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Review Article

Biodegradation of Petroleum Hydrocarbons and The Factors Effecting Rate of Biodegradation

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Abstract

In the recent decade, environmental pollution of petroleum and petrochemical products has attracted much attention. In the modern world, contamination of natural environment with oil products causes soil pollution. Therefore, petroleum hydrocarbons have been shown harmful effects on the environment and particularly to human beings. Earlier, many traditional techniques were used to treat such hydrocarbons on large scale. However, bioremediation technique is considered environment friendly due to relatively low cost and utilizing a natural microbial to reduce the concentrations and toxicity of various chemicals, aromatic, and aliphatic hydrocarbons. In this context, certain bacterial strains have been demonstrated to be able to breakdown chemical compounds present in petroleum products. The goal of this review paper is to discuss the most possible ways for biodegradation of petroleum hydrocarbons and highlight the environmental conditions which affecting the rate of biodegradation. In conclusion, by optimizing the environmental conditions such as oxygen, temperature, pH, moisture etc and other nutrients will maximize the hydrocarbon quickly in the natural environment.

Keywords: Petroleum, Bioremediation, Oxygen, Temperature, Natural Environment

Introduction

Worldwide, petroleum is used as the main source of energy in industry and daily life. However, the word petroleum is used as a synonym for crude oil, a dark-brown sticky liquid mixture consisting of varying molecular weight hydrocarbons that are used as the main source for the preparation of various petroleum products. Crude oil consists of over 30% poly-aromatic hydrocarbons (PAHs) and hundred other various hydrocarbons such as nephthenes, paraffins, aromatic compounds as well as organic nitrogen compounds, sulfur compounds, and oxygen-containing hydrocarbons (phenols) [1]. Crude oil is a complex mixture of solid or gaseous hydrocarbons that ranges from 16 to 1800 in molecular weight, dissolved in liquid hydrocarbon, and therefore has a liquid appearance. Petroleum hydrocarbons are classified into four major groups, saturated aromatic-ringed hydrocarbons (branched, unbranched and cyclic structures) such as polyaromatic hydrocarbons (PAHs), mono aromatic hydrocarbons (MAHs), polar oil surface structures, resins dissipated in aromatics and saturates. Biodegradation is a treatment method used to remove a wide range of contaminants, including petroleum hydrocarbon-contaminants from soil [2].

Biodegradation is a treatment method used to remove a wide range of contaminants, including petroleum hydrocarbon-contaminants from soil. Bioremediation is an engineering process where natural biodegradation of petroleum-hydrocarbons is accelerated by indigenous soil, protozoa, fungi, and bacteria. Bioremediation seems to be an environment-friendly technique due to the effective microbial decontamination of groundwater, soil, or treatment of oil-contaminated sites [3]. Bioremediation has been defined as the addition of biomass to accelerate the natural biodegradation in a contaminated environment such as degradation of chemical substances i.e., complex petroleum hydrocarbons are accelerated by bacteria, fungi, and other microorganisms. Moreover, the US Environmental Protection Agency (EPA) reported microbial culture and nutrient or enzyme as bioremediation agents that significantly enhance the biodegradation rate to reduce the impact of discharges. Bioremediation technology is preferred on other conventional technologies due to potential advantages such as being economical, few labor needs on contaminated sites, and producing eco-friendly products at the end [4].

Many of the methods are used to eliminate the contamination produced by petroleum or petroleum products such as biological, physical, and chemical treatment. Biological treatment method is the main process responsible for the elimination of contaminants, microbes such as fungi, bacteria can degrade the polyaromatic hydrocarbons (PAHs)into other low toxic organic chemicals [5]. Bioremediations degrade the complex organic chemical compounds into low toxic compounds by employing living organisms such as bacteria and fungi. Bioremediation may be in situ or ex-situ, in situ bioremediation oil is remediated in the contaminated site and in ex-situ, the oil is treated outside from the contaminated site. Several processes such as chemical, physical, or biological processes are employed for remediation while bioremediation in oil-contaminated soils completed by Remediation by natural attenuation (RENA) technique. Natural attenuation dissolves and degrades the contaminants in groundwater or soil by relying upon natural processes passively and soil conditions can also control or increase the contaminate degradation rate. These natural processes include chemical, physical, or biological transformations such as dispersion, oxidation/ reduction, aerobic/ anaerobic biodegradation, and adsorption.

