

An Overview on Microbial Degradation of Petroleum Hydrocarbon Contaminants

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Abstract— Microorganisms use to reduce/minimize the hazardous waste concentration from contaminated sites. Presently accepted disposal methods of incineration or burial insecure landfills have an expensive when amounts of contaminants are large. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites are limited effective and expensive. But the Bioremediation is now used extensively for the biodegradation of hydrocarbon contamination resulting from the activities related to the petrochemical industry, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds, by biological agents like microorganisms. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants. This paper presents an updated overview of petroleum hydrocarbon degradation by microorganisms.

Index Terms— Microorganisms, hydrocarbon, Bioremediation

I. INTRODUCTION

Petroleum is a liquid mixture consisting of complex hydrocarbons. It is the main source of energy for industry and daily life. During the exploration, production, refining, transport, and storage of petroleum and petroleum products the amount of natural crude oil seepage was estimated to be 600,000 metric tons per year [1]. The main cause of water and soil pollution is the release of hydrocarbons into the environment whether accidentally or due to human activities [2]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [3]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion and washing are expensive and can lead to incomplete decomposition of contaminants. The process of bioremediation defined as the use of biological agents, such as bacteria, fungi, or green plants, to remove or neutralize contaminants, as in polluted soil or water. Bacteria and fungi generally work by breaking down contaminants

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such as petroleum into less harmful substances [4]. It is a noninvasive and relatively cost-effective technology [5]. Oil spill bioremediation depends on presence of microorganisms with the appropriate metabolic capabilities. The optimal rates of growth and hydrocarbon biodegradation can be sustained by the adequate concentrations of nutrients, oxygen & pH values (6-9). Oil and oil surface area are also important factors for bioremediation success. Oil spill bioremediation can be done by two processes namely Bioaugmentation and Biostimulation. Bioremediation has been used on very large scale application, as demonstrated by the shore-line clean-up efforts in Prince William Sound, Alaska, after the Exxon Oil spill. Although the Alaska oil-spill cleanup represents the most extensive use of bioremediation on any one site, there have been many other successful application on smaller scale. This paper provides extensive information on microbial degradation of petroleum hydrocarbon contaminants towards the better understanding in bioremediation challenges.

II. MICROBIAL DEGRADATION OF PETROLEUM HYDROCARBONS

Rates of biodegradation depend greatly on the composition, state, and concentration of the oil or hydrocarbons, with dispersion and emulsification enhancing rates in aquatic systems and absorption by soil particulates being the key feature of terrestrial ecosystems. Hydrocarbon degradation may be influenced by different factors such as their limited availability to microorganisms. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes branched alkanes small aromatics cyclic alkanes [6,7]. Microbial degradation is one of the most promising mechanisms for removing the petroleum hydrocarbon pollutants from the environment [8-10]. There are different types of microorganisms such as bacteria, fungi & yeast, which are responsible for degradation of petroleum hydrocarbon pollutants. The microorganisms, namely, *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, and *Rhodococcus* were found to be involved for alkyl aromatic degradation in marine sediments which was reported by Jones et al. Individual population can't degrade complex mixture of hydrocarbon such as crude oil in soil, fresh water & marine environment, but mixed population with overall broad enzymatic capacities enhanced the rate. Bacteria are the most active & primary agents in petroleum degradation. Several bacteria are even known to feed exclusively on hydrocarbons [11]. Floodgate [12] listed 25 genera of hydrocarbon degrading bacteria and 25 genera of hydrocarbon degrading fungi, which were isolated from marine environment. Das and Mukherjee

reported the crude petroleum oil from petroleum contaminated soil from North East India. There are various bacterial genera namely, *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Dietzia*, *Burkholderia*, and *Mycobacterium* isolated from petroleum-contaminated soil, which have the capability for hydrocarbon degradation [15]. Some hydrocarbons such as poly-aromatic hydrocarbon may not be degraded at all but now it can be done by *Sphingomonas*. Fungal genera, namely, *Amorphoteca*, *Neosartorya*, *Talaromyces*, and *Grappium* and yeast genera, namely, *Candida*, *Yarrowia*, and *Pichia* were isolated from petroleum-contaminated soil and proved to be the potential organisms for hydrocarbon degradation [15].

