Ecological Questions 33 (2022) 2: 43–52 http://dx.doi.org/10.12775/EQ.2022.012

Bioremediation of Petroleum Contamination: A Short Review

Saimah Khan[1], Ayush Kumar Tripathi[1], Rohan Srivastava[1], Mohd Saad Saleem[1], Sergiy Yeremenko[2*], Volodymyr Sydorenko[2]

[1]Integral University Lucknow, UP, India
[2]Institute of Public Administration and Research in Civil Protection, Kyiv, Ukraine
*corresponding author's e-mail: esamns71@gmail.com

Received: 13 January 2022 / Accepted: 21 February 2022

Abstract. The pros and cons of using the bioremediation method for the removal of petroleum pollutants are discussed in this review article. Other methods along with bioremediation have been used to remediate petroleum hydrocarbon contaminants in the past. Bioremediation is cheap and efficient method than any other because major constituents of the crude oils are biodegradable. Despite the fact that, as compared to physicochemical strategies, longer periods are normally required, complete pollutant degradation can be achieved, and no further containment of the contaminated matrix is required. According to hydrocarbon present in the contaminants different strategies and organism are used for the bioremediation.

Common strategies include controlling environmental factors such as oxygen availability, hydrocarbon solubility, nutrient balance and managing hydrocarbon degrading bacteria by eliminating the rate limiting factors that may slow down the bioremediation rate. Microorganism dynamics during bioremediation is most important for understanding how they respond, adapt and remediate pollution. However, bioremediation can be considered one of the best technologies to deal with petroleum product contaminants.

Keywords: bioremediation, petroleum, pollutant, degradation.

1. Introduction

The demand for petroleum as a source of energy and as a primary source of raw material in many industries like a lubricant and petroleum jelly has been increasing. In 2016 world consumes 35,442,913,090 barrels of oil which is equivalent to 97,103,871 barrels per day. Global oil consumption per capita is 5 barrels of oil (199 gallons) per person each year (based on the 2016 world population of 7,464,022,049 people) or 0.5 gallons per capita per day. (www.worldometers.info/oil) Oil spills came into the picture in 1967, when approx. 96-165 million liters of oil were released by the Torrey Canyon super tanker into the south-west coast of the United Kingdom. According to data from the U.S. National Oceanic and Atmospheric Administration (NOAA), there had been 137 oil spills in 2018, about 11 per month in the U.S. only (There Were 137 Oil Spills in the US in 2018. See Where They Happened – Resource Watch Blo). It also noted that increase in oil pollution is basically due to the reason that major oil- producing countries (Saudi Arabia and Iraq) are not the major oil-consuming countries (China and India). In addition, environmental pollution with oil products may be due to the irrational activities of society (Malovanyy et al., 2018; Vambol et al., 2017). Petroleum is composed of three hydrocarbons, primarily paraffin and naphthene's, with a smaller number of aromatic compounds, with at least one aromatic ring. Ocean contaminated with oil spills damage plants such as seaweeds and kelp. It also limits the presence of sunlight in the ocean that can lead to stop or slow down the photosynthesis in the marine plant. Soil contaminated with oil spills causes organic pollution of groundwater that can decrease the agriculture activity of soil. For the clean-up of the oil-polluted sites, many techniques have been applied (Zelenko et al., 2019; Afzal et al., 2019; Cherukupally et al., 2016), and one of them is bioremediation. Bioremediation is used to detoxify the pollutants present in the environment (water, soil, and sediments) which can threaten public health and nature. The bioremediation techniques are cost-effective, environmentally friendly, and have ability to achieve complete degradation (Ziarati et al., 2020). The main problem that arises with this technique that limits their use is that less solubility of hydrocarbon, which restricted the growth of bacteria. Microorganisms serve as a biocatalyst in bioremediation and can be used as a source of carbon and energy. Therefore, microbes used for bioremediation have to be optimized in order to improve bioremediation.

1.1. Hydrocarbons as pollutants

The petroleum hydrocarbon is the organic pollutants with complex composition, wide distribution and toxicity. The most common petroleum hydrocarbon includes branched, aliphatic, cycloaliphatic alkanes, monocyclic and polycyclic aromatic hydrocarbon (PAH). PAH includes naphthalene, fluorene and anthracene etc. Petroleum does not have defined composition rather it's a combination of different hydrocarbon result in different volatility, toxicity, persistence and degradability.

Presence of oil in water can block oxygen, light and nutrients access to aquatic plants and animals. The availability and toxicity of hydrocarbon present in petroleum depend on their physical and chemical nature. Lethal effect of petroleum hydrocarbon depends on three factors a) duration of susceptibility, b) way of susceptibility and c) nature of petroleum fraction. Damage caused by the petroleum hydrocarbon may take several months and years. The aromatic compounds present in petroleum are environmental pollutants naphthene-aromatic, polyaromatic compounds are in heavy fractions. This compound has a carcinogenic effect, may lead to tumors, and damages the nervous system. The petroleum hydrocarbon contains polar organic compounds which can easily drift from the contamination sites into the ground water which ultimately result in the increase of petroleum hydrocarbon in the drinking water and make them unsafe for human beings and aquatic plants.

