DEGRADATION OF MARINE PETROLEUM COMPOUNDS BY BACTERIA AND ALGAE (REVIEW ARTICLE)

[1]SAMIRA MEHRDAD, [2]KEIVAN SAEB, [3]LOBAT TAGHAVI, [4]MASOOD GHANE

Azad University of Science & Research Branch,Tehran,Iran ,Azad university ,Tonekabon,
E-mail: [1]IranSamira.mehrdad93@gmail.com , [2]keivansaeb@gmail.com , [3]taghavi_lobat@yahoo.com,
[4]masoodghane@gmail.com

Abstract - Water pollution caused by crude oil or oil compounds is one of the environmental problems in the world. The aim of this article is introduction of some bacteria and algae that can degrade oil compounds in marine ecosystems. Bioremediation is a simple and economical method to treat contaminated water from these pollutants. This method utilizes the natural ability of microorganisms to degrade the toxic compound into simpler and non-toxic form. Some algae and bacteria can use petroleum as carbon source and hence, break them down to harmless end products. Successful bioremediation depends on having the right bacteria in the right place with the right environmental factors for degradation. Right bacteria are bacteria which have the physiological and metabolic capabilities to degrade the pollutants. Typical bacterial groups already known for their capacity to degrade hydrocarbons include Pseudomonas, Marinobacter, Alcanivorax, Microbulbifer, Sphingomonas, Micrococcus, Cellulomonas, Dietzia, and Gordonia groups. There are still some persistent organic pollutants that are difficult to break down by the microorganisms. The genetic engineering can solve this problem and improve the absorption and bioremediation of many organic pollutants and it is necessary to study about the best condition of environment such as pH, nutrients, temperature and etc. to increase the biodegradation and reduce the time of bioaccumulation.

Index Terms - Algae, Bacteria, Environment, Petroleum.

I. INTRODUCTION

Release of petroleum hydrocarbons into the environment due to accidental spills or human activities, such as oil exploitation, transportation, and storage are the main causes of water pollution [1], [2]. The main source of oil into the sea is related to Natural seeps (47%) and other sources are allocated to consumption (38%), transportation (12%) and production (3%) [3]. Oil pollution causes irreparable damage to surface water such as sea, lake and river. Oil contaminates fish, marine plants and other microorganisms and disrupts important food cycle and reduces crustacean food products. Oil pollution affects on the ecosystem of the aquatic environment, the fishing industry, tourism and recreation and sport, and landscape of natural environment [4]. The traditional treatment of oil compounds such as collection using floating booms and adsorption by natural or synthetic materials, etc., cannot degrade the crude oil thoroughly [5]. In the event of leakage of oil at the surface of the water normally caused by the accident, ships collect oil using special filters. This method is an incomplete method for faced with such incidents. This technology has its own limitations. The basic drawback is that floating filters cannot prevent leakage of oil. Also, small oil spills cannot be collected by these mechanical methods, so streaks of oil remain on the water. In recent years, in oil producing countries, this substance is considered as the main environmental pollutant and also conservation of natural ecosystems is very important. Thus, this is necessary to find the best solutions to biological decomposition of petroleum compounds by microorganisms [6]. Oil pollution is cleaned up in three ways: chemical, physical and biological. Bioremediation is more efficient than the other two methods. Bioremediation is one of the major environmental purification methods in which living organisms, especially bacteria, fungi, plants and algae, are used to decompose environmental pollutants and convert them into non-toxic compounds [7]. In order to break down the oil compounds, the bacteria carry out the digestive process of hydrocarbons by adding the hydroxyl group to the aromatic unsaturated rings, producing carbon dioxide, water and Eco-friendly products. Bioremediation is include of two main processes: biostimulation and bioaugmentation. Biostimulation is the process of addition of nutrients into the contaminated site to increase the growth and metabolic activities of microorganisms for degradation of pollutants. Bioaugmentation is the process of addition of specific microorganisms into the contaminated site to degrade pollutants. This process is faster and better than previous one. In bioremediation process some environmental conditions are important such as temperature, pressure, nutrients, moisture, oxygen and pH. Also the choice of appropriate microorganisms is essential for degradation of pollutants [8], [9]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. Figure 1 shows degradation rate in different environments [10].

Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [11]. Also according to scientific studies, algae can be effective for removal of petroleum.

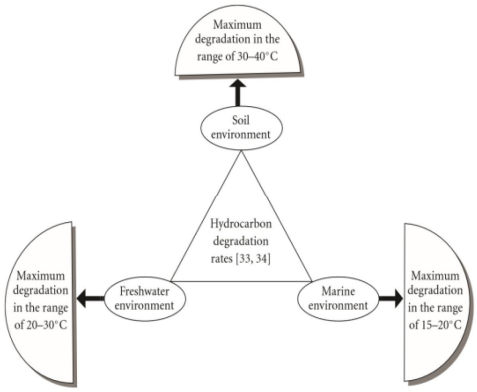

Fig. 1. Degradation rate of Hydrocarbon in different environments

Several microorganisms including bacteria, fungi and yeasts have been investigated in terms of hydrocarbon decomposition ability [12]. Appropriate metabolic capabilities are important requirement for bioremediation of microorganisms. Biodegradation of petroleum compounds depends on the nature and on the amount of the hydrocarbons present [9]. Cooney et al [10] have been studied different factors influencing hydrocarbon degradation in freshwater lakes such as: environment condition and hydrocarbons type (for example degradation of polycyclic aromatic hydrocarbons (PAHs) is difficult). Jones et al [13], reported degradation of petroleum-derived aromatic hydrocarbons in marine sediments. They used some microorganisms such as Arthrobacter, Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, and Rhodococcus for alkylaromatic degradation. Adebusoye et al [14] used nine bacteria namely Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffi, Flavobacterium sp., Micrococcus roseus, and Corynebacterium sp to degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria. The efficiency of biodegradation is ranged from 0.003% [15] to 100% [16] for marine bacteria.

Several bacteria are even known to removal of oil and petroleum hydrocarbons [17]. Though algae and protozoa are the important members in aquatic ecosystems, but they involved in hydrocarbons biodegradation rarely. Walker et al [18] studied on an alga namely Prototheca zopfi for bioremediation of crude oil and aromatic hydrocarbons. Cerniglia et al [19] found five green algae, one red alga, and one brown alga could degrade naphthalene. Guo-liang et al [20] investigated about biodegradation of crude oil by a specific bacteria called Pseudomonas aeruginosa. They concluded this strain of bacteria could degrade most of crude oil. Deviram et al [21] were used three algal strains Ulva sp, Cladophora sp and Chlorella sp for bioremediation in water systems. The results show the potential of chlorella sp is more than the other two. Binark et al [22] evaluated five algae for determination of oil on the surface and inside of algae: green; Ulva lactuca L, Enteromorpha linza J. Agard, brown; Cystoseira barbata J. Agard, red; Ceramium rubrum (Huds.) J. Agard, Pterocladia capillacea (Grev.). The results demonstrated that there is the highest amount of oil on the surface and inside of Ceramium rubrum alga. So this alga can use as an indicator of seawater pollution. Hasan Shahiyan and Emtiazi [23] studied on ability of Alcanivorax dieselolei bacteria for removal of petroleum pollution. The bacteria were enriched in the ONR7a medium and the colonies were screened for crude oil degradation. Eventually, two strains showed higher growth rates on crude oil. The crude oil breakdown was 85% by these two strains after 5 days. Roy et al [24] studied on some bacteria such as Mycobacterium, Pseudomonas, Nocardia, Micrococcus and Acinetobacter for degradation of oil in Sunderban coastal waters in india. They concluded two percent of heterotrophic bacteria are able to use hydrocarbons as the only source of energy and carbon. Chen et al [25] studied on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. They used five strains of bacteria, namely, Exiguobacterium sp. ASW-1, Pseudomonas aeruginosa strain ASW-2, Alcaligenes sp. ASW-3, Alcaligenes sp. ASS-1, and Bacillus sp. ASS-2. The results showed performance of biodegradation for normal alkanes is better than PAHs.

