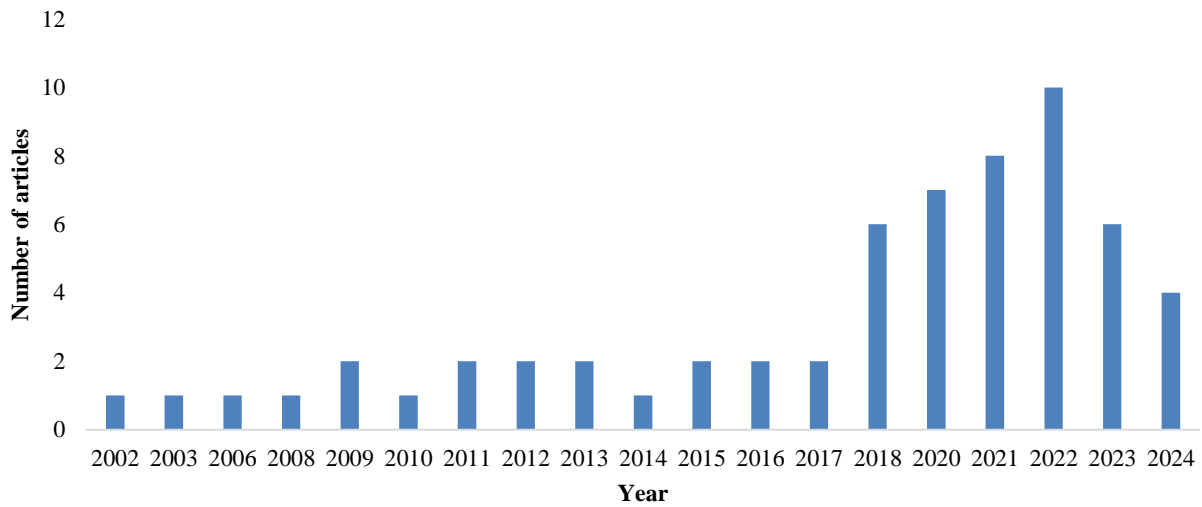
**Results**

**The result of search**

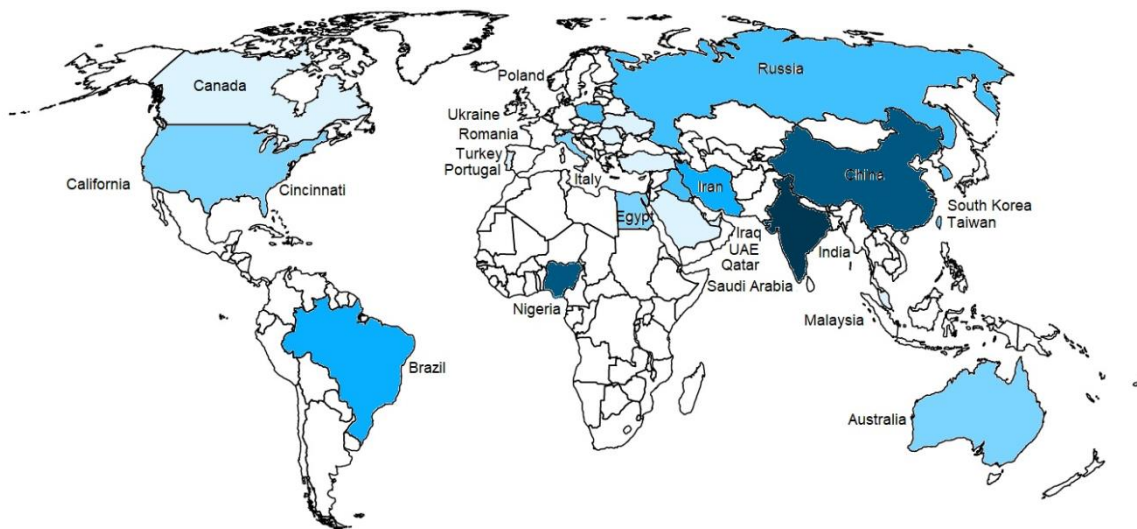
A total of 1,349 articles were retrieved from the databases. Duplicate articles were removed using Endnote software. An initial screening was performed, during which the titles and abstracts of the manuscripts were reviewed in accordance with the inclusion and exclusion criteria. After this screening, 291 articles were selected for a full review, and ultimately, 61 studies were chosen for inclusion.