1.2. Principal of bioremediations

Petroleum is a mixture of thousands of different hydrocarbons of various types. There are several ways to treat them with microbes. Microbes added or present in the soil can attach themselves to the hydrocarbon and use them as a source of carbon and energy. The low solubility of high molecular weight hydrocarbon can limit the bioremediation rate which can be improved by the addition of biosurfactants. Due to the difference in chemical nature of different hydrocarbons present in petroleum, some of them can be readily degraded, some resist degradation, and some are non-degradable. The Bioremediation of these compounds occurs simultaneously but at a different rate, which results in the removal, of individual components of petroleum over a certain period. Enzymes are produced by microorganisms, which are responsible for the bioremediation of petroleum. Many pathways and different types of enzymes are involved in this process. It may also note that absence of any effective enzymes (that are produced by microbes), can act as a barrier or result in incomplete degradation.

1.3. Bacteria

Freeze-dried bacteria are commercially available for the decomposition of hydrocarbon, which can be used, for bioremediation after propagation to a minimum of 2×108 CFU/mL (Thapa et al., 2012). Bacteria that can degrade the petroleum hydrocarbon are are-Pseudomonas, Aeromonas, Moraxella, Beijerinckia, Flavobacteria, Nocardia, Corynebacteria, Acinetobacter, Mycobacteria, Modococci, Streptomyces, Bacilli, Arthrobacter, Aeromonas, Cyanobacteria, etc. Although many microorganisms can degrade the petroleum hydrocarbon, the use of mixed culture bacteria instead of pure culture bacteria is found efficient, and their degradation also occurs at a higher rate. The growth and activity of the microorganism must be stimulated apart from carbon as an energy source microorganism also require a small amount of nitrogen and phosphorus.

1.4. Hydrogen degradation

The main hydrocarbon degraders are bacteria, yeast, and filamentous fungi (Van Beilen & Funhoff, 2007). The bioremediation process is depending on the genetic potential of the microorganisms, which act by introducing molecular oxygen into the hydrocarbon. Oxygen is necessary for the breakdown of hydrocarbons and it help in complete degradation of oil but if large quantity of oil is spills then consumption of oxygen is also very high, that can decrease the hydrocarbon degradation rate as well. In order to overcome this problem iron, nitrate, and sulphur rich fertilizers are added. Rate of degradation in anaerobic environment is less than that of aerobic. The availability of oil to bacteria is limited as it is hydrophobic in nature which leads to the slow degradation. Bioavailability can be increase by reducing surface tension. Surface tension can be reduced by surfactants, secreted by microbes called biosurfactants. Biosurfactants can be glycolipids, fatty acids, and lipoprotein. Oxygenase is the key enzyme in the hydrocarbon degradation, which catalyse the addition of molecular oxygen to the substrate (Rojo, 2009). Anaerobic degradation can be achieved by coupling carbon dioxide to hydrogen.

1.4.1. Alkanes

Unbranched alkanes are the major constituents of the petroleum hydrocarbon. The initial step of alkane degradation is oxidation by oxygenase. Alkane is first oxidized into an alcohol then into an aldehyde, which is transformed into fatty acids. Fatty acids are further oxidized by β-oxidation into odd chain alkanes (acetone and propionate). For example, Pseudomonas putida PbG6 (Oct) grows on alkanes of six to ten carbons in chain length (Nieder & Shapiro, 1975), whereas Acinetobacter sp. HO1-N is capable of growth on long-chain alkanes (Singer & Finnerty, 1984). Long chains secondary alcohols and ketones may be formed by the oxidation of subterminal alkanes (Markovetz, 1971).

1.4.2. Aromatics

Single ring like (benzene, toluene and xylene) and polycyclic aromatic compounds (naphthalene, anthracene) can be oxidized by both prokaryotic and eukaryotic microorganisms to from cis-dihydrodiol. Further oxidation of cis-dihydrodiol leads to the formation of catechol, trans-dihydrodiol can be formed by the oxidation of aromatic hydrocarbon with fungi and eukaryotic microorganism. Catechol can be oxidized either by meta pathway which involves cleavage between one of the carbons with a hydroxyl group and the adjacent carbon or by ortho pathway, which involves cleavage of the bond between the two carbon atoms bearing the hydroxyl groups to form cis-muconic acid. Substituted aromatics, like toluene, can either be initially oxidized by bacteria at the methyl to make benzoic acid, or on the aromatic ring to make a substituted dihydrodiol. Benzoic acid is converted to catechol and therefore the substituted diol is converted to 3-methylcatechol. The resistance of PAHs towards bioremediation increases with increase in molecular weight because higher the molecular weight results in low solubility and bioavailability of PAHs.

1.5. Research Methodology

This scientific review is conducted to highlight recent advances in bioremediation for the removal of oil pollutants, present an analysis of the information in the reviewed sources, as well as identify gaps in this area and prospects for future research. In this regard, the search for sources was carried out in Scopus, PubMed, ScienceDirect, ResearchGate, Google Scholar on the totality of the keywords of this study in various combinations. Since the keywords are in English, English-language sources were studied. After receiving the search results, scientific publications were selected containing the following information about:

- Interaction of microorganisms with hydrocarbons
- Nutrient requirements
- Factors affecting the degradation of oil hydrocarbons
- Mechanisms of oil hydrocarbons degradation
- Enzymes involved in the degradation of hydrocarbons and bioremediation agents available on the market
- Bioremediation strategies.

If there were duplicate publications, they were excluded. This sorting was done manually. At this stage, the primary database of publications was formed. Then, a search for sources among the references of selected publications was carried out, which made it possible to identify additional literary sources.

The information obtained was analyzed, verified by comparing with information (results, conclusions, conclusions) in other sources containing similar studies, and structured using synthesis, generalization and deduction.