Principles of Bioremediation

Crude oil is a complex mixture of several hydrocarbons, each type of oil has a different composition there are numerous ways to deal with it using microorganisms and flora. Bioremediation can happen naturally or with the help of bacteria and fertilizers. The oil and its constituents are initially recognized by biosurfactants and bio emulsifiers in the soil first, then microbes attach themselves and utilize the hydrocarbon present in the petroleum as a source of energy. Microorganisms have limited access to high molecular weight hydrocarbons due to their low solubility and adsorption. Biosurfactants improve the solubility and elimination of these contaminants, resulting in faster oil biodegradation [6].

The volatility and susceptibility to biodegradation of oil components varied significantly, some compounds are easily degraded but some compounds are persistent microbial degradation. Biodegradation of various compounds of petroleum occurs simultaneously while specific microbial species attack different compounds with different degradation rates [7]. Biodegradation of petroleum hydrocarbon is carried out via enzymes that are produced by the microbes in the presence of carbon source while lacking appropriate enzyme act as a barrier in hydrocarbon degradation process [8].

Bioremediation strategies

Bioremediation of petroleum hydrocarbons or oil spills is removed by bioaugmentation or biostimulation.

Bioaugmentation

Contaminated soils were treated by adding indigenous microbial strains or genetically modified microbes where natural existing microorganisms cannot metabolize the bioremediation process. Several microorganisms can degrade the petroleum hydrocarbons such as fungi bacteria and yeast, over 200 bacterial, fungal, and yeast species were known as hydrocarbon degraders and degrade simple hydrocarbon methane and a complex hydrocarbon containing more than 40 carbons. decontamination rate is a primary factor during seeding decrease the period to initiate the bioremediation process [9].

Six bio pile batches with different remediation strategies were examined for biodegradation of petroleum hydrocarbon oil (14,000 mg/kg), biostimulation (added with rhamnolipid, low level, high-level nutrient), bioaugmentation (introduction of kitchen waste and selected microbial consortiums), and biostimulation plus bioaugmentation (added both bacteria consortia and rhamnolipid) [10]. Low nutrient level (NEL) and kitchen waste (KW) baches result in the highest petroleum hydrocarbons greater than 80 percent after the operation of 140 days. Hydrocarbon analysis showed that the bioaugmentation strategy is more beneficial and effective for removing aromatic compounds (64 and 68 percent), KW and NEL techniques could eliminate the poor components positively, 11 percent and 21 percent respectively [11].

Biostimulation

Biostimulation is the process of accelerating biodegradation by adding rate-limiting nutrients. The major goal of the bench-scale test ability study is to establish the type and frequency of amendments involved in optimum stimulation in most extensively contaminated coastal ecosystems where the hydrocarbon nutrients are likely limiting factors in oil biodegradation [12]. Moreover, Turgay, *et al.* investigated the influence of bioaugmentation and biostimulation (powdered gyttja and its humic fulvic extract-HFA) on the degradation of hydrocarbons in oil-contaminated soil [13]. After a 60-days incubation period, it was concluded that biostimulation is a more effective strategy, resulting in 51-56 percent oil remodel whereas bioaugmentation resulted in 50 percent oil remediation by using a commercial bioremediation product. Only nutrition was used in the control treatment that resulted in 46% performance. Turgay et al., found that biostimulation of contaminated soil with friendly organic compounds like humic compounds can be as helpful as bioaugmentation, the hydrocarbon-degrading microorganisms can achieve a maximal growth rate of pollutant absorption by providing these additional nutrients at the right concentration [14].

Hazards of Oil Spills

Many research reports revealed a vast amount of organic and inorganic compounds discharge into environment due to human activities, dangerous for our ecosystem. Oil contaminated sites and waste of industries as well as other activities causing significant environmental risk [15]. Globally petroleum hydrocarbon is used as a primary source of energy, resulting from significant in environmental pollution. Petroleum is made from a complex combination of hydrophobic and non-aqueous components, most of them are carcinogenic, mutagenic, and poisonous [16].