III. FACTORS INFLUENCING PETROLEUM HYDROCARBON DEGRADATION

The fate of petroleum hydrocarbons in the environment is mainly determined by abiotic factors, which influence the Weathering, including biodegradation of the oil. Factors which influences rates of microbial growth & enzymatic activities affect the rates of petroleum pollutants depends on the quantity & quality of the hydrocarbon mixture & on the properties of the affected ecosystem. In one environment petroleum hydrocarbon can persist indefinitely, where as under another set of conditions the same hydrocarbons can be completely biodegraded within a relatively few hours or days. Brusseau discussed the number of limiting factors that affect the biodegradation of petroleum hydrocarbons [16]. Among physical factors, temperature plays an important role in biodegradation of hydrocarbons. Atlas [17] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation. Temperature also affects the solubility of hydrocarbons [21]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature.

Nutrients (such as nitrogen, phosphorus & in rarely iron) also play a very main role in biodegradation of hydrocarbon pollutants. Some of these nutrients could become limiting factor thus affecting the biodegradation processes. Atlas [11] reported that when a major oil spill occurred in marine and freshwater environments, the supply of carbon was significantly increased and the availability of nitrogen and phosphorus generally became the limiting factor for oil degradation. In marine environments, it was found to be more pronounced due to low levels of nitrogen and phosphorous in seawater [12]. Freshwater wetlands are typically considered to be nutrient deficient due to heavy demands of nutrients by the plants [22]. Therefore, additions of nutrients were necessary to enhance the biodegradation of oil pollutant [23,24]. On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity [25]. Several authors have reported the negative effects of high NPK levels on the biodegradation of hydrocarbons [26,27] especially on aromatics [28].

IV. MECHANISM OF PETROLEUM HYDROCARBON DEGRADATION

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Oxygenases & Peroxidases are the main enzymes, which are used, in intracellular attack of inorganic pollutants and activation as well as incorporation of oxygen in the enzyme key reaction. Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosyntheses and growths are synthesized by gluconeogenesis. The degradation of petroleum hydrocarbons can be mediated by specific enzyme system.

Cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous Heme-thiolate Monooxygenases which play an important role in the microbial degradation of oil and other compounds. Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 form. Cytochrome P450 enzyme systems was found to be involved in biodegradation of petroleum hydrocarbons. Yeast species are capable to use n-alkanes & other aliphatic hydrocarbons as a source of C and energy & it is governed by multiple microsomal cytochrome P-450 forms isolated from yeast species such as *Candida maltosa*, *Candida tropicalis*, and *Candida apicola*.

V. UPTAKE OF HYDROCARBON BY BIOSURFACTANTS

Biosurfactants are heterogeneous group of surface-active chemical compounds produced by a wide variety of microorganisms [18-20, 31-33]. Surfactants enhance solubilization and removal of contaminants [34,35]. Biodegradation is also enhanced by surfactants due to increased bioavailability of pollutants. Bioremediation of oil sludge using biosurfactants has been reported by Cameotra and Singh [36]. Pseudomonads are the best known bacteria capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants [13,36,37]. Among Pseudomonads, *P. aeruginosa* is widely studied for the production of glycolipid type biosurfactants. However, glycolipid type biosurfactants are also reported from some other species like *P. putida* and *P. chlororaphis*. Biosurfactants increase the oil surface area for bacteria to utilize it [38].

VI. COMMERCIALY AVAILABLE BIOREMEDIATION AGENTS

Microbiological cultures, enzyme additives, or nutrient additives that significantly increase the rate of biodegradation were defined as bioremediation agents by USEPA [39]. Bioremediation agents are classified as bioaugmentation agents and biostimulation agents based on the two main approaches to oil spill bioremediation. Numerous bioremediation products have been proposed and promoted by their vendors, especially during early 1990s, when

bioremediation was popularized as “the ultimate solution” to oil spills [40]. Studies showed that bioremediation products may be effective in the laboratory but significantly less so in the field [41]. This is because laboratory studies cannot always simulate complicated real world conditions such as spatial heterogeneity, biological interactions, climatic effects, and nutrient mass transport limitations. Therefore, field studies and applications are the ultimate tests or the most convincing demonstration of the effectiveness of bioremediation products.