2. Interaction of microorganism with hydrocarbons

Degradation of hydrocarbon involves the direct contact of hydrocarbon substrate with bacteria. Two methods can be used to increase the contact between the bacteria and the water insoluble hydrocarbons: adhesion and desorption, and b) emulsifier.

2.1. Adhesion and desorption

Growth of microorganism involves the adhesion of microorganism on the water insoluble hydrocarbon caused by the hydrophobic interaction, higher the non-polarity in the hydrocarbon substrate higher the hydrophobic interactions. The component of the bacteria which contribute to the cell-surface hydrophobicity termed as hydrophobins, such as fimbriae, fibrils, outer membrane and other surface proteins and lipids. Bacteria lacking these fimbriae failed were unable to grow on the hydrocarbon. Desorption from the used hydrocarbon is most critical and important part of the hydrocarbon degradations. Petroleum has the vast variety of hydrocarbons and particular bacteria only uses specific part of the hydrocarbon, so the relative amount of the non-utilizable hydrocarbon increases and bacteria were unable to grow. To overcome this, bacteria must be able to move from the used one hydrocarbon to the new one. When the bacteria became starved on the ‚used hydrocarbon, they release their capsule. The capsule is composed of an anionic heteropolysaccharide, with fatty acid side-chains, referred to as emulsan (Rosenberg, 1986). Each used hydrocarbon is then covered with a monomolecular film of emulsan. The re-attachment of bacteria is prevented by the hydrophilic outer surface of emulsan-coated hydrocarbon.

2.2. Emulsifier

Hydrocarbon degrading microorganism produce a wide range of surface-active agents called biosurfactants. These biosurfactants produce by microorganism can be divided into two categories a) low molecular weight surfactants, and b) high molecular weight surfactants. Mixture of glycolipids, fatty acids, and lipopeptides are the low molecular weight surfactants, complexes of hydrophilic and hydrophobic polymers and amphipathic polymers are the high molecular weight surfactants. Biosurfactants increases the growth of bacteria on the petroleum hydrocarbon by two methods a) increasing the hydrocarbon surface area, and b) desorbing the bacteria from the used hydrocarbon. When the bacteria adhere to the hydrocarbon surface, they begin to multiply on the surface. After some time, the surface will be full with bacteria and growth will be limited by the free surface. Now if bacteria can split the oil droplets (emulsification), new surface will become available and bacteria can grow. Desorption from used hydrocarbon is more critical faction of emulsifier. After the alkanes within the petroleum droplet are consumed, bacteria become starved, although it's still attached to the hydrocarbon enriched in aromatic and cyclic paraffins. Starvation of bacteria causes release of the mini capsule of emulsan. This discharged emulsan forms a polymeric film on the n-alkane-depleted oil droplet, thereby desorbing the starved cell. In effect, the ‚emulsifier' frees the cell to seek out a fresh substrate. At an equivalent time, the depleted oil droplet has been ‚marked' as getting used by having a hydrophilic outer surface to which bacteria cannot attach. Presumably, other microorganisms which may attach to hydrophilic surfaces and degrade aromatic and cyclic hydrocarbons continue the method of petroleum biodegradation.

3. Nutrient requirements

The bioremediation depends on the availability of oxygen, and utilizable sources of nitrogen and phosphorus. The availability of oxygen is not a problem in the case of oil spill in water. However, they can act as a limiting factor in oil spill on land in these circumstances; hydrogen peroxide has been used as a source of oxygen (Huling et al., 1991). Availability of nitrogen and phosphorus is the limiting factor in bioremediation of hydrocarbon on land and water. In theory, approximately 148 mg of nitrogen and 32 mg of phosphorus are consumed within the conversion of 1 g of hydrocarbon to cell material which may be satisfied by the ammonium phosphate and with a mixture of other salts, such as ammonium sulphate, ammonium chloride, potassium phosphate, sodium phosphate and calcium phosphate. When ammonium salts of strong acids are used, the pH of the medium generally decreases with growth, using urea as the nitrogen source will solve this problem. All of these compounds have a high-water solubility, which reduces their effectiveness in open systems because of rapid dilution.

4. Factors affecting petroleum hydrocarbon degradation

Among physical factors, temperature plays an important role in the biodegradation of hydrocarbons directly affect the chemistry of pollutants and effect the physiology and diversity of the microbial flora. Atlas found that at low temperatures, the oil viscosity increases, and the toxic lowers (Brusseau, 1998) the molecular volatility the weight of hydrocarbons is reduced, thereby delaying biodegradable. The rate of biodegradation generally decreases with decreasing temperature. In ingredients nitrogen are the most important for biodegradation of hydrocarbons related pollutants and also for some cases of Iron. Excessive nutrients can also lead to the biodegradation (Floodgate, 1984).

5. Mechanism of petroleum hydrocarbon degradation

Oxidative process is an initial intercellular attack of the organic pollutants and oxygenase and peroxidase (Hommel, 1990) catalyze the enzymatic reaction for activation and incorporation of Oxygen. Several degradation pathways convert pollutants into Intermediary metabolism intermediate. Degradation can be carried or achieved by specific use of enzyme (Fig. 1) shows the initial attack on Oxygenase.

