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Review on bioremediation of oil spillage and plastics through effective microorganisms

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Abstract

The worldwide increased demand of crude oil (also known as petroleum hydrocarbon) has elevated the obligation of clean-up technologies for the oil-spillage treatments. Bioremediation, an effective measure for eradication of this hazardous contaminant is exploited unanimously but there arise major challenges because the process proceeds in a sluggish way and is time consuming which cannot meet up the instant requirements of clean-up of the marine environment. This review article is focused on the different sources of microorganisms studied in the last two decades and their beneficial involvement in the biodegradation of hydrocarbons. Moreover, the immeasurable consumption of plastic in our daily life is becoming life threatening for the environment. This review is principally aimed to study the different bacterial species predominantly the *Bacillus* strains and fungal isolates acting as predominant protagonists in the biodegradation process of complex hydrocarbons such as plastic.

Keywords

Crude oil, hydrocarbons, plastics, biodegradation, bioremediation, microorganisms

Introduction:

Over the last three decades, bioremediation is used as an alternative source for clean-up technology for immediate restoration of affected areas. Crude oil is composed of aromatic and aliphatic hydrocarbons, which are released naturally, accidentally or purposefully to the aquatic

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environment especially to the marine ecosystem. Moreover, the increased consumptions of petroleum products by human activity is becoming life threatening for the aquatic life. The oil enters into the feathers of birds and fur of the animals which decreases their buoyancy due to variation in temperature [1]. This is hazardous and often prove fatal to the marine flora and fauna.

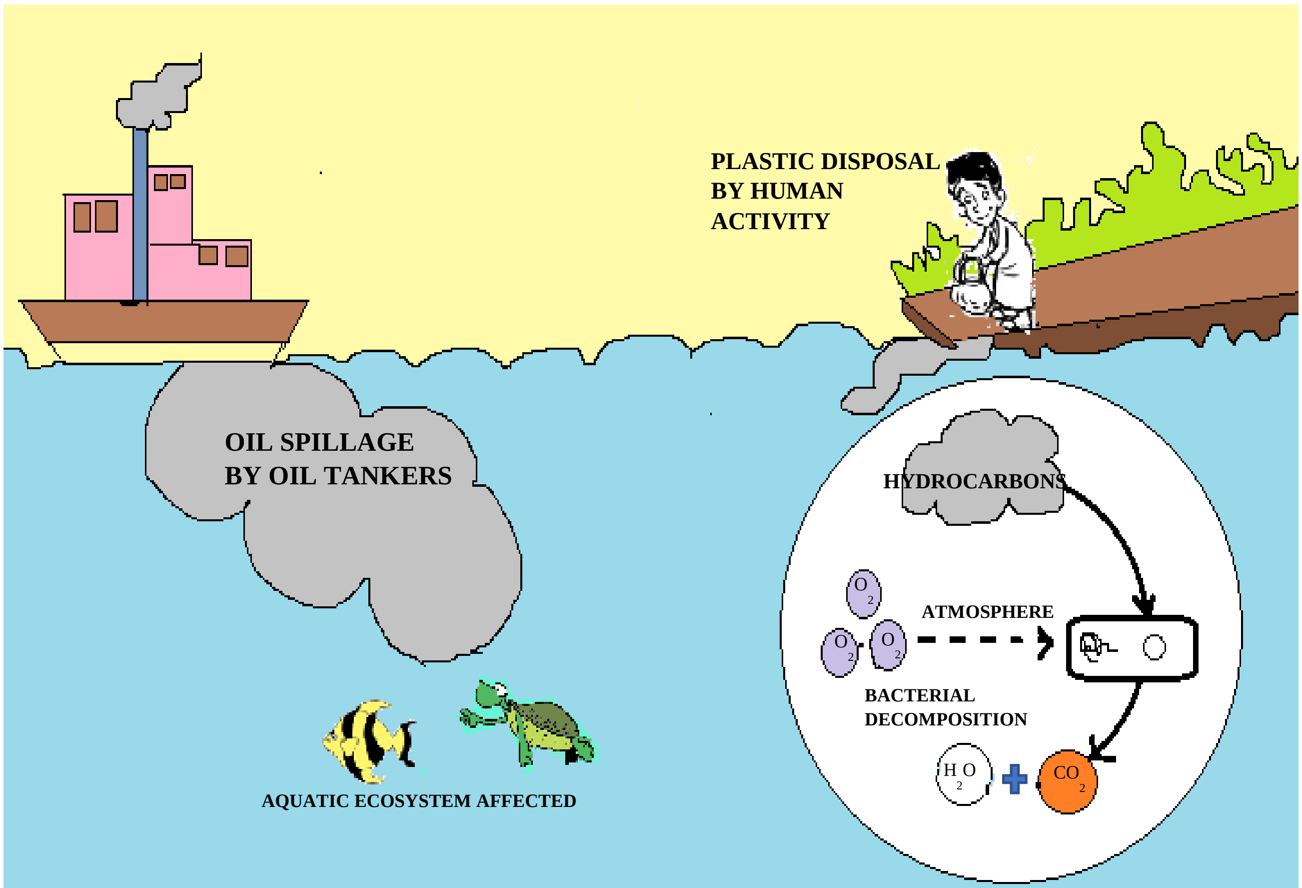

Figure 1 Marine ecosystem is regularly affected with oil spillage and human activity where the hydrocarbons are degraded therough the Bioremediation process by microorganisms.