VII. PHYTOREMEDIATION AND GENETICALLY MODIFIED BACTERIA

Phytoremediation is an emerging technology that uses plants to manage a wide variety of environmental pollution problems, including the cleanup of soils and groundwater contaminated with hydrocarbons and other hazardous substances. The different mechanisms, namely, hydraulic control, phytovolatilization, rhizoremediation, and phytotransformation. Could be utilized for the remediation of a wide variety of contaminants. Phytoremediation can be cost-effective (a) for large sites with shallow residual levels of contamination by organic, nutrient, or metal pollutants, where contamination does not pose an imminent danger and only “polishing treatment” is required; (b) where vegetation is used as a final cap and closure of the site. Phytoremediation could be applied for the remediation of various contaminated sites. Genetically engineered microorganisms (GEMs) are useful to improve the degradation of hazardous wastes under laboratory conditions. The genetically engineered bacteria showed higher degradative capacity. However, ecological and environmental concerns and regulatory constraints are major obstacles for testing GEM in the field. These problems must be solved before GEM can provide an effective clean-up process at lower cost. The use of genetically engineered bacteria was applied to bioremediation process monitoring, strain monitoring, stress response, end-point analysis, and toxicity assessment.

VIII. CONCLUSION

- Bioremediation has high ecological significances that depend on the indigenous microorganisms to mineralize the organic contaminants.
- Microorganism has enzymes systems to degrade and utilize different hydrocarbons as a source of carbon and energy.
- Bioremediation is a more promising technology than mechanical, burying, evaporation dispersion and washing because these technologies are expensive and can lead to incomplete decomposition of contaminants.
- The use of genetically modified bacteria represents research frontier with broad implications.