Figure 2 illustrates the distribution of reviewed

and selected articles by publication year, highlighting a notable increase in research articles over the past five years. This trend likely reflects the growing awareness of the need for petroleum pollution remediation in recent years. Figure 3 shows a map of published studies selected in this study. According to this figure, most studies were related to 3 countries: Nigeria, India, and China. Nigeria is the largest oil producer in Africa, and China and India are among the largest oil producers in Asia.



**Figure 2:** Published articles on the bioremediation of petroleum pollution using microorganisms.



**Figure 3:** Map of the published studies.

The results from reviewing 61 articles are summarized, categorized, and detailed in the following sections:

### **Sources of Wastewater Contaminated by Oil**

Global production of crude oil reaches approximately 60 million barrels per day<sup>13</sup>. Oil-derived compounds are prevalent in wastewater of many industries, such as metalworking, food production, transportation, textiles, leather, oil and gas extraction, petrochemical, and refining sectors. A significant portion of oily wastewater released into the environment comes from petrochemical and refining processes, which are key contributors to environmental pollution. Substantial amounts of water are utilized in oil refining activities, including distillation, water treatment, desalination, and cooling. The volume and nature of wastewater produced are determined by specific processes used in each refinery<sup>14</sup>.

Wastewater in refineries is typically generated at various stages and in different sections, including:

- The introduction of barium sulfate, iron oxide, and sodium silicate into wastewater during the slurry process for oil well casing, which is intended to reinforce well structure.
- Crude oil enters surrounding waters during the extraction phase.
- The release of chemicals such as hydrogen sulfide, phenol, ammonia, cyanides, and copper acetate during oil processing in refineries.
- The entry of colloidal particles, detergents, and other substances into wastewater during cleaning of platforms and refinery areas, which introduce various organic and inorganic materials.

Furthermore, storage tanks, pipelines, and petroleum transportation networks are significant sources of organic pollutants and petroleum compounds entering the environment, especially water bodies, due to leaks or accidental incidents. Effective management of these pollutants following a spill requires identifying specific contaminants and tracing the source of pollution, such as the leak location. A major source of low-level oil pollution in urban runoff is the use of petroleum-based materials on roads and during

dust control operations<sup>15</sup>. Oil pollution can also stem from tanker accidents, bombings, oil spills and derivatives, human negligence, and similar factors. In recent decades, industrial equipment manufacturers and the expansion of industrial activities have played a significant role in the degradation of water quality, both through failing to treat wastewater properly before discharge and by accidental release of pollutants into aquatic environments. Moreover, neglect of safety standards and routine maintenance of equipment (such as pipelines, terminals, and platforms) further intensifies water pollution issues related to the oil industry.

### **Environmental Effects of Oil Pollution**

When oil compounds enter the environment, they can lead to several significant impacts:

- Water pollution, affecting both surface and groundwater, can have direct or indirect effects on human health.
- Contamination of seafood, since certain organic compounds accumulate in marine organisms, particularly fish, and may enter the human food chain.
- Health risks from direct human contact with petroleum products.
- Air pollution.
- Soil contamination, which can affect agricultural yields and pollute plants.

Oil pollution is often associated with PAHs, which can accumulate in fat tissues. Some PAHs, such as benzo[a]pyrene, are known to be mutagenic and carcinogenic. Exposure to petroleum compounds primarily affects the central nervous system and kidneys. Studies have demonstrated that laboratory rats exposed to these compounds develop kidney issues, including cancer. The International Agency for Research on Cancer (IARC) has classified gasoline as a carcinogen for humans. Cyclohexane exposure in rabbits has also been linked to eye irritation as well as kidney and liver damage<sup>16</sup>. The carcinogenic potential of substances like BTEX, MTBE, and benzene has been proven in humans. MTBE, widely used as a gasoline additive to enhance fuel

efficiency, has replaced lead in gasoline in recent years. It is highly soluble in water, contributing to contamination of surface runoff and groundwater. MTBE is a resilient compound, found in significant quantities in the groundwater of large cities <sup>17</sup>. Leaks from underground fuel storage tanks, pipelines, and other distribution systems contribute to the contamination of groundwater, increasing the cost of water treatment and posing a risk to public health <sup>17</sup>. Heavy crude oil, with its higher resin and asphaltene content compared to light crude oil, can persist in soil for many years, although certain plants can break down petroleum contaminants in soil.

