

Prospects of Petroleum Microbiology for Bioremediation and Cost Effective Petroleum Recovery

*Ekta Khare, Department of Microbiology, School of Life Sciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur-208024, UP India

Kalpna Upadhyay, Anziam Bio Pvt. Ltd., Faridabad, Haryana, India

Hiba Kausar, Department of Microbiology, School of Life Sciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur-208024, UP India

*Email: ektakhare@csjmu.ac.in, ekhare.cnu@gmail.com

Abstract

Environmental pollution from petroleum hydrocarbons has increased with the rapid growth in population and modernization of civilization, necessitating urgent repair. Recovery, transportation, and refining of petroleum all pose major environmental pollution problems. Petroleum hydrocarbons pose a major threat to the environment since they are poisonous, mutagenic, and carcinogenic. One of the most promising treatments involving microorganisms has emerged as bioremediation. Another use of microorganisms connected to petroleum is microbial enhanced oil recovery (MEOR), which entails changing the function, structure, or both, of microbial habitats found in oil reservoirs. It has become a successful and economical cleanup and oil recovery strategy since it does not use intensive chemical and mechanical treatments. This review gives an introduction of petroleum pollution, discusses how microbial technologies are being used for bioremediation and oil recovery, and offers ideas for further research.

Key words: Bioremediation, bioaugmentation, biostimulation, petroleum, microbial enhanced oil recovery

Introduction

An organic compound comprising hydrogen and carbon is called a hydrocarbon. Crude oil naturally contains the majority of the hydrocarbons that may be found on Earth, whereas decomposing organic matter is a rich source of carbon and hydrogen. Group 14 hydrides include compounds like hydrocarbons. Numerous types of hydrocarbons include alkanes, cycloalkanes, alkyne-based compounds, and aromatic hydrocarbons (arenes). All single bonds in saturated hydrocarbons make up their structure, and they are saturated with hydrogen (Callaghan 2013). Oil can be destroyed by microorganisms like bacteria, fungus, yeast, and microalgae depending on the source of the oil. Petroleum hydrocarbons make up 50–98% of crude oil, and alkenes make up 20–50% of oil. Hydrocarbons are linked to two main problems. First off, when conventional oil extraction techniques are used, a large percentage of this priceless and non-renewable resource is left in the ground. Second, spills of petroleum gasoline from various incidents are thought to be the most common organic pollutant of soil and ground water and are categorised as hazardous waste. These spills pose a major threat to both human and animal health and are also mutagenic and carcinogenic (Connellan 2017). Hydrocarbon residues are often made of heated, highly viscous water with a high pH, which are frequently utilised to recover oil from sands. A wide variety of microorganisms have the capacity to release enzyme systems that allow them to use hydrocarbons as a source of energy (Parthipan et al. 2017).

Over the past few decades, oil spills have escalated into severe environmental problems in industrialised and developing nations. The Taylor energy well disasters in the Gulf of Mexico (USA), which were brought on by a hurricane on September 16, 2004, and whose oil leakage is still ongoing, are one of several major ongoing oil spills in the world. There are many ways that oil can return to humans following accidental spills, including accumulation in fish and shellfish and intake of contaminated groundwater. Crude oil and petroleum compounds are common water and soil pollutants resulting from marine and terrestrial spillages. There are different types of hydrocarbons, and depending on how susceptible they are to microbial attack, they can be ranked as follows: Cyclic alkanes are followed by tiny aromatics, branched alkanes, and linear alkanes (Perry 1984).

The primary and most effective natural method for removing petroleum hydrocarbon contaminants from the environment is microbial degradation. At many hydrocarbon-

contaminated locations, biodegradation—which takes use of microorganisms' capacity to break down or detoxify organic contaminants—is employed as a treatment option. One of the main methods for removing petroleum and diesel products from the environment on a budget is biodegradation of hydrocarbons by natural populations of microorganisms, which enables the transformation of dangerous compounds into less toxic or nontoxic forms (Ismail et al. 2017). This review introduces petroleum pollution, explores the employment of microbial technology in bioremediation and oil recovery, and provides suggestions for further research.