Moreover, hydrocarbon discharge into the environment is carefully regulated and divided by US EPA (United States Environmental Protection Agency), environmental contaminants cause negative effects on the ecosystem and human health [17]. Crude oil has negative impact on soil qualities, soil organisms, and plants, it is making soil unsuitable for plant growth due to increasing toxic level of specific element included zinc, iron and decrease nutrient. Globally now a day's oil spills cause most hazardous effect on environment, poses risk to ecosystem but oil spills have had serious problems for decades [18]. Oil contamination currently observes in Mumbai (India), caused due to drain in Mumbai-urban pipeline during January 2011, leaking 55 tones oil into Arabian sea [19].

One of the most important products of oil, diesel engine oil, is the main cause the ecosystem. Oil spills on agriculture level normally decrease soil fertility, plant growth, and decrease germination due to undesirable's soil condition, because diesel engine oil displace air between soil particle there is deficient aeration of soil [20]. Crude oil leakage was estimated to be 600,000 metric tons every year through exploration, petroleum storage, refining petroleum product transportation, and production [21]. However, a major cause of environmental pollution is due to the addition of hydrocarbon in air unintentional or owing human activities because pollutants collect in plant and animals' tissue, they can cause mutation or death, soil pollution mixing with hydrocarbon generates severe harm on ecosystem. Globally soil contamination is main problem because of oil drilling because its poses a severe threat to human health, economic problem, reduce agriculture productivity, cause groundwater pollution, limiting its usage and environmental problem [22].

Moreover, the main problem of worry is a danger of illness caused due to direct contact by soil pollution, vapor from the soil, pollution of water sources inside and under the soil. It is commonly known that petroleum hydrocarbon is harmful to humans, animals, plants, and microorganisms. Petroleum hydrocarbon harms Tri-Star plants and its usage as a weed killer by enabling cell content and dissolving in lipids part of cytoplasm membrane [23]. There are different sources of oil pollution such as industrial waste, oil tanker spills, and accidents of oil tankers during transportation and refining facilities. Crude oil transported from a distance through the water tank and land pipelines both methods because pollutant, those way oil-producing countries is not a consumer of oil to avoid pollution. Petroleum transportations take place from high production area to high consumption area.

During transportation hydrocarbon reach food chain can become hazardous and other carcinogenic compounds such as PAHs (polycyclic aromatic hydrocarbon) and PCBs (polychlorinated biphenyls) [24]. A huge amount of oil is consumed by transportation, storage, and processed all over the world. Due to road accidents, ship accidents oil blasts, work at garages etc. A large amount of oil is spread in the water as well as soil environment which can further affect the main flora and funnel. The most common chemical involved in soil contamination are petroleum hydrocarbon like benzopyrene, solvent, pesticide, lead, chlorinated hydrocarbon (CHF), heavy metal (Cr, Cd, Pb, Mn, Fe, Zn, and as) are widely dislocated and separated through ecosystem because of insufficient combustion of organic compounds [25].

PAH are ubiquitous petroleum concentrations found in the environment that are thought to be carcinogenic and mutagenic, while in living organism PAHs and benzopyrene harm DNA has been linked to breast cancer [26]. Oil spill effect living organisms may be direct, indirect, and chronic such as suffocation (nasal channel and clogging of lungs), anoxia and restriction of animal's movement in ocean, river, and soil, that observation after comparing sea and land environment [27]. On the other side, indirect effect includes reduced development (animals and plants) physical abnormalities, trophic cascade, and reproduction. During 1981 in Norway little oil spill of oil washing from ships killed 30,000 birds because this leakage occurs in area where numerous seasons [28]. Similarly, another disaster take place in 1989 in Exon Valdez spilled oil approximately 245,000 barrels oil killing 250,000 birds on ground, later in 1993 oil spilled around 595,000 barrels killing just 1500 birds [29].

Kingston studies that variation in biodiversity reaction to oil contamination might be due to some factors include mating seasons at oil spill soil in oceans environment affecting fish and birds. The toxicity of pollutants to biological processes facilitated through soil microbes can have major harmful impact on soil environment resulting in a huge decrease of soil quantity [30]. Contaminated soil studies revealed increased level of pollutants because low bacterial population, mineralization of organic material, decrease biomass and reduce leaf litter biodegradation. Oil contaminated soil is caused due to unbalanced in nitrogen and carbon reaction at spill site, because pollutant oil is containing a combination of hydrogen and carbon. As a result, nitrogen storage occurs in oil-soaked soil slowing the rate of bacterial development and carbon consumption. Moreover, a high concentration of organic biodegradable material reduces oxygen level in top layer of soil cause delay of oxygen absorption in the lower layer [31].