A. Degradation pathways and mechanism

There are three process for removal of petroleum compounds: 1- the first step is absorption of oil compounds onto microbial surface. 2- Then these compounds are transferred to microbial cell membrane and 3- the compounds are degraded into small molecules by microorganisms [26]. However, the degradation pathways of different hydrocarbons due to different structures is different. Because there are diverse hydrocarbons such as saturated, aromatic, resin and asphaltene fractions hydrocarbon in petroleum compounds [27] For example alkane is saturated hydrocarbon with all single bonds in the structure and Cycloalkane has several carbon rings in the structure. Degradation pathway of alkanes and cycloalkane, aromatic hydrocarbon and polycyclic aromatic hydrocarbons (PAHs) are shown in Figure 2, 3 and 4 respectively [28].

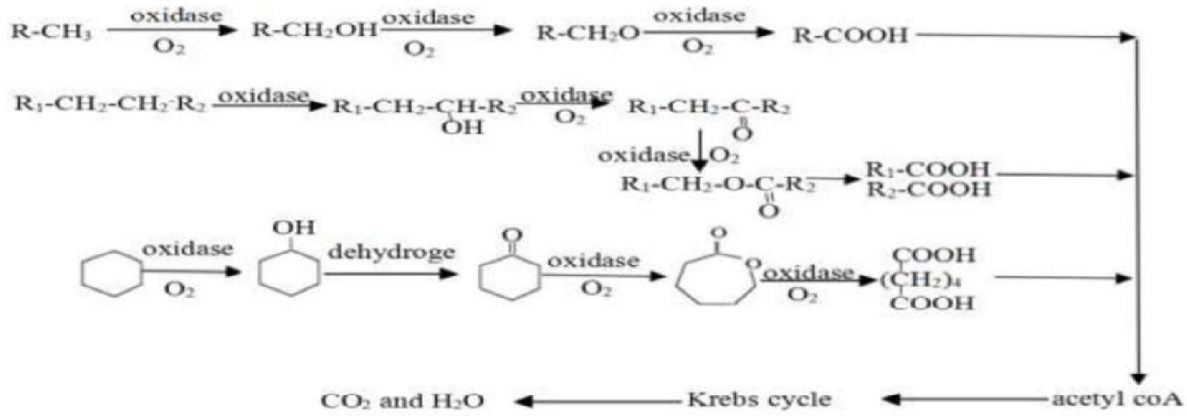

Fig. 2. Degradation pathway of alkanes and cycloalkane

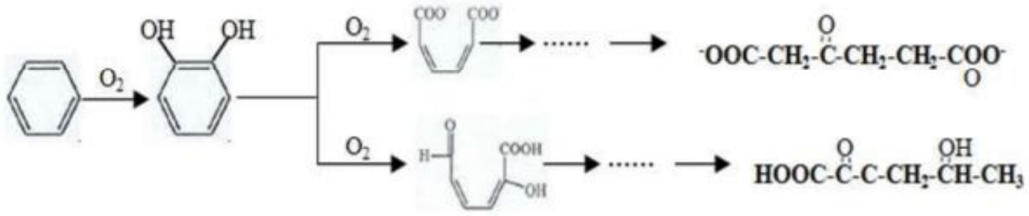

Fig. 3. Degradation pathway of aromatic hydrocarbon

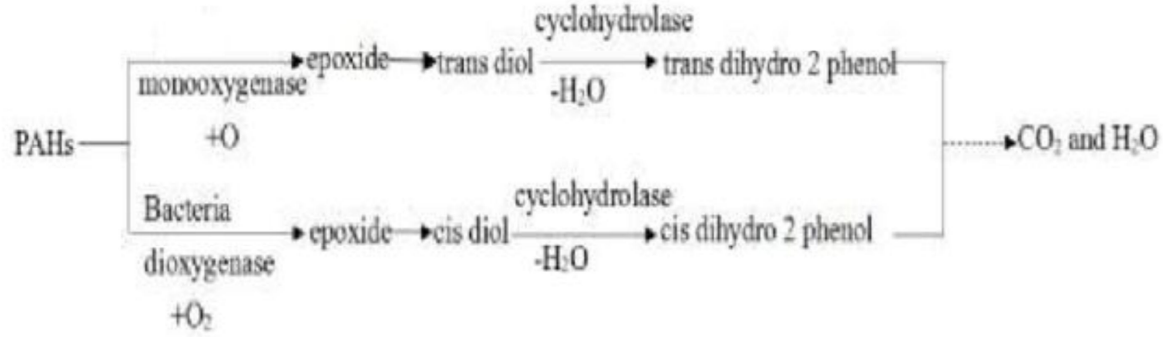

Fig. 4. Degradation pathways of polycyclic aromatic hydrocarbons (PAHs)

II. METHODS (literature review)

It has been reported many studies that investigated about different bacteria and algae for remediation of pollutants in marine ecosystems. In this review article, we summarize them

.