As a result, these waste materials must not be discharged into the environment without undergoing pre-treatment, even if they are significantly diluted. By utilizing appropriate treatment methods and ensuring proper and timely management, the spread of pollution both at the surface and deeper levels can be controlled, allowing for effective decontamination of the affected area at a reasonable cost.

### Characteristics of Oily Wastewater

The characteristics of wastewater are shaped by the types of pollutants introduced during different stages, from drilling and extraction to processing and refining. The nature of oily wastewater produced also varies depending on the specific crude oil refining process. Oily wastewater typically contains a wide array of organic and inorganic compounds. Inorganic components include cations and anions, while organic pollutants encompass oil, grease, phenol, and various hydrocarbons such as benzene, toluene, ethylbenzene, xylene, and PAHs <sup>4</sup>. Oil-in-water emulsions, within the range of 100-1000 PPM, are considered major pollutants. The U.S. Clean Water Act (CWA) and the European Union have mandated that oil and grease concentrations in treated water should not exceed 10 PPM and 5 PPM, respectively <sup>18</sup>. Table 1 outlines the characteristics of oily wastewater as studied by several research groups.

**Table 1:** Characteristics of Oily Wastewater

Parameter	Range	Sources
COD (mg/L)	500 - 4000	19, 20
BOD (mg/L)	50 - 600	21, 22
TSS (mg/L)	130 - 700	19, 22
pH	6 - 9	23, 24
TOC (mg/L)	60 - 950	25, 26
Phenol (mg/L)	0.5 - 11	23, 27
Oil, Grease (mg/L)	15 - 390	25, 28
Ammonia (mg/L)	4 - 100	24, 27
BTEX (mg/L)	1 - 120	29, 30
Sulfides (mg/L)	1 - 400	31, 32
Total Petroleum Hydrocarbons (TPH) (mg/L)	2 - 320	26, 33

## Discussion

### Bioremediation

Bioremediation refers to the process by which contaminants are broken down by living organisms, primarily microorganisms, under controlled conditions to either neutralize them or reduce their concentration to levels those regulatory authorities consider safe. This technique is commonly employed to eliminate chemicals from soil, groundwater, wastewater, sludge,

industrial waste, and gases. One of the key advantages of bioremediation over physico-chemical methods is its cost-effectiveness. Some of the key benefits include:

- It can be carried out directly on the contaminated site, eliminating the need for transportation.
- The impact on the site surroundings is minimal.
- It is generally well-accepted by the public.
- Bioremediation can be integrated with other

treatment methods.

- It is environmentally safe and sustainable.
- It operates continuously, around the clock.

However, the process is slower than chemical methods, and high concentrations of toxic substances can hinder microbial activity. Additionally, certain chemicals cannot be degraded by biological organisms<sup>34</sup>.

In the 1970s, bioremediation became an accepted technology and experienced rapid growth<sup>35</sup>. For over 80 years, it has been recognized that certain microorganisms are capable of degrading petroleum hydrocarbons, using them as their primary source of carbon and energy for growth, a concept first briefly mentioned by Davis in 1967<sup>9</sup>. Bioremediation techniques have since had considerable success in addressing oil pollution. In 2020, Kim et al. examined the efficiency and microbial communities in a full-scale wastewater treatment plant (WWTP) that processed petroleum refinery wastewater (PRW) containing toxic and carcinogenic hydrocarbons. The WWTP achieved a stable process performance with over 70% efficiency in removing soluble chemical oxygen demand (SCOD) and benzene. Moreover, more than 30 bacterial genera were identified, with *Sulfuritalea*, *Ottowia*, *Thauera*, and *Hyphomicrobium* being the dominant ones, potentially responsible for breaking down aromatic compounds like benzene and phenol<sup>36</sup>. Kumar et al. (2022) found that microbial consortia, genetically modified microbes and plants, plant-microbe combinations, and specialized wetlands have shown promising results for the treatment of petrochemical wastewater<sup>37</sup>.