Among physical factors, temperature plays an important role in the biodegradation of hydrocarbons directly affect the chemistry of pollutants and effect the physiology and diversity of the microbial flora. Atlas found that at low temperatures, the oil viscosity increases, and the toxic lowers (Floodgate, 1984) the molecular volatility the weight of hydrocarbons is reduced, thereby delaying biodegradable. The rate of biodegradation generally decreases with decreasing

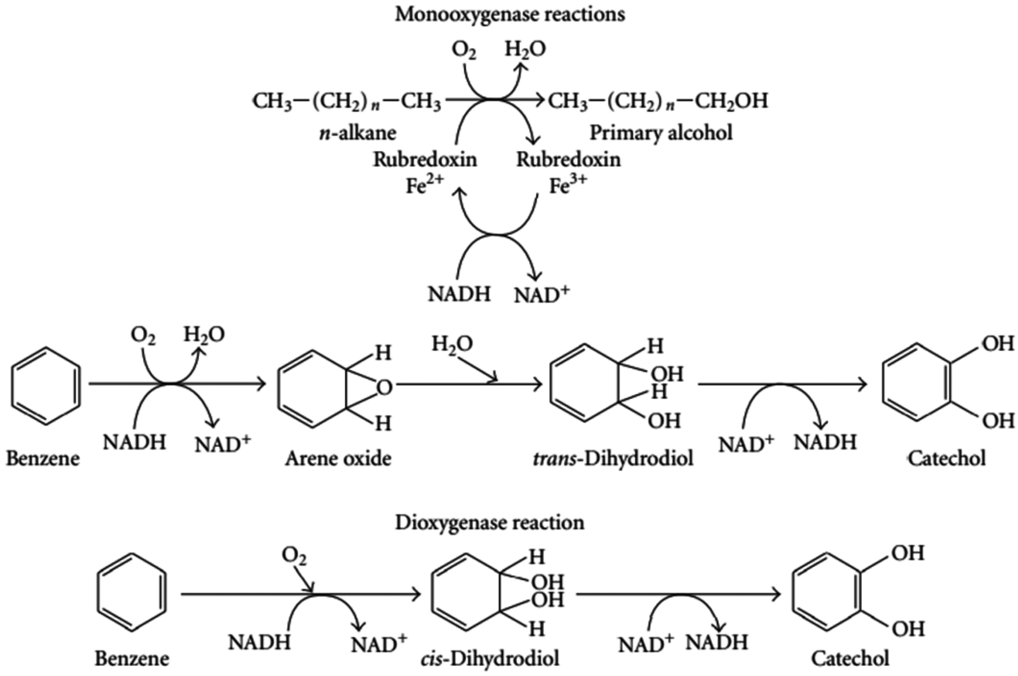

Figure 1. Enzyme reactions for hydrocarbon degradation (Leahy & Colwell, 1990)

temperature. In ingredients nitrogen are the most important for biodegradation of hydrocarbons related pollutants and also for some cases of Iron. Excessive nutrients can also lead to the biodegradation (Hommel, 1990).

6. Enzymes involved in hydrocarbon degradation

Cytochrome P450 (Hommel, 1990) has a ubiquitous Heme-thiolate Monooxygenase which pays key role in microbial degradation of hydrocarbons, oils, fuel additives and others. The main role or we can say function of enzymes attached systems that to introduce Oxygen just only to initiate biodegradation. In microorganisms such P450 (Van Beilen & Funhoff, 2007) multiplicity can only be found in few species. Cytochrome P450 enzyme systems were found to be involved in biodegradation of petroleum hydrocarbons (Table 1) (Zimmer et al., 1996).

In the global market, the demand of bio lubricants is growing as a strong alternative for engine oils, hydraulic oils and marine oils. 56% of the demand for bio-lubricant comes from the engine oil. In India, research on bio lubricants as an alternate for different tribological applications is on priority

Table 1. Enzymes involved in biodegradation of petroleum hydrocarbons

Enzymes	Substrate	Microorganisms	References
Soluble Methane Monooxygenas es	C_1-C_8 alkanes alkenes and cydoalkanes	Methylococcus, Methylosinus, Methylocystis, Methylomonas, Methylocella	McDonald and Portier (2003)
Particulate Methane Monooxygenas	CI-Cs (halogenated) alkanes and cycloalkanes	Methylobaaer, Methylococcus, Methylocystis	McDonald and Portier (2003)
AlkB related alkane hydroxylase	Cs-C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	Pseudomonas, Burkholderia, Rhodococcus, Mycobacterium	Moody et al. (2001)
Eukaryotic P450	Cio-C_{16} alkanes, fatty acids	Candida maltose, Candida tropicalis	Hanano et al. (2015), Iida et al. (1998)
Bacterial P450 oxygenase system	C_5-C_{16} alkanes, cydoalkanes	Acinetobacter, Caulobacter, Mycobacterium	Van Beilen and Funhoff (2007)
Dioxygenases	Cio-C_{30} alkanes	Acinetobacter sp.	Maeng et al. (1996)

as eco-friendly lubricants which are gaining importance in global market. Vegetable oils and oils made from palm, canola, and sunflower seeds provide the base oil for the more commodity products. North American manufacturers are instead developing innovative biosynthetic base oil solutions, which may represent the future of bio-lubricant technology. Increasing the supply of synthetic bio-based lubricant characterized by high performance standards and offering good value for money is bringing the bio- lubricants industry to the next level, but high costs the next thing to be overcome. The size of the global market for bio-lubricants, according to one analysis, is estimated between 250 and 300 kilotons in 2016. North America and Europe are having the highest consumption of bio-lubricants due to environmental policies.