Petroleum

Petroleum, also known as fossil fuel, is created when ancient creatures and microbes, primarily plankton and basic plants, that were buried deep under the ground during geologic time, fail to fully decompose biologically (Goldstein 2015). Due to high temperatures and pressure, these organic sediments underwent some physical, chemical, and biological reactions as they slowly decomposed in anaerobic circumstances, producing oil. Petroleum is still being formed, even though it takes a very long time, on the scale of millions of years. Yet, the chemical and physical characteristics of petroleum from various reservoirs vary greatly (Van Hamme et al. 2003). Almost all petroleum products and crude oils are complicated combinations. Jet fuels, for instance, can have more than 300 distinct hydrocarbons.

Exploration of petroleum

Early Mesopotamian civilizations are known for the world's first discovery of petroleum as early as 20,000 B.C., when they used it to light torches made of wood. The Chinese are thought to have begun approximately 5,000 BC when they discovered oil and began using it in medicine, waterproofing, and warfare. Then, by 3,000 BC, petroleum use had spread widely in the Middle East, where there were numerous oil seeps on the surface of the earth. Colonel Edwin Drake, a train conductor, drilled the world's first oil well at Titusville, Western Pennsylvania in 1859. Since then, the industry has grown to provide the raw ingredients for petrochemicals and roughly half of the world's energy. The Persian Gulf, the United States, the Soviet Union, northern and western Africa, Mexico, Indonesia, and Venezuela are the top oil-producing nations in the world.

Petroleum compounds

Saturates (alkanes), aromatics, resins, and asphaltenes are the four kinds of hydrocarbons that make up petroleum (Widdel and Rabus 2001) (Fig. 1). Alkanes and cycloalkanes are composed of saturated hydrocarbons, which lack a double bond in their structure. Alkanes are extremely hydrophobic and, depending on their molecular weight, can be found in solid (C₁₈-C₃₈), liquid (C₅-C₁₇), or gaseous (C₁-C₄) states at physiological temperatures (Rojo 2009). The primary ingredients of crude oil and natural gas are alkanes. One or more aromatic rings are often substituted with different alkyl groups to form aromatic hydrocarbons.

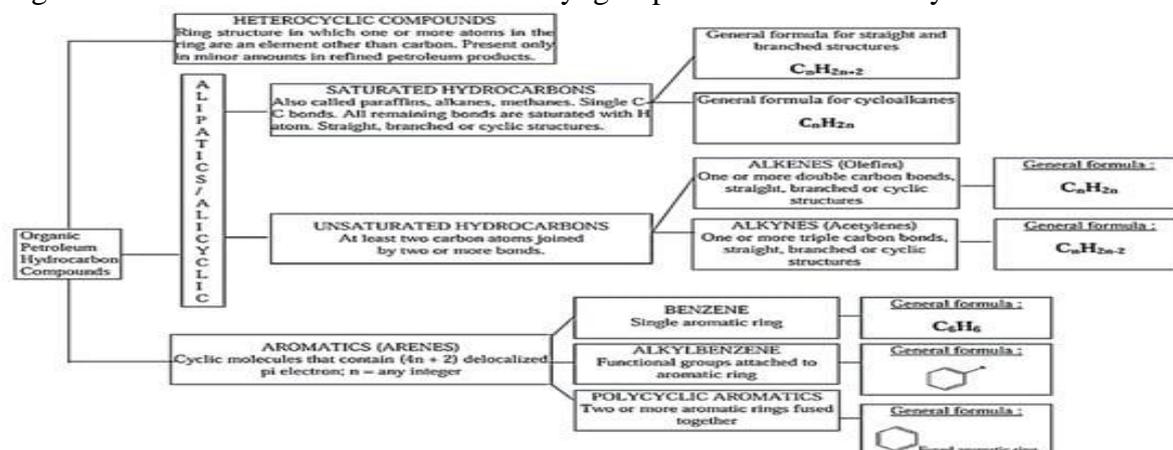


Figure 1. A list of the many categories and subcategories of all petroleum hydrocarbons (Hidayat and Turjaman 2016).