A.Different algae for removal of oil products

The algae play an important role in controlling and biomonitoring of organic pollutants in aquatic ecosystems.

1. Cellana

Cellana is a type of algae that can be used for removal of crude oil. Cellana is in the process of evaluating commercial algae facility locations in Hawaii, the mainland U.S., the Middle East, North Africa region and in Southeast Asia. This alga is popular for removal of oil and its compounds.

2. Scenedesmus obliques

Rajasulochana et al [29] studied on bioremediation of oil refinery effluent by using Scenedesmus Obliquus. Micro algal populations were collected at different places from where the effluent was collected; isolated and identified by using the standard manual and were maintained in Bold Basal Medium (BBM). It is observed that Scenedesmus obliquus proved to be an efficient removal of most of the parameters including COD and BOD.

3. Aphanocapsa sps.,

Chlorella autotrophica, Coccochloris elabens, Dunaliella tertiolecta,

4. Oscillatoria sps.,

Scenedesmus obliques, Synechococcus elongates and Volvox

Cao et al [30] studied on effects of Micro-Algae characteristics on the bioremediation rate of deep water horizon crude oil. Algal growth in heavy crude conditions was analyzed via spectrophotometry and computer image processing techniques. They stated these algae were helpful in the degradation of 35% of the crude and they remain alive also in high crude condition abilities that are necessary for bioremediation. Results demonstrate that properly controlled algae are feasible agents for crude oil bioremediation.

5. Scenedesmus obliquus GH2

Tang et al [31] demonstrated a specific type of alga called Scenedesmus obliquus GH2 could degrade crude oil from aquatic ecosystems with appropriate efficiency.

6. Chlorella vulgaris

Algae constitute the first chain in different groups of organisms. Chlorella Vulgaris alga is a group of unicellular and non-mobile green algae found on the surface of wet soil, within sweet waters and saline water. El-Sheekh et al [32] studied on Biodegradation of crude oil by Scenedesmus obliquus and Chlorella vulgaris growing under heterotrophic conditions. They found this alga degrade oil effectively when incubated with low concentrations of oil.

7. Selenastrum capricornutum

Gavrilescu [33] studied ability of Selenastrum capricornutum alga on degradation of some petroleum products such as Benzene, toluene, naphthalene, phenanthrene and pyrene. It has been concluded that Organic pollutants can be accumulated by this type of alga.

8. Prototheca zopfi

Walker et al [18] isolated an alga, Prototheca zopfi which was capable of utilizing crude oil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and isoalkanes as well as aromatic hydrocarbons.

B. Different bacteria for removal of oil products

Certain bacteria belonging to the Bacillus and Pseudomonas species have these desirable characteristics: they consume organic materials thousands of times faster than other types of bacteria. they grow and reproduce easily, are non-pathogenic, and do not produce foul odors or gas [34] Almost 79 genera have been identified from bacteria that have the potential to use hydrocarbon as a source of carbon and energy.

1. Pseudomonas aeruginosa

Strain of Pseudomonas aeruginosa can be used for degradation of crude oil in water ecosystems [20]. The biomass of Pseudomonas aeruginosa was first isolated from 3-day-culture broth by centrifugation at 350 g for 20 min. The pellets were first immersed in 1% sodium hypochloride for 30 min before Pseudomonas aeruginosa was confirmed to be dead according to viability staining. Then the killed Pseudomonas aeruginosa were precipitated by centrifugation and washed three times with mineral medium. Also physical factors such as temperature, pH etc., was adjusted for this bacteria. The adsorption of crude oil by cell mass was implemented in a 1 L shaking flask at temperature of 35 °C and rotary speed of 150 r/min. Forty-eight hours later, the initial crude oil and the final crude oil were measured for determination of the adsorption capacity. It is concluded that the capacity of this bacteria for biodegradation of the crude oil is high.