7. Immobilized cell participation

It has been used since long for the study of bioremediation of petroleum pollutants and other toxic chemicals. Biotechnology Research International Volume 2011, Article ID 941810 state that "The study indicated that immobilization resulted in a combination of increased contact between cell and hydrocarbon droplets and enhanced level of rhamnolipids production. Rhamnolipids caused greater dispersion of water- insoluble n-alkanes in the aqueous phase due to their amphipathic properties and the molecules consist of hydrophilic and hydrophobic moieties reduced the interfacial tension of oil-water systems. This resulted in higher interaction of cells with solubilized hydrocarbon droplets much smaller than the cells and rapid uptake of hydrocarbon in to the cells" (Díaz et al., 2002). Immobilization can be done in continuous mode as well as bed mode, in continuous mode packed bed reactors are commonly used for hydrocarbon degradation. Several experiments done to understand the capacity of Immobilized bacteria to degrade hydrocarbon but there was no decline in the biodegradation activity of microbial consortium on the repeated use. At last, it was concluded that Immobilized cell are very promising for those places which are polluted from the petroleum hydrocarbons and they can be cleared by the help of Immobilized cell and their biodegradation action on the pollutants.

8. Bioremediation agents available in market

Several agencies have declared the Bioremediation agents as "microbiological culture, enzyme additives and nutrient additives that increases the rate of biodegradation" (Rahman et al., 2006; Atlas, 1981). Numerous bioremediations have been enlightened and promoted by the different vendors, for this several SOP has been declared by the US EPA along with the list of bioremediation agents as a part of National Oil and Hazardous Substances Pollution Contingency Plan (NCP), Product Schedule (Mearns, 1997; Hoff, 1993).

Table 2 contains list of 15 bioremediations agents (Nichols, 2001) that are defined by us EPA. Several tests have been defined in laboratory that clarifies that the essential trials have been done before submitting the final name of the agents the protocol is developed by EPA (Nicholas, 2001). Listing of products does not confirm that the agents are approved or certified and can be used for oil spill. Minimum 28 days Bioremediations should be cleared by the products is only the efficacy requirement for being listed. In comparison to the normal microbial products very few nutrient additives have been added to bioremediations product specially used for oil spill cleanup. It is probably because common fertilizers are inexpensive, readily available, and have been shown effective if used properly. However, due to the limitations of common fertilizers (e.g., being rapidly washed out due to tide and wave action), several organic nutrient products, such as oleophilic nutrient products, have recently been evaluated and marketed as bioremediation agents. Four agents, namely, Inipol EAP22, Oil Spill Eater II (OSE II), BIOREN 1, and BIOREN 2, listed on the NCP Product Schedule have also been put into this category (US EPA, 2002).

Table 2. Bioremediation agents in NCP product schedule (US EPA, 2002)

Name	Product Type	Manufacture
BET BIOPETRO	MC	BioEnviro Tech, Tomball, TX
BILGEPRO	NA	International Environment Products
LAND AND SEA RESTORATION	NA	Land and Sea Restoration, LLC TX
INIPOLE EAP 22	NA	Société, CECA S.A. France
MICRO-BLAZE	MC	Verde Environmental, Inc., Austin, TX
OIL SPILL EATER II	NA/EA	OSE International, Corporation Dallas, TX
STEP ONE	MC	B&SResearch, Inc, Embrass MN
BINUTRIX	NA	BioNutraTech, Inc, Houston, TX
WIMI-2000	MC	WMI Intl, TX

Abbreviation of product type: MC – Microbial Culture; EA – Enzyme Additive; NA – Nutrient Additive.

9. Bioremediation strategies

Bioremediation can be achieved either by situ or ex-situ treatments. Aim of choosing bioremediation strategies is to remove of any limiting factors and increase the bioremediation rates. Situ treatment involves the treatment at the polluted site only, whereas in ex-situ treatment polluted soil is transported to the place, where suitable treatment environment can be established. Four major problems that may be occurred during bioremediation of petroleum hydrocarbons are 1) limitation of nutrient such as nitrogen and phosphorus due to excess of hydrocarbon source, 2) insufficient oxygen during aerobic degradation, 3) low availability of hydrocarbon, 4) non-efficient microorganisms with can slow the degradation rates Bioaugmentation, biostimulation, and bioventing strategies are used in situ treatments for hydrocarbon polluted soils. Bioaugmentation is the addition of genetically modified microorganisms to treat the contaminated sites. It is effective where native microorganisms do not have the capability to perform the degradation. Biostimulation is the process of adding nutrients and optimizing environmental condition at the treatment sites to stimulate the bacteria involve in degradation. Bioventing is the process used to enhance the aerobic bioremediation by providing air using slotted pipes. Biopiles, and landfarming are the common strategies are used in ex-situ treatments for hydrocarbon polluted soils. Biopiles is the process of mixing polluted soil with organic materials this improves microbial activity by enhancing aeration, moisture, and soil texture (Jørgensen et al., 2000). Landfarming is the process in which contaminated soil is mixed with some fertilizers and rotated occasionally. It increases the degradation rate by enhancing the bacterial growth. Organic maters that have anti-oxidant property can increase the bacterial tolerance to toxicity of aromatics and the oxidative stress during their degradation (Ponce et al., 2011). Physiochemical process costs are commonly higher than the bioremediation, cost depend on the depth of pollution in the contaminated soils and soil type. In general, costs per soil volume increase when the site is small, the pollution is deep, and the soil particles are small, i.e., clay/silt soil is harder to remediate than sandy soils (US FRTR, 2014). In addition, the possibility of delivering microorganisms to the contaminated area should be taken into account, since contaminated places are not always easily accessible. In some cases, the delivery of microorganisms for bioremediation of significant contaminants can be achieved using nozzles similar to those used in the study (Vambol et al., 2019).