By using silica gel chromatography, petroleum can be divided into four categories: aliphatic, aromatic, asphaltic (Phenols and porphyrins), and resins (pyridines, quinolines, sulfoxides and amides). Compounds referred to as BTEX (Benzene, Toluene, Ethylbenzene, and ortho, para and meta xylene) are found in the mono aromatic fraction of crude petroleum.

Petroleum pollution

The most prevalent large-scale environmental contaminants are oil and oil-derived compounds. Petroleum hydrocarbons are becoming the most pervasive and harmful pollutant in the biosphere due to the widespread use and storage of petroleum fuels, which is endangering human health and the sustainability of the ecosystem. The most prevalent oil pollutants include petrol, diesel, fuel oil, and toxic petroleum chemicals like benzene, toluene, xylene, naphthalene, and certain polynuclear aromatics. Aquatic habitats have been impacted by oil pollution from two main sources. One of the sources is the spilling and dumping of waste oil, as well as oil tank leaks from moving vehicles onto the surfaces of the roads. Several human-related activities lead to oil contamination in many ways. Manufacturing, engineering products, cleaning, the improper disposal of oil brines, industrial oil spills due to accidents, domestic and industrial waste, runoff from land, and car industrial activities are some of these (El-Fadel and Houry 2001).

Petroleum is a major environmental contaminant as a result of its extensive production, transportation, use, and disposal. Water systems around the world are contaminated by spills and leaks of petroleum hydrocarbons from storage facilities and distribution systems (Adeniji et al. 2017). Infected soil contains hydrocarbons that can seep into lower layers, contaminate other habitats, such as water sources and agricultural fields, and possibly enter the food chain. The generally accepted physio-chemical treatment and disposal procedures can become unaffordable when the affected region is sizable. Thus, more advanced alternative technologies are required. Oil spilling into the water causes a great deal of public anxiety and emphasises the need for an economical method that is also environmentally friendly. Hence, creating methods for eliminating hydrocarbons from contaminated settings is of tremendous interest. Several researchers described the use of hydrocarbon-oxidizing bacteria, when assessed in surface soil samples, as a signal for oil and gas fields in the deeper subsurface. Despite the difficulty in treating oil pollution, bacteria that break down petroleum hydrocarbons have developed due to their closeness to these substances in the environment. These species are suitable for the remediation of oil pollution because it is inexpensive and environmentally beneficial, using bacteria to remove environmental toxins has recently gained popularity as a potential solution (Sajna et al. 2015).

Petroleum Microbiology

Petroleum microbial technology takes advantage of the incredible metabolic and adaptive powers of specialised microbes that degrade or change hydrocarbons (Van Hamme et al., 2003; Mbadanga et al. 2011). Petroleum biotechnology has been used to biologically treat refinery wastes and clean up oil spills in the environment (bioremediation). Oil exploration, microbial enhanced oil recovery (MEOR), biodesulfurization and biodenitrogenation of distillates, biodemetalization, bioupgrading of heavy crudes and refining residues, valorization of refining wastes, bioconversion of residual oil to methane, prevention of corrosion and souring in oil fields, formulation of petrochemicals, etc. are some other emerging applications (Ismail et al. 2017). Microorganisms have two most important functions in the world economy: bioremediation and increased oil recovery. Approximately 79 bacterial species that can degrade petroleum hydrocarbons have been found from recent studies (Tremblay et al. 2017).