2. Pseudomonas xanthomarina KMM 1447 and Pseudomonas stutzeri ATCC 17588

El-Sheshtawy et al [35] used two bacterial species (Pseudomonas xanthomarina and Pseudomonas stutzeri). They concluded these bacterial strains may be useful for the bioremediation process in the Gemsa Bay, Red Sea decreasing oil pollution in this marine ecosystem.

3. Bacillus subtilis CN2

Bezza and Chirwa [36] studied on production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by Bacillus subtilis CN2. The results reveal the potential of the bio surfactant for use in bioremediation of hydrocarbon contaminated environmental media.

4. Schizothrix vaginata ISC108

Safari et al [37] used Schizothrix vaginata ISC108 for removal of petroleum hydrocarbons. It was found that Schizothrix vaginata cyanobacteria has a high potential for crude oil biodegradation. Therefore, since oil is a toxic product for biological systems and one of the main pollutants of biological ecosystems, the results indicate the potential of this cyanobacteria to be used as an indicator for eliminating oil pollution in contaminated areas.

CONCLUSION

The release of contaminants, such as petroleum and its byproducts, into the environment is one of the main causes of global pollution [38]. Oil and petroleum products can pollute sources of water such as seas, oceans, rivers, or underground waters [39]. Water contamination with oil and its products is becoming a critical issue for environmental researchers because it can threaten human health, plant, birds, and aquatic life. Bioremediation is the act of adding materials to contaminated environments for natural biodegradation processes [40]. Bioremediation technology is a desirable alternative and believed to be noninvasive, relatively cost effective and important method for the treatment of oil-contaminated sites [41], [42], [43], [44]. There are widespread uses of bacteria and algae for bioremediation of petroleum and hydrocarbons

compounds. Requirements of a successful bioremediation process of petroleum contaminants are including of availability of microorganisms, environmental/nutritional factors include the physical nature of the crude oil, the chemical nature of the crude oil, availability of nutrients, concentration of oxygen, pH and water temperature and bio surfactants [45].

The aim of this paper is investigation of bacteria and algae application in bioremediation of oil compounds. To future, it is necessary to identify patterns of microbial diversity associated with optimum contaminant removal and environmental factors that promote the process. It has been concluded that however many microorganisms can be used for biomonitoring and restoration of aquatic systems, but there are still some persistent organic pollutants that are difficult to break down by the microorganisms. The genetic engineering can solve this problem and exhibits a promising tool to improve the absorption and bioremediation of many organic pollutants and increase micro-algal tolerance to these pollutants and it is necessary to study about the best condition of environment such as pH, nutrients, temperature and etc. to increase the biodegradation and reduce the time of bioaccumulation.

REFERENCES

[1] Stancu, M. M. Bacterial Degradation of Petroleum and Petroleum Products. Journal of Molecular Microbiology. Vol.2. No.1, 2018.

[2] Al-Wasify RS, Hamed SR. Bacterial biodegradation of crude oil using local isolates. Inte J Bacteriol: 863272, 2014.

[3] Prince, R.C. Lessard, R.R. and Clark, J.R. Bioremediation of Marine Oil Spills. Oil & Gas Science and Technology – Rev. IFP, Vol. 58, No. 4: 463-468, 2003.

[4] Su F S, Lu C S, Hu S K. Adsorption of benzene, toluene, ethylbenzene and p-xylene by NaOCl-oxidized carbon nanotubes. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 353 (1): 83–91, 2010.

[5] Ollis, D. Slick solutions for oil spills. Nature, 358:453-454,1992

[6] Megharaj, M., Ramakrishnan, B., Subashchandrabose, S.R., Venkateswarlu, K., Naidu, R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. Environment International. 51: 59-72, 2013.

[7] Mohammadi F. Akhavan A. Mohammadi F. Amini M. Bioremedation of water contaminated with crude oil per isolatin Bacillus from oily pound. Journal of Yazd School of Public Health. 11(2): 107-118, 2012.

[8] Singh, K. and Chandra, S. Treatment of petroleum Hydrocarbon polluted environment through bioremediation: A review. Pakistan journal of biological sciences 17(1): 1-8, 2014.

[9] Das, N. Chandran, P. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. SAGE-Hindawi Access to Research Biotechnology Research International. Pp 13., 2011.