REFERENCES

- [1] K. A. Kvenvolden and C. K. Cooper, “Natural seepage of crude oil into the marine environment,” *Geo-Marine Letters*, vol. 23, no. 3-4, pp. 140–146, 2003.
- [2] C. Holliger, S. Gaspard, G. Glod, C. Heijman, W. Schumacher, R. P. Schwarzenbach, and F. Vazquez, “Contaminated environments in the subsurface and bioremediation: organic contaminants,” *FEMS Microbiology Reviews*, vol. 20, no. 3-4, pp. 517–523, 1997.
- [3] P. J. J. Alvarez and T. M. Vogel, “Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries,” *Applied and Environmental Microbiology*, vol. 57, no. 10, pp. 2981–2985, 1991.
- [4] J. I. Medina-Bellver, P. Marín, A. Delgado, A. Rodríguez-Sánchez, E. Reyes, J. L. Ramos, and S. Marqués, “Evidence for *in situ* crude oil biodegradation after the Prestige oil spill,” *Environmental Microbiology*, vol. 7, no. 6, pp. 773–779, 2005.
- [5] T. M. April, J. M. Foght, and R. S. Currah, “Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada,” *Canadian Journal of Microbiology*, vol. 46, no. 1, pp. 38–49, 2000.
- [6] W. Ulrici, “Contaminant soil areas, different countries and contaminant monitoring of contaminants,” in *Environmental Process II. Soil Decontamination Biotechnology*, H. J. Rehm and G. Reed, Eds., vol. 11, pp. 5–42, 2000.
- [7] J. J. Perry, “Microbial metabolism of cyclic alkanes,” in *Petroleum Microbiology*, R. M. Atlas, Ed., pp. 61–98, Macmillan, New York, NY, USA, 1984.
- [8] R. M. Atlas, “Petroleum microbiology,” in *Encyclopedia of Microbiology*, pp. 363–369, Academic Press, Baltimore, Md, USA, 1992.
- [9] O. O. Amund and N. Nwokoye, “Hydrocarbon potentials of yeast isolates from a polluted Lagoon,” *Journal of Scientific Research and Development*, vol. 1, pp. 65–68, 1993.
- [10] B. Lal and S. Khanna, “Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*,” *Journal of Applied Bacteriology*, vol. 81, no. 4, pp. 355–362, 1996.
- [11] R. M. Atlas, “Effects of hydrocarbons on micro-organisms and biodegradation in Arctic ecosystems,” in *Petroleum Effects in the Arctic Environment*, F. R. Engelhardt, Ed., pp. 63–99, Elsevier, London, UK, 1985.
- [12] G. Floodgate, “The fate of petroleum in marine ecosystems,” in *Petroleum Microbiology*, R. M. Atlas, Ed., pp. 355–398, Macmillan, New York, NY, USA, 1984.
- [13] K. S. M. Rahman, T. J. Rahman, Y. Kourkoutas, I. Petsas, R. Marchant, and I. M. Banat, “Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients,” *Bioresource Technology*, vol. 90, no. 2, pp. 159–168, 2003.
- [14] M. M. Yakimov, K. N. Timmis, and P. N. Golyshin, “Obligate oil-degrading marine bacteria,” *Current Opinion in Biotechnology*, vol. 18, no. 3, pp. 257–266, 2007.
- [15] F. Chaillan, A. Le Flèche, E. Bury, Y.-H. Phantavong, P. Grimont, A. Saliot, and J. Oudot, “Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms,” *Research in Microbiology*, vol. 155, no. 7, pp. 587–595, 2004.
- [16] M. L. Brusseau, “The impact of physical, chemical and biological factors on biodegradation,” in *Proceedings of the International Conference on Biotechnology for Soil Remediation: Scientific Bases and Practical Applications*, R. Serra, Ed., pp. 81–98, C.I.P.A. S.R.L., Milan, Italy, 1998.
- [17] R. M. Atlas, “Effects of temperature and crude oil composition on petroleum biodegradation,” *Journal of Applied Microbiology*, vol. 30, no. 3, pp. 396–403, 1975.
- [18] K. Muthusamy, S. Gopalakrishnan, T. K. Ravi, and P. Sivachidambaram, “Biosurfactants: properties, commercial production and application,” *Current Science*, vol. 94, no. 6, pp. 736–747, 2008.