Bioremediation

The word "bio" stands for biological creatures, which frequently include microscopic organisms like fungi and bacteria, while the word "remediation" stands for correcting the problem. Hence, bioremediation is a method that converts toxic chemicals into harmless compounds using naturally occurring microorganisms (Lal and Khanna, 1996). Many kinds

of microorganisms fall into the main groups of bacteria, yeast, or fungi, and they break down the dangerous chemicals and compounds contained in oily sludge and waste water effluent sludge into simpler, less toxic, or nontoxic substances (Bartha and Bossert, 1984). Many types of soil bacteria consume and convert petroleum hydrocarbons into biomass and harmless byproducts, primarily carbon dioxide, water, and fatty acids, without harming the environment. Some of them which play pivotal role in bioremediation are *Achromobacter*, *Acinetobacter*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Dietzia*, *Enterobacter*, *Marinobacter*, *Mycobacterium*, *Pandora*, *Pseudomonas*, *Staphylococcus*, *Streptobacillus*, *Streptococcus*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Nocardia* and *Rhodococcus* (Xu et al. 2018). Fig. 2 illustrates the bacterial surface characteristics that are necessary for the efficient biodegradation of hydrophobic hydrocarbon substrates.

Approaches to bioremediation are typically categorised as in situ or ex situ. Ex situ bioremediation entails removing the polluted material from the site to be treated elsewhere, whereas in situ bioremediation treats the polluted material on the spot. When organic pollutants are organically broken down in their natural environments, either to carbon dioxide and water or an attenuated transformation product, this process is known as in situ bioremediation. For the remediation of polluted sites, it is a low-cost, low-maintenance, environmentally benign, and long-lasting strategy. Ex situ bioremediation techniques may be more expensive than in situ ones in terms of cost. Also, there are differences between in situ and ex situ bioremediation procedures in terms of the rate of biodegradation and the consistency of the process output. The various methods for bioremediation of crude oil polluted sites include (a) biostimulation, which entails altering the existing environmental conditions by adding substrates and oxygen to trigger microorganism activity and accelerate the degradation of petroleum hydrocarbons, and (b) bioaugmentation, which entails adding known oil-degrading bacteria with specific catabolic abilities to the environment to supplement the existing microbial population, (c) microbial consortium: is the mixed cultures of different hydrocarbon degrading microorganisms used together to clean the oil polluted sites, and (d) microbial cell immobilization systems (Ron and Rosenberg 2014).

According to several studies, members of the phyla Firmicutes, Actinobacteria, and particularly Proteobacteria are among the most significant bacterial taxonomic groupings involved in hydrocarbon breakdown. Either the activity of microorganisms already present at the site or bioaugmentation approaches, in which microorganisms with the necessary catabolic features are supplied to the site, are relied upon for the bioremediation of contaminated areas (Azubuikie et al. 2016).