The core of the bioremediation process lies in microorganisms that produce enzyme systems capable of breaking down, removing, or detoxifying pollutants. Microbes release enzymes that break the pollutants into digestible parts, which microorganisms then use as food. The only byproducts left behind are harmless biological residues<sup>38</sup>. This technology involves knowledge from disciplines such as microbiology, biochemistry, and environmental health engineering. The process can take place either

aerobically (with oxygen) or anaerobically (without oxygen). Anaerobic digestion, aerobic digestion, or a combination of both methods are commonly employed in biological treatment processes for refinery and petrochemical wastewater.

The removal efficiency of COD from refinery wastewater in aerobic systems typically ranges between 70% and 98%, whereas anaerobic systems achieve removal rates of 70% to 93%. While oil degradation is predominantly an oxidative process, some evidence also supports the anaerobic breakdown of hydrocarbons<sup>39</sup>. In the treatment of oily wastewater, the process usually starts with an oil and grease separator, followed by a biological treatment to completely eliminate the remaining organic pollutants<sup>40</sup>. In the Iranian industrial sector, particularly in refineries, activated sludge systems are widely used for treating oily wastewater. Biological methods, when combined with other treatment processes, tend to achieve higher efficiency. No single microorganism can fully degrade crude oil on its own<sup>41</sup>. The degradation of crude oil involves a chain of successive reactions, with specific microorganisms initially attacking oil compounds. This leads to the formation of intermediate compounds, which are then metabolized by other groups of microorganisms<sup>42</sup>.

While various microorganisms are capable of breaking down crude oil, the biodegradation abilities of bacteria have been widely acknowledged<sup>43</sup>. Microbial degradation has emerged as the primary natural mechanism for eliminating non-volatile hydrocarbon contaminants from the environment. Oil-degrading microorganisms are abundant, and numerous bacteria have been identified as playing a significant role in the bioremediation of petroleum products. These bacteria include *Pseudomonas*, *Aeromonas*, *Moraxella*, *Beijerinckia*, *Flavobacterium*, *Corynebacteria*, *Nocardia*, *Corynebacterium*, *Acinetobacter*, *Mycobacteria*, *Modococcus*, *Streptomyces*, *Bacillus*, *Arthrobacter*, *Cyanobacteria*, *Acetobacter*, *Actinobacteria*, etc.<sup>9</sup>.

Microbial degradation focuses on the aliphatic

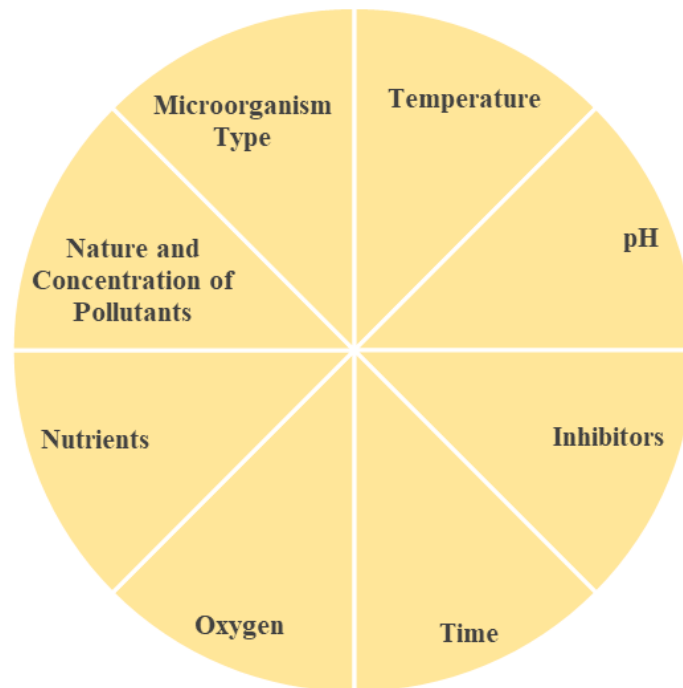
and aromatic components of oil. Microbes need to be soluble to act on oil. Since oil is insoluble in water and less dense, it floats on the surface as layers or films, where hydrocarbon-oxidizing microbes rapidly form<sup>44</sup>. Due to the complex composition of crude oil hydrocarbons, microbes have evolved various mechanisms to adapt to and utilize a wide range of hydrocarbons as substrates<sup>45</sup>. The initial breakdown of organic pollutants occurs intracellularly through an active oxidation process, with oxygen playing a critical role by facilitating enzymatic reactions through oxygenases and peroxidases. Secondary degradation pathways gradually decompose organic pollutants and direct them into central metabolic processes, such as the tricarboxylic acid (TCA) cycle. Biomass synthesis is derived from key metabolites like acetyl-CoA, succinate, and pyruvate, and sugars necessary for growth and biosynthesis are produced via gluconeogenesis. Petroleum hydrocarbon degradation can be mediated by specific enzyme systems. Additional mechanisms include (1) microbial cell attachment to substrates and (2) the production of biosurfactants. While the mechanism by which cells adhere to oil droplets remains unknown, biosurfactant production is well understood and documented<sup>15</sup>.