Factors Influence Petroleum Hydrocarbon Degradation

Presence of petroleum hydrocarbon in environment can be determined by a biotic factor, such as the degradation of oil. Factors that affect the growth of bacteria in environment, our ecosystem and enzyme which influence pollutants caused by petroleum depend directly on quality and quantity of hydrocarbon mixture and their properties. In our environment hydrocarbon of petroleum enter directly when suitable conditions are available that completely biodegrade hydrocarbon in relatively few hours or days [32]. Factors that affecting biodegradation of petroleum hydrocarbon is given below.

Temperature

Temperature is an important factor that effects on the biodegradation of petroleum hydrocarbon. Atlas revealed that the viscosity of low molecular hydrocarbon depends upon temperature; if the temperature is low its increase while volatility is inversely proportional to viscosity it reduces by increasing temperature. Temperature effect overall biodegradation of oil by changing its chemical and physical nature in soil, moreover, rate of metabolism of hydrocarbon can be changed by microorganism and their community. An increase in temperature above 30-40ºC, the metabolism of petroleum hydrocarbon also increased. Degradation of hydrocarbon take place at huge range of temperature, generally, metabolism rate decreases with decreasing temperature [33]. In seawater phenanthrene and naphthalene are observed in crude oil at 0ºC temperature that compares with manganese and laccase in ligninolytic Fungai observe at optimum temperature range 50ºC to >75ºC during PAHs (polycyclic aromatic hydrocarbon) degradation. More than 90% degradation of PAHs takes place at an optimum temperature [34].

Kuwait crude oil has different water-soluble environmental factor. Research results indicate PAHs degrade at 15ºC temperature and 4ppm oxygen level while an increase in temperature up to 40ºC decreases oxygen level to 0ppm [35]. Microorganisms that degrade petroleum hydrocarbon work at a specific range of temperatures such as thermophile (above 50ºC), mesophiles (15ºC-45ºC) and psychrophiles (below 20ºC). However, mostly microorganisms activated at mesothermal temperature range 20-35ºC provide maximum degradation [36]. In general, temperature effect on degradation of hydrocarbon mostly warm climate suitable than colder. Moreover, mesophiles have more variety of organisms which use for degradation, so thermophiles and mesophiles are a good choice of microorganisms for degradation.

Nutrients

Nutrients play important role in biodegradation such as iron, oxygen, nitrogen, and phosphorus. Nutrients require to start the biodegradation process by cleaning contaminated environments [37]. Previous studies indicate the presence of nutrients is important for increasing biodegradation through microbe's biomass. When liquid petroleum hydrocarbon release in an environment the amount of carbon increase and phosphorus or nitrogen quality reduce that effect oil degradation. Phosphorus and nitrogen in fresh water and seawater cause nutrient-deficient area and then plant require a huge amount of nutrients. It is necessary to increase amount of nutrient for degradation oil pollutant. While large concentration of nutrients inhabits process of biodegradation.

Several scientist work on nutrient level and reported NPK (nitrogen, potassium, and phosphorus) high level is inhibiting factor for degradation specially presence of aromatic hydrocarbon. Nutrient type and quality affect degradation mostly biodegradation of hydrocarbon [18]. Numerous studies indicate a sufficient supply of nutrients reduces the degradation of petroleum hydrocarbon. Aerobic microorganisms during degradation use different types of the nutrient include Sulphur, manganese, nitrogen, small amount of phosphorus and iron. Phosphorus and nitrogen are an important nutrient for natural degradation of hydrocarbon while lack anyone of them reduce the degrading process. Sea water has low amount of these nutrient in water. In seawater phosphorus is present in form of calcium phosphate, concentration of phosphorus and nitrogen compound depend upon temperature such as in sea water ranges from 0 to 0.7 mg/l and 0.1 to 1 mg /l respectively. If nutrient amount is not enough for biodegradation, then fertilizer is use as source of nutrient [38].

Oxygen

Oxygen is most important element for the biodegradation of hydrocarbon. Petroleum degradation's first step starts in presence of oxygen while it requires for overall degradation process as an important element. In aerobic conditions use the large amount of oxygen and their amount reduce in soil microbes uses molecular oxygen for respiration during the overall degradation pathway, so oxygen is sufficient for the degradation of hydrocarbon. Mostly 3-4 ml oxygen used in the degradation of 1ml hydrocarbon into water and carbon dioxide. The biodegradation process used large quantity of oxygen due to high amount of carbon and hydrogen in petroleum but low amount of carbon dioxide [39]. Water in lake, ocean and harbors have large concentration of oxygen on surface due to air, water, wave action and wind but oxygen concentration decrease in depth.