[10] Cooney, J. The fate of petroleum pollutants in fresh water ecosystems," in Petroleum Microbiology, R. M. Atlas, Ed., pp. 399–434, Macmillan, New York, NY, USA, 1984.

[11] Rahman, K. S. M. Rahman, T. J. Kourkoutas, Y. Petsas, I. Marchant, R. and Banat, I. M. "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.

[12] Chaillan, F., Gugger, M., Saliot, A., Coute, A., Oudot, J. Role of cyanobacteria in the biodegradation of crude oil by a tropical cyanobacterial mat. Chemosphere. 26: 1574-1582., 2006.

[13] Jones, D. M. Douglas, A. G. Parkes, R. J. Taylor, J. Giger, W. and Schaffner, C. "The recognition of biodegraded petroleum-derived aromatic hydrocarbons in recent marine sediments," Marine Pollution Bulletin, vol. 14, no. 3, pp. 103–108, 1983.

[14] Adebusoye, S. A. Ilori, M. O. Amund, O. O. Teniola, O. D. and Olatope, S. O. "Microbial degradation of petroleum hydrocarbons in a polluted tropical stream," World Journal of Microbiology and Biotechnology, vol. 23, no. 8, pp. 1149–1159, 2007.

[15] Hollaway, S. L. Faw, G. M. and Sizemore, R. K. "The bacterial community composition of an active oil field in the Northwestern Gulf of Mexico,"Marine Pollution Bulletin, vol. 11, no. 6, pp. 153–156, 1980.

[16] Phillips G. J. and Stewart, J. E. "Distribution of hydrocarbon utilizing bacteria in Northwestern Atlantic waters and coastal sediments," Canadian Journal of Microbiology, vol. 20, no. 7, pp. 955–962, 1974.

[17] Yakimov, M. M. Timmis, K. N. and Golyshin, P. N. "Obligate oil-degrading marine bacteria," Current Opinion in Biotechnology, vol. 18, no. 3, pp. 257–266, 2007.

[18] Walker, J. D. Colwell, R. R. Vaituzis, Z. and Meyer, S. A. "Petroleum degrading achlorophyllous alga Prototheca zopfii," Nature, vol. 254, no. 5499, pp. 423–424, 1975.

[19] Cerniglia, C. E. Gibson, D. T. and Van Baalen, C. "Oxidation of naphthalene by cyanobacteria and microalgae," Journal of General Microbiology, vol. 116, no. 2, pp. 495–500, 1980.

[20] Guo-liang, Z. Yue-ting, W. Xin-ping, Q and Qin, M. Biodegradation of crude oil by Pseudomonas aeruginosa in the presence of rhamnolipids. Journal of Zhejiang University SCIENCE. 6B (8):725-730, 2005.

[21] Deviram, G. Pradeep, K.V. and Prasuna, G. Purification of waste water using Algal species. European Journal of Experimental Biology, 1 (3): 216-222, 2011.

[22] Binark, N. Güven, K. C. Gezgin, T and Ünlü, S. Oil Pollution of Marine Algae. Bull. Environ. Contam. Toxicol. ,64:866-872, 2000.

[23] Hasan Shahiyan, M. Emtiyazi, G. Isolation and molecular identification of Alcanivorax dieselolei from Persian Gulf and Investigation of its biodegradability to clean up oil pollution. Journal of Biology of microorganisms, 1(1): 31-40, 2012.

[24] Roy S, Dipak H, Debabrata B, Dipa B, Ranajit K. Survey of petroleum-degrading bacteria in coastal waters of sunderban biosphere reserve World J Microbio Biotechno; 18: 575–581, 2002.

[25] Chen, Q. Li, J. Liu, M. Sun. H. and Bao, Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. PLOS ONE. 12(3): e0174445,2017.

[26] Atlas R.M. Petroleum biodegradation and oil spill bioremediation, Marine Pollution Bulletin 31:178-182, 1995.

[27] Øksenvåg, J. McFarlin, K. Netzer, R. Brakstad, O.G. Hansen, B.H. and Størseth, T, Biodegradation of Spilled Fuel Oil in Norwegian Marine Environments. The Norwegian Coastal Administration. pp. 58, 2018.