In addition to bacteria, plants, fungi, and yeasts also play a role in bioremediation. It has been shown that the degradation of most petroleum pollutants is performed by a consortium of microorganisms, with over 200 species of bacteria, fungi, and even algae capable of breaking down hydrocarbons<sup>46</sup>. Like other microorganisms, yeasts are a subject of numerous bioremediation studies due to their ability

to absorb hydrocarbons, though many questions remain about their specific role in biodegradation processes. Yeast species such as *Candida lipolytica*, *Rhodotorula mucilaginosa*, *Trichosporon mucoides*, and species of *Geotrichum* have been reported to degrade petroleum compounds<sup>46, 47</sup>. Hydrocarbon degradation by filamentous fungi has been extensively studied, and most fungal species have been found to be excellent hydrocarbon degraders<sup>48</sup>. Additionally, fungal mycelium can penetrate oil directly, increasing the contact surface area for bioremediation and enabling bacterial degradation. Fungi can also thrive under environmental stresses, such as low pH and nutrient-deficient conditions, where bacterial growth may be inhibited. Fungal genera like *Amorphotheca*, *Neosartorya*, *Talaromyces*, and *Graphium* have been isolated from oil-contaminated soils and proven to have potential for hydrocarbon degradation<sup>48</sup>. Algae and protozoa are also important members of microbial communities in both aquatic and terrestrial ecosystems<sup>15</sup>. Njoku et al. (2016) indicated that the plant species *Glycine max* is one of the plants capable of growing in hydrocarbon-contaminated sites and reducing petroleum hydrocarbons<sup>49</sup>.

#### ***Factors Influencing Bioremediation Effectiveness***

Successful management of bioremediation requires careful planning of strategies that can regulate pollutant degradation activities to achieve the desired outcomes within a feasible timeframe. The removal of petroleum pollutants from wastewater is influenced by several factors (Figure 4).



**Figure 4:** Factors Influencing Bioremediation Effectiveness

**Microorganism Type:** The presence of microorganisms, especially bacteria, is vital in bioremediation, as they use petroleum-based organic matter as a carbon source. If their population is insufficient, the efficiency of the process will be compromised. These microorganisms need to have suitable metabolic capabilities to break down petroleum compounds into simpler substances at an efficient rate. The degradation process is linked to the genetic potential of specific microorganisms to supply molecular oxygen to hydrocarbons and their ability to produce intermediates that enter energy-producing metabolic pathways<sup>50</sup>. Not all microorganisms are capable of degrading petroleum hydrocarbons, so having a diverse microbial population is important, as no single species can degrade all oil compounds. Certain species of *Pseudomonas* are recognized as efficient degraders of alkanes and aromatic hydrocarbons, and their relative abundance is strongly correlated with higher degradation rates<sup>51</sup>. For instance, *Pseudomonas putida* and *Pseudomonas fluorescens* are not only equipped with catabolic enzymes but also possess the metabolic flexibility to adapt to a

wide range of hydrocarbons<sup>52</sup>. Other genera, including *Acinetobacter*, *Marinobacter*, *Stenotrophomonas*, and *Vibrio*, have also been identified as PHC degraders<sup>53</sup>.