In deep water resource, degradation process is anaerobic due to low concentration of oxygen. If petroleum covered with sediments, scatter and go down to deep require longer time for degradation. The presence of oil polls on surface of water stops the reclamation of oxygen that require for degradation process, due to this different mechanical process used to remove oil and make boundary for oxygen penetration [40]. Oil movement depends on presence of oxygen, type of soil, concentration of moisture and microbes degrading ability that contribute to replacement and re-aeration. All these conditions are important for biodegradation of petroleum hydrocarbon. However, oxygen concentration will be high dependent on microorganism used in degradation. Aerobic bacteria use 3.1 mg/ml oxygen for the breakdown of 1 mg/ml hydrocarbon without taking an overall quantity of microbes [41].

Detergents

Petroleum is hydrophobic their access to microbes is low that way degradation process is slow. To increase the degradation adding detergent in oil-containing soil help in desorption of hydrocarbon by speeding up the repair process. Rhamnolapids are microorganism produce commonly utilized detergent that contain fatty acid and rhmanose moieties [42].

Anaerobic Degradation

Most bioremediation methods focus on increasing oxygen availability to contaminated locations, believing anaerobic respiration is a primary mechanism for hydrocarbon elimination. However, oxidation due to biological ammonia might increase the amount of ammonia and urea fertilizers, which are sometimes, employed oil spill remediation. On some sites, oxygen transfers not enough to restore oxygen depleted by microbial metabolism due to oil penetration in deeper sediments. Anaerobic degradation of petroleum hydrocarbon may be relevant in such circumstances [43].

Salinity

There are only few research papers that publish the effect of salinity on microbial degradation. Microorganisms present in world oceans normally manage with vast range of salinity. Only few evidence that indicate microbes are affected with hypersaline condition, like presence of salt in petroleum resources. Estuaries developed a unique situation because salinity level of oil well differs from oceans. Microorganism present in degrading environment is familiar if their compatibility is like saline quantity such as archaeal have ability to work in crude oil hypersaline environment. According Zvyagintseva, *et al.* Studies that Kazakhstan Kalamkass oil field use n-alkenes and isoprenoid for degradation of hydrocarbons using 10-25% salt from brine. Al-Mailem, *et al.* Reported halophilic strain of *halococcus, haloferax* and halobacterium in salt medium through oil vapour use as source of carbon in existence of >26% NaCl at 40-45ºc temperature in hyper saline area of Arabian gulf. In high salinity area microbes use aromatic and aliphatic hydrocarbon as source of energy and carbon [44].

Studies indicate that only few types of fungi help in the degradation process in high concentration of salt. Obuekwe, *et al.* observe that only *Papulaspora spp.,* Fusarium lateritious and Drechsler spp. play role in degradation. Saltmarsh decomposing crude oil as source of carbon in Kuwaiti dessert where salinity is 5to 10%. However, bacteria, eukaryotes and archaea degrade crude oil even at high range of salinity (0-30%). All archaea organism *(halococcus, haloferax and halobacterium)* and eubacteria *(Actinopolysporaspp., Streptomyces Albiaxialis, MarinobacterAquaeolei)* work at 20-30% salinity and these organisms use for cleaning of oil damage in hyper saline environment because natural attenuation is slow in such conditions [45].

pH

The majority of heterotrophic Fungai and bacteria prefer natural pH while Fungai work in acidic condition, Burtha and Dibble reported the optimum pH for mineralization of oil sediment ranges 5.0 to 7.8. Many hydrocarbons contaminated area does not have ideal pH for bioremediation such as grass work sites have notable quantity of brick and concreate. Microbiological condition less suitable pH when drain of element raise pH and created ground [46].

Moreover, leaching and oxidation of coal developed acidic condition due to oxidation and releasing sulfides. If pH of the contaminated area is increased to become pollutant, then microbes have not capability to degrade PAHs in such alkaline and acidic environment. The impact of pH was investigated on sodium phosphate (NO3PO4) degradation of an aromatic compound in pollutant soil. Overall degradation of polycyclic compound take place by using Na3PO4 powder and 2-methyl naphthalene (1.7%) at 2.0 pH on base of result obtain by spectroscopy and gas chromatography (GC) [47].