[28] Xue, J. Yu, Y. Bai, Y. Wang, L. Wu, Y. Marine Oil-degrading microorganisms and Biodegradation Process of Petroleum hydrocarbon in Marine Environments: A Review. Microbiology journal, v. 71, no. 2 pp. 220-228, 2015.

[29] Rajasulochana, P., Dhamotharan, R., Murgesan, S. and Rama Chandra Murthy A. Bioremediation of Oil Refinery Effluent by using Scenedesmus Obliquus. Journal of American Science; 5(4): 17-22, 2009.

[30] [30] Cao Xiuqi, Ying Xiong and Janelle Lund. The effects of micro-algae characteristics on the bioremediation rate of Deepwater Horizon Crude Oil. Journal of Emerging Investigators, 2013.

[31] Tang X, He LY, Tao XQ, Dang Z, Guo CL, Lu GN, Yi XY. Construction of an artificial microalgal-bacterial consortium that efficiently degrades crude oil. J. Hazard. Mater. 181:1158-62, 2010.

[32] El-Sheekh, M.M., Hamouda, R.A., Nizam, A.A. Biodegradation of crude oil by Scenedesmus obliquus and Chlorella vulgaris growing under heterotrophic conditions. International Biodeterioration & Biodegradation. 82: 67-72, 2013.
[33] Gavrilescu M. Environmental Biotechnology: Achievements, Opportunities and Challenges. Dynamic Biochem Process Biotech. Mol.Biol. 4:1-36, 2010.
[34] Efeovbokhan, V.E. Hymore, F.K. Ayoola. A.A. Adeeyo, O.A. Comparison of Aerobic and Anaerobic Bioremediation of Polluted Water Samples. American International Journal of Contemporary Research. Vol. 4 No. 4: 120-126, 2014.
[35] El-Sheshtawy, H.S. Khalil, N.M. Ahmed, W. and Abdallah, R.I. Monitoring of oil pollution at Gemsa Bay and bioremediation capacity of bacterial isolates with biosurfactants and nanoparticles. Marine Pollution Bulletin 87, 191–200, 2014.
[36] Bezza, F.A. Chirwa, E.N. Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by Bacillus subtilis CN2. Biochemical Engineering Journal 101, 168–178, 2015.
[37] Safari, M. Ahmadi, S. and Soltani, N. Investigation of the ability of Schizothrix vaginata ISC 108 to bioremediation of crude oil. Journal of Health and Environment, 7(3): 363-374, 2014.
[38] Silva, R.D.C.F. Almeida, D.G. Rufino, R.D. Luna, J.M. Santos, V.A. Sarubbo, L.A. Applications of biosurfactants in the petroleum industry and the remediation of oil spills, Int. J. Mol. Sci. 15 (7) 12523–12542, 2014.
[39] Annunciado, T. R., Sydenstricker, T. H. D., and Amico, S. C., Experimental Investigation of Various Vegetable Fibers as Sorbent Materials for Oil Spills Marine Pollution Bulletin, Vol. 50, p. 1340-1346, 2005.
[40] Swannell, R. Lee, K. McDonagh, M. Field Evaluations of Marine Oil Spill Bioremediation. Microbiological Reviews 60, 342-365, 1996.
[41] April, T.M.; Foght, J.M. and Currah, R.S: Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. Can. J. Microbiol., 46(1): 38–49, 2000.
[42] Zhu, X.; Venosa, A.D.; Suidan, M.T. and Lee, K: Guidelines for the bioremediation of oilcontaminated salt marshes. EPA/600/R-04/074, EPA Contract No. 68-C-00-159, Task Order No. 8, National Risk Management Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268, USA, 61p, 2004.
[43] Karhu, M.; Kaakinen, J.; Kuokkanen, T. and Rämö, J: Biodegradation of light fuel oils in water and soil as determined by the manometric respirometric method. Water Air Soil Pollut, 197(1-4): 3–14, 2009.
[44] Montagnolli, R.N.; Matos Lopes, P.R. and Bidoia, E.D: Assessing Bacillus subtilis biosurfactant effects on the biodegradation of petroleum products. Environ. Monit. Assess, 187:4116, 17p, 2015.
[45] Zaki, M.S. Authman, M. N. and Abbas, H.H. Bioremediation of Petroleum Contaminants in Aquatic Environments (Review Article). Life Science Journal; 12(5): 127-139, 2015.

★★★