10. Case studies

10.1. Bioremediation of Prince William Sound

It was probably the largest marine bioremediation project ever undertaken. Around 40,000 tons of oil is spilled into Prince William Sound, Alaska on 24 March, 1989 by oil tanker Exxon Valdez. The immediate action was to unload the cargo and collect as much oil as possible with skimmers from the sea and washed beaches. Fertilizer Inipol EAP22 (a microemulsion of urea, oleic acid, lauryl phosphate, 2-butoxy-l- ethanol and water) was chosen for most of the treatment. Initially there were remarkable removable of oil was observed in two weeks. A 'clean rectangle' was observed in the arial photograph, on the area where Inipol was applied, surrounded by a darker area of oiled cobblestones. Addition of fertilizer enhances the biodegradation rates of hydrocarbons in field as high as 12.5% per day. Degradation rates decreases even after the fertilizer was reapplied when the more readily component of the oil was depleted (Atlas & Hazen, 2011). During 1989-1991 more than 48 tons of nitrogen containing fertilizer was applied deposits analysis during 1989 shows that 25-30% of the petroleum hydrocarbon on shorelines was degraded within the first few days to weeks. Only 1.3% of the shoreline remained polluted by the 1992 (Bragg et al., 1994).

Monitoring biodegradation after the EVOS accident left lessons about the importance of nutrients balance for bioremediation. Appropriate nitrogen-containing fertilizer amounts showed to be critical. Oxygen, phosphorous, hydrocarbon bioavailability, and oil age were less important (Rosenberg et al., 1982).

10.2. Oil spill in Haifa beach

Approximately 100 tons of oil was spilled accidentally on August 1991, between Haifa and Akko in Israel. The sand contaminated with oil was collected into piles spread over the beach. Bacteria that were chosen for bioremediation can utilize hydrocarbon as their energy and carbon source, and extract utilizable nitrogen and phosphorus by enzymatically hydrolysing fertilizer. These types of bacteria are rare, so it is necessary to immunize the bacteria before using for bioremediation. Fertilizer F-l (38 kg, in the form of a fine powder) was then added as the source of nitrogen and phosphorus, the experimental plot was immunized with 20 liters of a mixture of three selected bacteria (5×10^8 cells/mL), and was watered from the adjacent sea (water temperature was 27°C). The control plot was left undisturbed. The experimental data are presented in Table 3. The core samples were taken on day 0 (September 1, 1991). At the start of the experiment, the experimental plot contained significantly more hydrocarbon (3.80 mg/g sand) than the control plot (2.30 mg/g sand). There was only a little decrease within the first day. However, by the fourth day, 30% of the hydrocarbon had been degraded. The biodegradation continued, reaching 50% on day 9 and 84.5% on day 25, when the experiment was concluded. The control plot showed 18% degradation by day 9 which remained relatively constant until day 25.

Table 3. Bioremediation of hydrocarbon-contaminated sand at Haifa beach (Rosenberg et al., 1982), Total petroleum hydrocarbon (TPH)g/Kg

Day	Control	%	Experiment	%
0	2.30	100	3.80	100
4	2.53	110	2.76	73
9	1.88	82	1.89	50
14	1.70	74	0.88	23
21	1.94	84	1.40	23.37
25	1.95	85	0.95	25

Analysis of alkane fractions using gas chromatography shows that at day 0, C_{20}-C_{30} were most abundant and was degraded to the extent of 94%, whereas the C_{14}-C_{18} and C_{36}-C_{40} were degraded to 80% and 75% respectively.

30,000 m^2 sand was treated in the field trial, since the treatment took place in winter temperature was about 5-10°C for a couple of months, it was not necessary to water. After four months, result of the experiment shows that 88% of oil was degraded, whereas no significant degradation was observed in control plots.

11. Conclusion

In nature aerobic process dominate the hydrocarbon breakdown. The main aerobic strategy for hydrocarbon activation is hydroxylation. Successive oxidation of alkane produces carboxylic acids that can be β-oxidation pathway. High diversity of mono- and dioxygenases for alkane hydroxylation has been reported. Aromatic hydrocarbons are oxidized by RHDs, which are less diverse than alkane oxygenases. Hydrocarbon degradation pathways expand the microbial metabolic versatility and the carbon source range for growth.

Bioremediation is usually case-specific; however, some general rules can be pointed out. Bioremediation is usually simple, and less labour is required. It is the most economical method for the treatment of contaminated soil if the soil to be treated is more. Rate of bioremediation can be increased by managing environmental conditions such as temperature, pH, dissolved oxygen, water content, and nutrient availability. The efficacy and safety of using bioremediation techniques will have to be convincingly demonstrated and communicated to the public.

The paper gives the state of art related for tribological behavior of Biolubricants with nanoadditives, performance of nanoparticles as an additives and mechanism of lubrication of nanoparticles additives. The research conducted notified that the nanoparticles as additives in Biolubricants enhance its performance. The lubrication mechanism of nanoparticles reported in the paper is complex to understand, because there are large number of nanoparticles and each nanoparticle work in a different manner for different applications. Nanoparticles performance parameters, is also reported in the paper and the most important performance parameters ie. Dispersion stability is important to be maintained for better performance of the lubricants. It was reported that different dispersion techniques, surface modification techniques and surfactants are used for complete dispersion of the nanoparticles in the Biolubricants. Biodegradability is an important issue and the global demand of Biolubricant is also reported in the paper which focuses on the development of biodegradable bio lubricants as the alternative for mineral oils. For the future, researcher must work on the development of new nano additives; chemically modified Biolubricants for different tribological applications.They should work on new lubrication mechanism and performance parameters which affect the tribological properties of the Biolubricants.