- [19] Mahmoud, Y. Aziza, A. Abdeltif, and M. Rachida, "Biosurfactant production by Bacillus strain injected in the petroleum reservoirs," *Journal of Industrial Microbiology & Biotechnology*, vol. 35, pp. 1303–1306, 2008.
- [20] M. O. Ilori, C. J. Amobi, and A. C. Odocha, "Factors affecting biosurfactant production by oil degrading *Aeromonas* spp. isolated from a tropical environment
- [21] J. M. Foght, D. W. S. Westlake, W. M. Johnson, and H. F. Ridgway, "Environmental gasoline-utilizing isolates and clinical isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable by chemotaxonomic and molecular techniques," *Microbiology*, vol. 142, no. 9, pp. 2333–2340, 1996.
- [22] W. J. Mitsch and J. G. Gosselink, *Wetlands*, John Wiley & Sons, New York, NY, USA, 2nd edition, 1993.
- [23] S.-C. Choi, K. K. Kwon, J. H. Sohn, and S.-J. Kim, "Evaluation of fertilizer additions to stimulate oil biodegradation in sand seashore mesocosms," *Journal of Microbiology and Biotechnology*, vol. 12, no. 3, pp. 431–436, 2002.
- [24] S.-J. Kim, D. H. Choi, D. S. Sim, and Y.-S. Oh, "Evaluation of bioremediation effectiveness on crude oil-contaminated sand," *Chemosphere*, vol. 59, no. 6, pp. 845–852, 2005.
- [25] F. Chaillan, C. H. Chaîneau, V. Point, A. Saliot, and J. Oudot, "Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings," *Environmental Pollution*, vol. 144, no. 1, pp. 255–265, 2006.
- [26] J. Oudot, F. X. Merlin, and P. Pinvidic, "Weathering rates of oil components in a bioremediation experiment in estuarine sediments," *Marine Environmental Research*, vol. 45, no. 2, pp. 113–125, 1998.
- [27] H. Chaîneau, G. Rougeux, C. Yéprémian, and J. Oudot, "Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil," *Soil Biology and Biochemistry*, vol. 37, no. 8, pp. 1490–1497, 2005.
- [28] L. M. Carmichael and F. K. Pfaender, "The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils," *Biodegradation*, vol. 8, no. 1, pp. 1–13, 1997.
- [29] F. Chaillan, A. Le Flèche, E. Bury, Y. H. Phantavong, P. Grimont, A. Saliot, and J. Oudot, "Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms," *Research in Microbiology*, vol. 155, no. 7, pp. 587–595, 2004.
- [30] U. Scheuer, T. Zimmer, D. Becher, F. Schauer, and W.-H. Schunck, "Oxygenation cascade in conversion of n-alkanes to α,ω -dioic acids catalyzed by cytochrome P450 52A3," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32528–32534, 1998.
- [31] M. O. Ilori, S. A. Adebuseye, and A. C. Ojo, "Isolation and characterization of hydrocarbon-degrading and biosurfactant-producing yeast strains obtained from a polluted lagoon water," *World Journal of Microbiology and Biotechnology*, vol. 24, no. 11, pp. 2539–2545, 2008.
- [32] G. S. Kiran, T. A. Hema, R. Gandhimathi, J. Selvin, T. A. Thomas, T. Rajeetha Ravji, and K. Natarajaseenivasan, "Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus ustus* MSF3," *Colloids and Surfaces B*, vol. 73, no. 2, pp. 250–256, 2009.
- [33] O. S. Obayori, M. O. Ilori, S. A. Adebuseye, G. O. Oyetibo, A. E. Omotayo, and O. O. Amund, "Degradation of hydrocarbons and biosurfactant production by *Pseudomonas* sp. strain LP1," *World Journal of Microbiology and Biotechnology*, vol. 25, no. 9, pp. 1615–1623, 2009.
- [34] M. L. Brusseau, R. M. Miller, Y. Zhang, X. Wang, and G. Y. Bai, "Biosurfactant and cosolvent enhanced remediation of contaminated media," *ACS Symposium Series*, vol. 594, pp. 82–94, 1995.
- [35] G. Bai, M. L. Brusseau, and R. M. Miller, "Biosurfactant-enhanced removal of residual hydrocarbon from soil," *Journal of Contaminant Hydrology*, vol. 25, no. 1-2, pp. 157–170, 1997.
- [36] S. S. Cameotra and P. Singh, "Bioremediation of oil sludge using crude biosurfactants," *International Biodeterioration and Biodegradation*, vol. 62, no. 3, pp. 274–280, 2008.
- [37] O. Pornsunthorntawe, P. Wongpanit, S. Chavadej, M. Abe, and R. Rujiravanit, "Structural and physicochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil," *Bioresource Technology*, vol. 99, no. 6, pp. 1589–1595, 2008.
- [38] M. Nikolopoulou and N. Kalogerakis, "Biostimulation strategies for fresh and chronically polluted marine environments with petroleum hydrocarbons," *Journal of Chemical Technology and Biotechnology*, vol. 84, no. 6, pp. 802–807, 2009.
- [39] W. J. Nichols, "The U.S. Environmental Protection 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*, pp. 1479–1483, American Petroleum Institute, Washington, DC, USA, 2001.
- [40] R. Z. Hoff, "Bioremediation: an overview of its development and use for oil spill cleanup," *Marine Pollution Bulletin*, vol. 26, no. 9, pp. 476–481, 1993.
- [41] K. Lee, G. H. Tremblay, J. Gauthier, S. E. Cobanli, and M. Griffin, "Bioaugmentation and biostimulation: a paradox between laboratory and field results," in *Proceedings of the International Oil Spill Conference*, pp. 697–705, American Petroleum Institute, Washington, DC, USA, 1997.