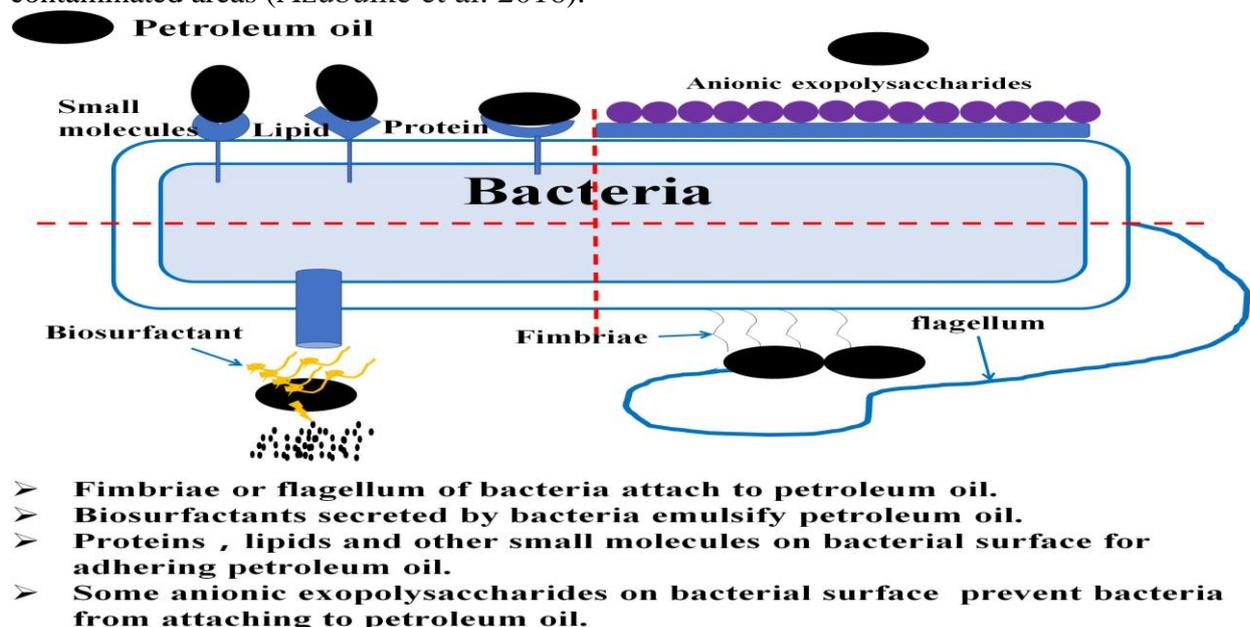


Figure 2. Schematic representation of the interaction between bacteria and petroleum hydrocarbons (Xu et al. 2018).

Microbial Enhanced Oil Recovery

During traditional approaches, about 30–40% of crude oil is retrieved while rest remains confined in the reservoir (Babadagli 2007, Bao et al. 2009, Shibulal et al. 2014). Oil that has been trapped is the focus of enhanced oil recovery (EOR). With an American Petroleum Institute (API) gravity of less than 20, heavy crude oil is more resistive to flow due to its high density or specific gravity. Heavy crude oil extraction requires a greater energy input. Open-pit mining, steam stimulation, sand addition to oil, and air injection into wells to start underground fires that burn heavier hydrocarbons to provide heat are all examples of current extraction techniques. Certain forms of crude oil are very challenging to transport through pipelines and need specific dilution agents. Occasionally mixtures of heavy and light crude oils are used in pipeline transit. The light crude will become contaminated as a result, decreasing its value (Holliger et al. 1997).

Microorganisms or their metabolites are used to improve the recovery of residual oil in the tertiary recovery process known as microbial enhanced oil recovery (MEOR) (Xu et al. 2009, Sivasankar and Kumar 2017). By infusing live microorganisms and nutrients into the reservoir, MEOR differs from traditional EOR techniques like CO₂ injection, steam injection, chemical surfactant and polymer flooding in that it allows bacteria and their metabolic byproducts to mobilise the remaining oil. As it doesn't use any hazardous chemicals and doesn't require any alterations to the current water-injection facilities, it is thought to be a more environmentally friendly method (Brown 2010). MEOR occurs through a variety of mechanisms, including the production of surfactants, which reduces oil-water interfacial tension and alters wettability, microorganisms and their metabolites selectively plug pores, gas production responsible for reduction of oil viscosity, the breakdown or biotransformation of long-chain saturated hydrocarbons, and acid production, which increases absolute permeability by dissolving rock minerals (Nielsen et al. 2010). Biosurfactants, biopolymers, acids, solvents, gases, and enzymes are some of the metabolic byproducts produced by microorganisms. The bacteria utilised in MEOR are typically non-pathogenic, hydrocarbon-utilizing, and occur naturally in petroleum reserves.