One way to increase the rate of biodegradation of pollutants is by introducing large populations of specific microorganisms into contaminated waters, which artificially boosts the concentration of pollutant-degrading microbes. Another modern approach is the integration of key genes from various biodegrading microorganisms into a single host species, creating a genetically modified organism with enhanced bioremediation capacity. Genetically engineered microorganisms can be developed for one or more of the following objectives:

1. To enhance or alter the degradation capabilities of a specific strain.
2. To equip bacteria with resistance to harsh environmental conditions.
3. To track the presence of the introduced bacteria.
4. To assess the bioavailability of pollutants.

The first documented case of a genetically modified microorganism capable of degrading multiple hydrocarbons was reported in 1976 by

Freiloy, Milroy, and Chakrabarti. The organism used in this study, *Pseudomonas* species, demonstrated the ability to oxidize aliphatic, aromatic, terpenic, and PAHs<sup>54</sup>.

**Nature and Concentration of Pollutants:** To be effectively biodegraded, target compounds need to be accessible to microorganisms. Organic pollutants in wastewater can only be removed if microorganisms can utilize them as a source of carbon and energy. If pollutants are not usable by microorganisms, bioremediation will not be successful. The molecular structure of the organic material must meet specific criteria. The number of carbon atoms in the pollutant molecular formula plays an important role. For example, wastewater containing large amounts of heavy petroleum compounds may not be easily biodegraded since molecules are too large and complex for microorganisms to break down. Similarly, highly concentrated oils and greases can inhibit the bioremediation process. The general ranking for microbial degradation sensitivity of hydrocarbons is: linear alkanes > branched alkanes > small aromatics > cyclic alkanes<sup>55</sup>.

Aliphatic hydrocarbons are typically degraded by a wide range of microorganisms. Medium-chain n-alkanes (C10-C24) degrade the fastest, while short-chain alkanes (under C9) tend to evaporate quickly into the atmosphere. Long-chain alkanes are generally resistant to biodegradation. The more branched a hydrocarbon is, the slower it biodegrades. Cyclic hydrocarbons may undergo partial oxidation but are metabolized by only a few bacteria. PAHs degrade very slowly. Microorganisms can only function in contaminated environments if the pollution concentration is not toxic to them.

**Inhibitors:** Any factor that is toxic to microorganisms or disrupts their function is considered an inhibitor. Contaminated sites must be free from concentrations or chemical compounds that inhibit the activity of degrading organisms. Heavy metals, chlorinated organic compounds, certain pesticides, and high levels of inorganic salts can inhibit microbial activity (although some inorganic salts are essential for

biological processes). The particle size can interfere with the contact between microorganisms and pollutants, especially if the particles are uneven. Salinity is a crucial factor in the degradation of hydrocarbons by microorganisms. Excessive salinity can significantly inhibit microbial enzyme activity and impact the composition of microbial consortiums. Sharp increases in salinity can exert osmotic pressure on bacterial cell membranes, leading to dehydration and eventually plasmolysis. Ward and Brock (1978b) found that hydrocarbon metabolism rates dropped by 4% to 28% as salinity increased, attributing this to an overall reduction in microbial metabolic rates<sup>56</sup>. The toxic effects of salinity can be mitigated by gradually acclimating bacteria to high-salinity conditions. In recent years, many studies have focused on using microbial consortia capable of degrading hydrocarbons while tolerating saline environments to optimize the treatment of saline oily wastewater<sup>57</sup>.