It has been found that several factors affecting the bioremediation which includes:

Microbial population-Microorganisms make their availability almost everywhere and make themselves active for the pollution to get interact with them.

Temperature-Temperature has its specific role ln the activity of microorganisms, after reaching at high temperature the activity of microorganisms' increases (Leahy & Colwell, 1990).

Moisture-Water is essential for microorganisms to work as degradants. Low water availability reduces the intracellular water potentials resulting in decrease of hydration and activity of enzyme (Abatenh et al., 2017).

pH-pH determines the acidity and basicity of different compounds. In general, soil whose pH is 7.5 exhibit greatest and fastest rate of biodegradation (Pawar, 2015).

Pollutants even in small volume but spread in a large area can also easily disturb the balance of ecosystem, substances that are accumulated at a specific place for a long period or for a long unused period is considered as pollutant the pollutant substrate is calculated by matching the values pf substrate with the different parameters considering concentrations, pH, temperature change, acidity and alkalinity (Leahy & Colwell, 1990).

Enzymes are used to remove the excess nutrients present in form of pollutants, which are actual hazards for the ecosystem and also for the human beings. Enzymes used as bioremediation are cost-effective, self-reliable, less toxic, and handier and also, it's a cost effective.

References

Abatenh E., Gizaw, B., Tsegaye, Z. & Wassie M., 2017, The role of microorganisms in bioremediation-A review. Open Journal of Environmental Biology 2(1): 030-046.

Afzal M., Rehman K., Shabir G., Tahseen R., Ijaz A., Hashmat A.J. & Brix H., 2019, Large-scale remediation of oil-contaminated water using floating treatment wetlands. NPJ Clean Water 2(1): 1–10.

Atlas R.M., 1981, Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiological Reviews 45(1): 180–209.

Atlas R.M. & Hazen T.C., 2011, Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. history. Environ Sci Technol 45: 6709–6715. doi:10.1021/es2013227.

Bragg J.R., Prince R.C., Harner E.J. & Atlas R.M., 1994, Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 368: 413– 418. doi:10.1038/368413a0

Brusseau M.L., 1998, The impact of physical, chemical and biological factors on biodegradation, [in:] R. Serra (ed.), Proceedings of the International Conference on Biotechnology for Soil Remediation: Scientific Bases and Practical Applications, p. 81–98. C.I.P.A. S.R.L., Milan, Italy.

Cherukupally P., Chu R.K.M., Bilton A. & Park C.B., 2016, October. Oil diffusion properties of acoustic foams for oil contaminated water treatment, [in:] AIP Conference Proceedings (Vol. 1779, No. 1, p. 050011). AIP Publishing LLC.

Díaz M.P., Boyd K.G., Grigson S.J.W. & Burgess J.G., 2002, Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. Biotechnology and Bioengineering 79(2): 145–153.

Floodgate G., 1984, The fate of petroleum in marine ecosystems, [in:] R.M. Atlas (ed.), Petroleum Microbiology, p. 355–398. Macmillan Publishing Co., New York, NY.

Hanano A., Shaban M., Almousally I. & Al-Ktaifani M., 2015, Saccharomyces cerevisiae SHSY detoxifies petroleum n-alkanes by an induced CYP52A58 and an enhanced order in cell surface hydrophobicity. Chemosphere 135: 418–426. https://doi.org/10.1016/j.chemosphere.2014.11.011

Hoff R.Z., 1993, Bioremediation: an overview of its development and use for oil spill cleanup. Marine Pollution Bulletin 26(9): 476–481.

Hommel R.K., 1990, Formation and phylogenetic role of biosurfactants. Journal of Applied Microbiology 89(1): 158–119.

Huling S.G., Bledsoe B.C. & White M.G., 1991, The feasibility of utilizing hydrogen peroxide as a source of oxygen in bioremediation, [in:] R.E. Hinchee and R.F. Olfenbuttel (eds), Bioreclamation, p. 83–103. Butterworth-Heinemann, Stoneham, MA.

Iida T., Ohta A. & Takagi M., 1998, Cloning and characterization of an n-alkane-inducible cytochrome P450 gene essential for n-decane assimilation by Yarrowia lipolytica. Yeast 14(15): 1387–1397. https://doi.org/10.1002/(SICI)1097-0061(199811)14:15<1387::AID-YEA333>3.0.CO;2-M

Jørgensen K.S., Puustinen J. & Suortti A.M., 2000, Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. Environ. Pollut. 107: 245–254. doi:10.1016/S0269-7491(99)00144-X

Leahy J.G. & Colwell R.R., 1990, Microbial degradation of hydrocarbons in the environment. Microbiological Reviews 54(3): 305–315. https://doi.org/10.1128/mr.54.3.305-315.1990

Maeng J.H., Sakai Y., Tani Y. & Kato N., 2021, Isolation and characterization of a novel oxygenase that catalyzes the first step of n-alkane oxidation in Acinetobacter sp. strain M-1. Journal of Bacteriology 178(13): 3695–3700. https://doi.org/10.1128/jb.178.13.3695-3700.1996