Conclusion and Prospects

Petroleum chemicals pose serious risks to both human and environmental health. Especially poisonous low molecular weight compounds like naphthalene and phenanthrene are present, while large molecular weight compounds that are mutagenic and/or have the potential to cause cancer are a severe danger (Abdel-Shafy and Mansour 2016). Since the majority of them are refractory by nature, the presence of hydrocarbons in the soil and water is a serious issue. Oil spills do the most damage, both immediately and economically, because they not only disrupt the ecosystem but also the isolated location. Many of these accidents involve tankers or offshore oil wells, some of which catch fire and release significant amounts of toxic ash that are harmful to human health as they burn. Moreover, hydrocarbons have been demonstrated to have negative effects on the environment, including the death of marine animals, seabirds, and large fish, as was shown during the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska. This caused an oil film to accumulate on the shorelines, which in turn caused land deterioration and water contamination (Peterson et al. 2003).

The kind and quantity of oil or hydrocarbon present determines the quantitative and qualitative features of the complex biodegradation process that occurs when petroleum and other hydrocarbons are present in the environment. Only bacteria are thought to be potential Microbial Enhanced Oil Recovery candidates. Molds, yeast, and protozoa are not suitable because of their size or because they cannot flourish in reservoirs' environmental conditions. A more environmentally friendly and cost-effective method of upgrading heavy oil is through biological processing, which offers a higher selectivity to particular processes. A significant benefit of using pre-selected microorganisms isolated from contaminated sites is that they are more likely to live and thrive there than transient strains or microbes that aren't adapted to the environment (Khan et al. 2012). Humanity will greatly benefit from these advances in our

understanding of petroleum microbiology and the use of microorganisms; however, adequate exploitation of microorganism capabilities is required.

Although extremely difficult, microbial technology has enormous potential for the fossil fuel business, and the field will advance significantly over the next few decades. Advances in pertinent fields including metabolic engineering, bioprocess technology, biochemical engineering, and biocatalysis will fuel this. A particularly active area of research that can be used to create novel hydrocarbon biotransformations is the topic of artificial metalloenzymes. Alongside scientific developments, close cooperation between academia and the fossil fuel sector is necessary to ensure the effective creation and application of biotechnology applications that address particular technological, financial, and environmental problems. Nevertheless, it is obvious that petroleum is still of utmost importance to industry and the environment and will continue to be a hotbed of basic and applied research for decades to come, despite its deep roots in the history of microbiology.

Acknowledgements

The author Ekta Khare is grateful to Vice Chancellor, Chhatrapati Shahu Ji Maharaj University, Kanpur, India for providing facilities.

References

1. Abdel-Shafy HI, Mansour MSM (2016) A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egypt J Pet* 25(1):107–123.
2. Adeniji AO, Okoh OO, Okoh AI (2017) Petroleum hydrocarbon profiles of water and sediment of Algoa Bay, Eastern Cape, South Africa. *Int J Environ Res Public Health* 14(10):1263.
3. Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques-classification based on site of application: principles, advantages, limitations and prospects. *World J Microbiol Biotechnol* 32(11):180.
4. Babadagli T (2007) Development of mature oil fields—A review. *J Pet Sci Eng* 57:221–246.
5. Bao M, Kong X, Jiang G, Wang X, Li X (2009) Laboratory study on activating indigenous microorganisms to enhance oil recovery in Shengli Oilfield. *J Pet Sci Eng* 66:42–46.
6. Bartha R, Bossert I (1984) The treatment and disposal of petroleum refinery wastes. In: *Petroleum Microbiology*, RM Atlas (ed.), Macmillan, New York, pp. 553-577.
7. Brown LR (2010) Microbial enhanced oil recovery (MEOR). *Curr Opin Microbiol* 13:316–320.
8. Callaghan A (2013) Enzymes involved in the anaerobic oxidation of n-alkanes: from methane to long-chain paraffins. *Front Microbiol* 4.
9. Connellan S (2017) Lung diseases associated with hydrocarbon exposure. *Respir Med* 126: 46-51.
10. El-Fadel M, Khoury R (2001) Strategies for vehicle waste-oil management: a case study. *Resour Conserv Recycl* 33(2):75–91.
11. Goldstein A (2015) Formation of Oil [Online]. Available: http://f03.classes.colgate.edu/fsem037-oil/formation_of_oil.htm
12. Hidayat A, Turjaman M (2016) Biological degradation of crude oil contaminants and its application in Indonesia. In: *Microbes for Restoration of Degraded Ecosystems*. DJ Bagyaraj, Jamaluddin (eds.), New India Publishing Agency: New Delhi, India.
13. Holliger C, Gaspard S, Glod G, Heijman C, Schumacher W, Schwarzenbach RP, Vazquez F (1997) Contaminated environments in the subsurface and bioremediation: organic contaminants. *FEMS Microbiol Rev* 20(3-4):517-23.
14. Ismail WA, Van Hamme JD, Kilbane JJ, Gu J-D (2017) Editorial: petroleum microbial biotechnology: challenges and prospects. *Front Microbiol* 8:833.