Using 4g Na_3PO_4 as catalyst to identify degradation of PAHs at pH 2.0-4.0. pH sites adjustment is common by adding lime, *Burkholderiacocovenenas* bacteria present in contaminated soil that help degradation of phenanthrene at 5.5 to 7.5 pH. Bacteria that grow in contaminated soil are not affected with phenanthrene 40% remove at 5.5 pH after every 16 days, while at high pH it removes 80%. *Sphingomonas paucimobilis* is degradation microbes help in PAHs degradation that sensitive to pH and work only 5.2-7, while Fungai strain work at low temperature. Microbes use for degradation such as Fungai, bacteria and yeast work at 2 pH. Some bacteria are alkaliphiles present in 7.5-10 pH and salinity seawater. The fact that hydrocarbon is degrading with 0.1-2 M NaCl while maximum at 0.4M is about like natural water [34].

Land farming

The method uses to separate pollutant soil from natural by using fertilizer and rotated contaminated soil. Landfarming increase the bacterial activity and degradation of petroleum. The following criteria were used to identify the right location, normally 3 feet distance is use for separation between groundwater and ground surface, when slop of ground is less than 8%. Land farming is a process in which contaminated soil is separated and their thickness is 0.3-1.0 m, adding some nutrients and mixing soil on regular basis. Total petroleum hydrocarbon (TPH) in land farming is lost by biodegradation. Moreover, land farming merger of two process, however land farming depending on environment, temperature, location, and type of soil. The concentration of hydrocarbon kept around 5% then degradation is maximum in upper layer of soil (10-15 cm) [48].

To increase degradation of hydrocarbon of petroleum different components added in sludge soil such as surfactant, inorganic and organic fertilizer, bulking agent, and inoculate. During land farming process microbes in soil is not affected with pH, moisture, temperature, and oxygen mining, but land farming requires long time and is successful only in a warmer climate such as southern countries. An experiment performed in Australia for 12 months, in found TPH level reduced from 4644 to 10 ppm. Moreover, bioremediation practice for petroleum industry is not acceptable because large clean area of land contaminated first. Another major environmental disadvantages of land farming relate to quantity of organic material present in contaminated soil, monitoring chemical is critical because of their role in tropospheric zone and harmful influence on health [49].

Composting

Composting is method that used organic compound present in pollutant soil to form fertilizers. Organic fertilizer helps in development of microorganism due to this process of degradation also stimulated for short time. Composting is phenomena where recycle nutrients and carbon from microbes present in soil. Composting use to manage organic waste include explosive, coal tar, it's also uses to handle soil effected with bulking agent (bark and other structuring). During the degradation process microbes' activities produces heat that keep in pile, while bulking agent expands permeability holding capacity of water and cultivated land [10].

By enhancing the characterization rate of biodegradation of waste accelerated. As result composting work for hydrocarbon-rich material includes tank bottom and sludge soil. Composting is mostly done in four different way a static pile, an enclosed space, a window, and a vessel. Composting methods work on aerobic degradation of organic material. Biodegradation use a combination of organic matter while water concentration is roughly 55%, organic matter greater than 70% facilitates successful biodegradation. Moisture content greater than 60% reduce temperature, oxygen level and porosity but low moisture less than 50% slows the composting process. Microorganism activity impacted through pH with ideal value 5.5-8 on scale [50].

Moisture

Moisture is an important factor of a biological process that transport waste product, food nutrient to microorganism. Moisture present in water surface, lakes and oceans create no problem but in soil it helps degradation process. If water quantity increases hinder soil re-aeration, then the process become anaerobic. The optimum range of moisture in soil due to environment and soil type. Some studies indicate that these ranges are 30-990% but in other studies its 12-32% while in aerobic process ranges 50-70% on base of water capacity in soil. Whereas tidal and waves are important in the transfer of water to marches and beaches, while rain fall applicable in the degradation of oil by transfer oxygen and moisture to microbes [51].