Malovanyy M., Zhuk V., Sliusar V. & Sereda A., 2018, Two stage treatment of solid waste leachates in aerated lagoons and at municipal wastewater treatment plants. Eastern-European Journal of Enterprise Technologies 1(10): 23–30. doi.org/10.15587/1729-4061.2018.12242

Markovetz A.J., 1971, Subterminal oxidation of aliphatic hydrocarbons by microorganisms. CRC Critical Reviews in Microbiology 1: 225–38. https://doi.org/10.3109/10408417109104482

McDonald J.A. & Portier R.J., 2003, Feasibility studies on in-situ biological treatment of drilling muds at an abandoned site in Sicily. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology 78(6): 709–716. https://doi.org/10.1002/jctb.847

Mearns A.J., 1997, Cleaning oiled shores: putting bioremediation to the test. Spill Science and Technology Bulletin 4(4): 209–217. https://doi.org/10.1016/S1353-2561(98)00026-7

Moody J.D., Freeman J.P., Doerge D.R. & Cerniglia C.E., 2001, Degradation of phenanthrene and anthracene by cell suspensions of Mycobacterium sp. strain PYR-1. Applied and Environmental Microbiology, 67(4): 1476–1483. https://doi.org/10.1128/AEM.67.4.1476-1483.2001

Nichols W.J., 2001, The U.S. Environmental Protect Agency: National Oil and Hazardous Substances Pollution Contingency Plan, Subpart J Product Schedule (40 CFR 300.900), [in:] Proceedings of the International Oil Spill Conference, p. 1479–1483. American Petroleum Institute, Washington, DC, USA.

Nieder M. & Shapiro J., 1975, Physiological function of Pseudomonas putida PpG6 (Pseudomonas oleovarans) alkane hydroxylase: monoterminal oxidation of alkanes and fatty acids. Journal of Bacteriology 122: 93–100. DOI:10.1128/JB.122.1.93-98.1975

Pawar R.M., 2015, The effect of soil pH on bioremediation of polycyclic aromatic hydrocarbons (PAHS). Journal of Bioremediation & Biodegradation 6(3): 291–304. https://doi.org/10.4172/2155-6199.1000291

Ponce B.L., Latorre V.K., González M. & Seeger M., 2011, Antioxidant compounds improved PCB-degradation by Burkholderia xenovorans strain LB400. Enzym Microb Technol 49: 509–516. doi: 10.1016/j.enzmictec.2011.04.021

Rahman R.N.Z.A., Ghazali F.M., Salleh A.B. & Basri M., 2006, Biodegradation of hydrocarbon contamination by immobilized bacterial cells. Journal of Microbiology 44(3): 354–359.

Rojo F., 2009, Degradation of alkanes by bacteria. Environ. Microbiol. 11: 2477–2490. doi:10.1111/j.1462-2920.2009.01948.x

Rosenberg E., 1986, Microbial Surfactants. CRC Critical Reviews in Biotechnology 3(2): 109–132. https://doi.org/10.3109/07388558509150781

Rosenberg M., Bayer E.A., Delaria J. & Rosenberg E., 1982, Role of thin fimbriae in adherence and growth of Acinetobacter calcoaceticus RAG-1 on hexadecane. Applied and Environmental Microbiology 44: 929–37.

Singer M.E. & Finnerty W.R., 1984, Microbial metabolism of straight-chainandbranchedalkane, [in:] R.M. Atlas (ed.), Petroleum Microbiology, p.1-60. Macmillan Publishing Co., New York, NY.

Thapa B., KC, A. K., & Ghimire A., 2012, A Review On Bioremediation Of Petroleum Hydrocarbon Contaminants In Soil. Kathmandu University Journal of Science Engineering and Technology 8(1): 164–170. https://doi.org/10.3126/kuset.v8i1.6056

U.S. EPA, 2002, Spill NCP Product Schedule, http:// www.epa.gov/oilspill

US Federal Remediation Technologies Roundtable (US FRTR), 2014, http://www. frtr.gov/matrix2/top_page.html [Accessed 20 Jan 2014].

Vambol S., Vambol V. & Al-Khalidy K.A.H., 2019, Experimental study of the effectiveness of water-air suspension to prevent an explosion, [in:] Journal of Physics: Conference Series (Vol. 1294, No. 7, p. 072009). IOP Publishing.

Vambol S., Vambol V., Suchikova Y. & Deyneko N., 2017, Analysis of the ways to provide ecological safety for the products of nanotechnologies throughout their life cycle. Eastern European Journal of Enterprise Technologies 1/10(85): 27–36. https://doi.org/10.15587/1729-4061.2017.85847

Van Beilen J.B. & Funhoff E.G., 2007, Alkane hydroxylases involved in microbial alkane degradation. Appl Microbiol Biotechnol 74: 13– 21. doi:10.1007/s00253-006-0748-0

Zelenko Yu., Malovanyy M. & Tarasova L., 2019, Optimization of heat-and-power plants water purification. Chemistry & Chemical Technology 13(2): 218–223. https://doi.org/10.23939/chcht13.02.218

Ziarati P., Vambol S. & Vambol V., 2020, Use of inductively coupled plasma optical emission spectrometry detection in determination of arsenic bioaccumulation in *Trifolium pratense* L. from contaminated soil. Ecological Questions 31(1): 15–22. https://doi.org/10.12775/EQ.2020.003

Zimmer T., Ohkuma M., Ohta A., Takagi M. & Schunck W.-H., 1996, The CYP52 multigene family of Candida maltosa encodes functionally diverse n-alkane-inducible cytochromes p450. Biochemical and Biophysical Research Communications 224(3): 784–789.