15. Khan S, Al-Qurainy F, Nadeem M (2012) Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia. Saudi J Biol Sci 19(1):1-11.
16. Lal B, Khanna S (1996) Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*. J Appl Bacteriol 81:355-362.
17. Mbadanga SM, Wang L-Y, Zhou L, Liu J-F, Gu J-D, Mu B- Z (2011) Microbial communities involved in anaerobic degradation of alkanes. Int Biodeterior Biodegradation 65:1–13.
18. Nielsen SM, Shapiro AA, Michelsen ML, Stenby EH (2010) 1D Simulations for microbial enhanced oil recovery with metabolite partitioning. Transp Porous Media 85:785–802.
19. Perry JJ (1984) Microbial metabolism of cyclic alkanes. In: Petroleum microbiology. Macmillan Publishing Co. RM Atlas (eds.), New York, pp. 61-98..
20. Peterson ChH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB (2003) Long-term ecosystem response to the Exxon Valdez oil spill. Science 302:2082–2086.
21. Parthipan P, Preetham E, Machuca LL, Rahman PKSM, Murugan K and Rajasekar A (2017) Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium *Bacillus subtilis* A1. Front Microbiol 8:193.
22. Rojo F (2009) Degradation of alkanes by bacteria. Environ Microbiol 11(10):2477–2490.
23. Ron EZ, Rosenberg E (2014) Enhanced bioremediation of oil spills in the sea. Curr Opin Biotechnol 27:191–194.
24. Sajna KV, Sukumaran RK, Gottumukkala LD, Pandey A (2015) Crude oil biodegradation aided by biosurfactants from *Pseudozyma* sp. NII 08165 or its culture broth. Biores Technol 191:133–139.
25. Shibulal B, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi SJ (2014) Microbial enhanced heavy oil recovery by the aid of inhabitant spore-forming bacteria: An insight review. Sci World J 2014:1-12.
26. Sivasankar P, Kumar GS (2017) Influence of pH on dynamics of microbial enhanced oil recovery processes using biosurfactant producing *Pseudomonas putida*: Mathematical modelling and numerical simulation. Bioresour Technol 224:498–508.
27. Tremblay J, Yergeau E, Fortin N, Cobanli S, Elias M, King TL, et al. (2017) Chemical dispersants enhance the activity of oil-and gas condensate-degrading marine bacteria. ISME J 11:2793–2808.
28. Van Hamme JD, Singh A, Ward OP (2003) Recent advances in petroleum microbiology. Microbiol Mol Biol Rev 67(4):503–549.
29. Widdel F, Rabus R (2001) Anaerobic biodegradation of saturated and aromatic hydrocarbons. Curr Opin Biotechnol 12(3):259–276.
30. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Gao X, Li F, Li H, Yu H (2018) Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. Front Microbiol 9:2885.
31. Xu T, Chen C, Liu C, Zhang S, Wu Y, Zhang PA (2009) novel way to enhance the oil recovery ratio by *Streptococcus* sp. BT-003. J Basic Microbiol 49:477–481.