**pH:** Microorganisms require a specific pH range to thrive and reproduce. Some microorganisms can tolerate a broad pH range, while others are sensitive to even small fluctuations. Studies and practical conditions from various biological processes suggest that a neutral pH is ideal for bioremediation. Most bacteria and fungi grow best in environments with a pH near neutral. The USEPA (1993) recommends an optimal pH range of 6 to 8 for successful bioremediation<sup>58</sup>. Lu et al. (2002) reported that the maximum degradation of BTEX occurred between pH 7.5 and 8<sup>59</sup>. Deviating from neutral pH, particularly increasing it, significantly reduces hydrocarbon degradation, while in some cases, acidic pH levels can completely inhibit biodegradation<sup>60</sup>. Inefficiencies in the bioremediation of oil wastewater have been reported due to the accumulation of high levels of volatile fatty acids, which lower pH and inhibit the methanogenesis process<sup>61</sup>. Additionally, pH affects the availability of certain nutrients, affecting their accessibility to microorganisms.

**Temperature:** Temperature plays a crucial role in affecting both chemical properties of pollutants and the physiology and diversity of microbial

communities<sup>62</sup>. While both low and high temperatures can hinder microbial activity, certain microorganisms have been found to survive in extreme temperatures. Studies have shown that biological activity increases significantly by rising temperature. As temperature rises, the solubility of pollutants increases, making them more accessible to microorganisms. In general, for every 10°C increase in temperature between 5°C and 25°C, the metabolic rate of bacteria doubles. However, this response can vary depending on the type of bacteria. Some microorganisms, called thermophiles, exhibit better enzyme activity at higher temperatures. Parr et al. (1994) identified the optimal temperature range for microbial growth as 20°C to 35°C<sup>63</sup>, a range in which most microorganisms achieve maximum hydrocarbon degradation, and it was further demonstrated by Hong et al. (2007)<sup>64</sup>. Venosa and Zhu (2003) found that ambient temperature affects both properties of oil and microbial activity<sup>65</sup>. Cold winter temperatures have been noted as a limiting factor for the biodegradation of PAHs in estuary sediments and for various hydrocarbons in freshwater lakes<sup>66, 67</sup>.

For oil refineries, wastewater temperature is a key consideration. High water temperatures pose drawbacks for reuse (in industrial and agricultural contexts) or for discharge into water bodies. When used for cooling, higher temperatures increase water consumption, and sometimes water does not cool sufficiently. When used for irrigation, excessively high water temperatures can disrupt plant growth. Elevated temperatures in discharged water reduce dissolved oxygen (DO) levels, accelerate chemical reactions, and increase biological metabolism. If temperatures rise too much, the survival of aquatic organisms is at risk. Moreover, high temperatures increase the toxicity of hydrocarbons to bacteria<sup>68</sup>.

**Nutrients:** Microbial activity ceases if sufficient nutrients are not available. The most important nutrients include carbon, nitrogen, phosphorus, and sulfur, which are often present in limited quantities in contaminated environments. As a result, in many cases, these nutrients must be added to the

system to meet biological needs. Hydrocarbon-degrading bacteria require a stable nitrogen source, such as NH<sub>3</sub>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> (inorganic), and some organic nitrogen sources. Microorganisms use phosphorus to synthesize ATP, nucleic acids, and components of cell membrane<sup>69</sup>.

The ratio of carbon to nitrogen to phosphorus (C:N:P) is a critical factor. Studies have shown that microorganisms achieve optimal growth under specific nutrient ratios. Thomas et al. identified the optimal C:N:P ratio for microbial growth as 120:10:1<sup>70</sup>. Due to the high carbon content of crude oil in wastewater, this ratio is often disrupted, requiring the supplementation of nitrogen and phosphorus. When a major oil spill occurs in aquatic environments, carbon levels significantly increase, and the availability of nitrogen and phosphorus becomes the limiting factor for oil degradation<sup>71</sup>. Adequate nitrogen levels are crucial for promoting microbial cell growth, reducing the adaptation phase, and maintaining high microbial activity. On the other hand, an excess of nutrients can inhibit microbial biodegradation processes<sup>72</sup>. Oudot et al. (1998) reported that high levels of NPK have negative effects on the biodegradation of hydrocarbons, especially aromatic compounds<sup>73</sup>.