Microbial Remediation

Microbes are extremely helpful in the restoration of polluted environments. Bioremediation process consists of a variety of microbes including aerobes and fungus. Different microorganisms are eliminated in different bio-mediation stages such as already removed hydrocarbons. Structure of alkenes is transfer to aromatic hydrocarbons by microbial populations. There is different method to treat pollution caused by petroleum and their products such as chemical, physical, and biological remediation. Biodegradation is known to be an important process of hydrocarbon removal in soils. Bacteria and fungi are microorganisms that convert toxic organic compounds to inorganic final products like carbon dioxide (CO2) and water [52]. Bioremediation is more economical methods compared to other such as burning and washing of soil.

Bioremediation is an effective and efficient method for attaining the maximum biodegradation conditions in which microorganisms break down hydrocarbons as a source of energy and carbon. The scope of current knowledge of oil bioremediation is restricted because most of these domain studies and analyses have relied on the evolution of bioremediation technology to cope with large oil spills on coastal shorelines. Bacteria, yeast, and fungi are major bio-degraders of hydrocarbons in the environment. Biodegradation efficacy has been reported ranging from 6-2 percent for soil fungus, 0.1-50 percent for soil bacteria, and 0.003-100 percent for marine bacteria. In the soil and aquatic environments mixed populations with different enzymatic capacities are required to decompose a complex mixture of hydrocarbons such as crude oil and metal [53].

Microorganism such as Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and *Rhoaococcus* are utilized to break down petroleum hydrocarbon pollutants in the environment. Commercially available bacteria for hydrocarbon breakdown are freeze dried bacteria that can be utilized for bioremediation after being cultivated to a minimum of 2*10 8 CFU/ml. Bacterial Species such as *Pseudomonas, Aeromonas, Moraxella, Bajerinckia, f. Lavobacteria, Chrobacteria, Nocardia, Corynebacteria, Atinebacter, Mycobacteria, Modococci, Streptomyces, Baclliarthobaccter,* and *Cyanobacteria* can decompose petroleum products [54]. Floodgate reported 25 varieties of hydrocarbon-degrading bacteria and fungi from maritime environment. According to Das and Mukhergee, crude oil was extracted from gasoline soil in north-eastern India. Different bacteria genera *Burkholderia, Gordonia, Acromicrobium, Brevibacterium, Deutzia,* and *Mycobacterium* are identified from petroleum-contaminated soil and can degrade hydrocarbons [55].

Polyaromatic hydrocarbons only are degraded with Sphingomonas; fungal genera such as Amorphoteca, Neosartorya, Talaryomyces and group such as Pichia and hum are isolated from crude petroleum soil and have potential hydrocarbon degraders. Sorkhoal et al. identified a gradual change in the composition of oil-degrading bacteria in sand samples contaminated with oil over time. Venkateswaran and Harayama observed a similar result in sequential extraction in media having remnant crude oil. Bacteria and fungi are main contributors to oil pollution mineralization. Commonly, most bacteria undergo gram negative alpha Proteobacteria species such as *Sphingomonas, Pseudomonas, Alcaligens, Acinetobacter, Proteus, Moraxella,* and other major group is low G+C, gram positive especially *Actinomycetes* [56]. Hydrocarbon-contaminated sites and hydrocarbon-degrading cultures are common sources of Pseudomonas species. Chemical substances such as Alkenes, thiophene, alicyclic and aromatic, polycyclic aromatic hydrocarbons (PAHs) are among crude oils, most resistant components with members of this genus having a significant affinity for hydrocarbons and ability to decompose a variety of hydrocarbons [57].

After 20 days of incubation, a bacterial consortium indicates a maximum percentage of 78 percent crude oil degradation. Chatre *et al.* confirmed almost 60% of crude oil deterioration. *Bacillus sp.,* such as *Penebacilus Polymyxa, Paenibacillus Lautus, and Brevi bacillusagri* were isolated during the research for nitrogen-fixing hydrocarbon degradation. In nature, these gram-positive spores forming, *Bacillales* have shown significant availability and variation in physiology and phylogeny. Degradation of most materials obtained from animal and plant sources having ability to decompose a variety of macromolecules such as protein and polysaccharides are among common features [58]. Sporogenisis allows *Paenibacillus, bacillus*, and related organisms to survive in severe environmental conditions for longer durations, these are two genera of bacteria that fix nitrogen. They were isolated from different sources such as soil, water, rhizosphere, food, and infected insect larvae. Brevibacterium and Bacillus species are identified abundantly in hydrocarbon-contaminated soil. However, lack of data on Brevibacillus' hydrocarbon degradation, while information on its ability to handle high concentrations of a variety of organic solvents is available which cannot be used as a carbon source [59].