**Oxygen:** Hydrocarbons, being highly reduced substrates, require an electron acceptor, and molecular oxygen is the most commonly used. Although most studies indicate that hydrocarbon biodegradation is an aerobic process, anaerobic degradation has also been observed. Oxygen availability is crucial for effective aerobic degradation<sup>74</sup>. The first microbial attack on hydrocarbons always involves an oxygenase enzyme, which catalyzes biochemical reactions in the presence of oxygen. Monitoring oxygen consumption is a fast and efficient method to track aerobic biodegradation<sup>75</sup>. Oxygen concentration has been identified as the rate-limiting factor in the biodegradation of diesel in groundwater<sup>76</sup>. Oxygen can be supplied through the injection of pure oxygen, air, or H<sub>2</sub>O<sub>2</sub>. However, as H<sub>2</sub>O<sub>2</sub> can be toxic to microbial flora, it is recommended to add it gradually to prevent any harmful effects.

**Time:** Time is a critical factor in the efficiency of treatment processes, as it aids in improving system performance. Most microorganisms need time to adapt to new conditions, and during the initial transitional phase, they do not immediately start degrading hydrocarbons. Over time, as the transitional phase ends, microorganisms begin breaking down hydrocarbons more effectively. If the retention time is too short, this phase may not be completed, resulting in lower efficiency. As time increases, the microbial population grows, leading to greater enzyme production and improved system performance. Longer retention times give microorganisms more time to acclimate to pollutants and degrade a larger percentage of compounds. However, if the retention time is too long, the significant reduction in TPH levels can cause microorganisms to enter a self-destructive respiration phase. Therefore, in biological systems, achieving 100% efficiency solely by extending retention time is not feasible. Razavi and Miri found that complex oily industrial wastewater requires longer retention times for effective treatment and microbial adaptation<sup>77</sup>.

Biodegradation is a process that requires a careful balance between these factors. Controlling one factor without considering the others may lead to disruption of the overall system performance. Proper management of these factors and understanding their interactions can help improve the efficiency of biological treatment systems and reduce operational problems. System design should be based on a thorough understanding of these interactions. For example, temperature changes can affect pH, which in turn changes the enzymatic activity of microorganisms. At high temperatures, the rate of biological activities increases, but if the pH is outside the optimal range, this activity stops. Also, some toxic substances may directly or indirectly affect biological activity (by changing pH or reducing DO). Also, if sufficient nutrients are not available, microorganisms cannot grow, even if the hydraulic retention time is long. Conversely, if sufficient nutrients are provided but the retention time is short, microorganisms will not have enough time to decompose organic matter.

## Conclusion

The environmental health challenges posed by wastewater containing petroleum compounds are among the most critical issues facing the global environment. With industrial growth and the rising demand for energy from fossil fuels, pollution from these compounds is also increasing. While there are various methods to reduce the concentration of these pollutants, the focus is on eco-friendly approaches that harness natural processes for pollutant removal.

Currently, nature employs biological control strategies to eliminate hazardous pollutants from the environment. When pollutant levels are high, microbial treatment provides a safe and effective method to enhance environmental health, offering advantages such as low cost, simplicity, and widespread public acceptance for cleaning ecosystems contaminated with oil. Recent advancements in biotechnology confirm that various hydrocarbons in crude oil can be utilized by microorganisms as a carbon source. These hydrocarbons are both a target and a byproduct of microbial metabolism. Many researchers have explored the use of microbes to break down petroleum products, highlighting this as a promising alternative technology. As a result, research in this area continues to grow. In this systematic review, key parameters affecting the success of bioremediation were identified and discussed based on selected studies. This allows decision-makers to evaluate these factors before implementing and investing in this method, ensuring its effectiveness in reducing petroleum pollution concentrations. They can also model a biological treatment plant for petroleum wastewater based on these factors. Further research on unknown microorganisms (that can be effective in degradation), biochemical pathways, interaction of factors with each other, and also comparison of these factors under aerobic and anaerobic degradation conditions can be carried out, which will help in the success of biological treatment. Also, pilot-scale studies are necessary to minimize problems related to practical application, especially for the treatment of large volumes of petroleum wastewater.

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The authors declare that there is no competing interest.

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### Ethical Considerations

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that the submitted work is original and has not been published elsewhere in any language.

### Code of Ethics

This article is open scientific research and has not yet been registered as a research project at the university, but all issues related to research ethics have been observed.

### Authors' Contributions

All authors of this study have a complete contribution for data collection, data analyses, and manuscript writing.

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