The earlier results effectively demonstrated the vulnerability of hydrocarbons to biodegradation which varies depending on the type and size of hydrocarbon molecule. On crude oil, kerosene, diesel, and liquid aromatic hydrocarbons isolate effectively including n-hexadecane (C16H26). Linear and branched Alkenes with distinct chain lengths and aromatic compounds are used to synthesize kerosene and diesel. Especially, linear alkenes are known as biodegradability chemicals [60]. Saturated aliphatic hydrocarbons for instance hexane (C6H14) and decane (C10H22) could sustain the isolate's growth, although it's not an optimal carbon source which indicated slow growth in aromatic-hydrocarbon essentially in xylems and benzene. Cyclic hydrocarbons including cyclohexane and motor oil that are complex molecules of long-chain hydrocarbons ranging from (C16-C36), cyclic olefins and some essential metals components showed slow growth, there is no utilization of phenol. Recalcitrant to biodegradation is an aromatic-hydrocarbon (C16-C36) and cyclic-alkene. In general, the vulnerability of hydrocarbons to pathogen attack decreases in order listed below N-Alkenes > branched-alkenes > low-molecular weight aromatic > cyclic-alkenes.

Commonly, we isolate a huge number of hydrocarbon-degrading microbes as results of different bio-degraders are present in the environment which involved in the degradation of hydrocarbons. Bacteria possess a regulating system that produces enzymes capable of initiating an attack on this chemical only when essential. Petroleum hydrocarbon residues are degraded by naturally occurring bacteria in soil and water [61].

Furthermore, Fungal genera including *Amorphoteca, Neosar toryagraphium and Talaromyce* and yeast genera i.e., *Yarrowia, Candida, and Pichia* were obtained from petroleum-contaminated earth and revealed to be significant hydrocarbon degradation microbes. Singh a family of terrestrial fungi known as *Aspergillus, Pencillium and Cephalosporium* also was reported being a potential crude oil hydrocarbon degrader [62]. Yeast species such as *Candida lipolytica, Geotrichum sp., Rhodotorula Mucilaginous, Trichosporon mucoides and Rhodosporidium Toruloides* were found to decompose petroleum substances after being isolated from contaminated areas. Hidayat and Tachibana revealed that *Fusarium so* [63]. *F092* can destroy chrysene and aliphatic portion of crude oil when contaminated with artificial seawater in liquid culture (35 %) algae and protozoa are essential components of the microbial population in both terrestrial and aquatic ecosystems, but only a few observations on their role in hydrocarbon biodegradation are reliable. walker et al.., obtained an alga, *Prototheca zopfi* that used crude oil and combined hydrocarbon substrate and degraded n- alkenes and aromatic hydrocarbons effectively. Cerniglia *et al.* reported microbes that oxidize naphthalene such as one red alga, two diatoms, five green Algae, and nine cyanobacteria [64].

Gems For the Removal of Petroleum Hydrocarbon

Genetic engineering has enhanced the degradation process of hazardous through laboratory condition from last 20 year by generating GMO (genetically modified organisms). DNA recombinant and genetic engineering are both used to assemble recombinant microbes. Genetic engineering used to develop microbes having degradation gene, for this we identified specific gene and insert in bacteria developed recombinant bacteria against pollutants [65]. GEMS demonstrated bioremediation capability in soil, sludge environment, ground water with increase detractive power for wide spectrum of chemical in contamination. First time GEMS was observed in field for bioremediation through university of Tennessee cooperation with oak ridge national laboratory. In the contaminated environment they are reporting genetically modified *Pseudomonas fluorescent* HK44GEM strain [66].

Conclusions

Globally, eight million tons of petroleum is discharged into the environment. Bioremediation of petroleum polluted sites has become a major concern in cities, industrial sites, and in areas where crude oil and natural gas are drilled. Bioremediation is a popular remediation strategy for petroleum hydrocarbons contaminated soils have several advantages due to low cost compared to other techniques. From the present study, it can be concluded that soil and sludge contain some bacteria and fungi that can bring about biodegradation of petroleum hydrocarbon. Mostly bacteria and fungi are attached to the surface of oil and bring biodegradation of petroleum hydrocarbon. There is a great possibility to find the microbes of extreme harsh environment which can degrade petroleum hydrocarbon.

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