Microorganisms are omnipresent which utilize these hydrocarbons present in the petroleum oil and decompose it to release hydrocarbons and energy. Bioremediation is the use of specific microorganisms which metabolize to remove harmful substances from the environment or add pollutant specific microbes to the affected area. Abundant species of microorganisms are present in nature but only a limited number of archaea, bacteria and fungi are present which degrade petroleum (Figure 1).

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Biodegradation:

The recent developments in the offshore oil transportation industry led to a high risk of chances of pollution in coastal waters due to oil leakage [2]. Crude oil is made up of mixtures of aromatic and aliphatic compounds and each microbe can break down and utilize only a part of the hydrocarbons as its substrate. Hence, a diverse range of micro-organisms play a key role in the degradation of hydrocarbons [3,4]. There are various bacterial strains that can degrade or transform the various products of crude oil into nontoxic, nonhazardous, biodegradable and environmentally friendly components. This process is known as the biodegradation of crude oil.

Biodegradation is the method to remove the oil pollutants from the environment with the most eco-friendly method without damaging the environment. The most primary and suitable method to eliminate the effect of crude oil with the help of microorganisms is through the procedure of biodegradation [5]. The significant role is played by bacterial strain for degradation of hydrocarbons. In addition to this, it has the ability to completely absorb the hydrocarbon and provide enough energy to the cells for growth. Marine sources such as the coastal regions and the contaminated soil samples provide the main source for isolation of bacteria. These isolated bacterial strains provide a range of microorganisms which can consume and metabolize hydrocarbons and release CO_2 and H_2O to the environment [6].

Petroleum is used as an alternative source in terms of fossil fuel as it is a non-renewable source of energy. Petroleum is used as an energy source and plays an important role in daily life. Frequent worldwide transport has increased the chances of oil spillage in the coastal region or the soil leading to oil pollution.[7].

Biodegradation is the main process in order to break down the crude oil or to reduce the effect of oil pollution in the environment. Various microorganisms like bacteria and fungi can degrade a large range of crude oil. The renowned but most common names are *Bacillus sp, Pseudomonas sp, Corneybacterium sp* and biodegrading fungi such as *Aspergillus sp, Penicillium*

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sp and *Trichoderma* sp which can degrade a wide variety of hydrocarbons. Ding and co-workers investigated that the soil from contaminated sites showed the presence of *Bacillus sp* [8].

Various factors such as temperature, oxygen, and nutritional requirement, pH of the source of contamination is responsible for biodegradation of crude oil [9]. *Bacillus sp* has the ability to grow on different substrates. They have the ability to degrade different hydrocarbons by the production of Biosurfactants (BIO), which facilitates the hydrocarbon degradation. *Bacillus* has the ability to grow on crude oil hence degrading crude oil and reducing oil pollution. Apart from this, *Pseudomonas* species is considered one of the best species which can degrade crude oil [10].

Biodegradation of Petroleum hydrocarbon by beneficial micro-organisms

Biodegradation is a multifaceted process which is largely dependent on the natural habitat and mainly the temperature and soil conditions. Petroleum hydrocarbon can be alienated into four components mainly: the saturates, the aromatics, the aliphatics like asphaltenes (for instance: porphyrins and fatty acids), and the resins (such as pyridines, quinolones, sulfoxides) [11]. A large number of factors like pH condition, climate affect the hydrocarbon degradation. Due to urbanization, there is the limited availability to microorganisms which is one of the major limitations studied so far. Moreover, the chemical petroleum hydrocarbon binds to the soil in such a complex manner that it becomes a difficult process to be removed or biodegraded.

Microbial degradation is the most popularly used method and ultimate natural mechanism to clean up the crude oil pollutant from nature. Immense studies have been conducted on the breakdown of alkyl aromatic compounds present in the marine world. Different tropical areas of Lagos Nigeria were manifested with microflora in the soil and the main strains were *Pseudomonas fluorescens, P. aeruginosa*. *Bacillus subtilis, Bacillus sp*., *Acinetobacter iwoffi, Flavobacterium sp., Micrococcus eosus, and Corynebacterium sp* [12].

Yeast, fungi and bacteria are the naturally occurring group of micro-organisms widely distributed which assist in the degradation of hydrocarbons. The biodegradation range of soil fungi

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is from a lower percentage of 6 and extends largely upto 82 percentage. Soil bacteria extends from 0.13 to 50 percentage, and for marine bacteria the range is between 0.003 to 100. Many scientists come to the conclusion that the mixed population of all microorganisms are required to degrade the crude oil from soil, freshwater and aquatic habitat. Bacteria are the most extensively used agent in degradation of petroleum and they help in the degradation of all types of oil spillage in the environment. Floodgate listed about 25 bacterial genera and 25 fungal genera which degrade hydrocarbons and these were isolated from the marine habitat where it was found that *Acinetobacter sp*. utilize n- alkanes of chain length of C10 – C40 as the main carbon source [13].

Bioremediation:

Bioremediation is considered as the alternative source to treat the residual oils and considered for cleanup technology [14]. Bioremediation is proved to be the cheapest and most efficient method to resolve the oil spills from the discharge from the oil refineries, shipping or tankers spillage, accidental breakage of pipelines and offshore production which greatly affect the environment. Bioremediation based on the metabolic activity of microorganisms has certain advantages; also, it is cheaper than many other remediation technologies [15,16].

Bioremediation by bacteria

It is reported that *Acinetobacter sp* named as D-32 isolated from contaminated soil samples consume hydrocarbons as their sole energy source and act as a biosurfactant achieving production level of $0.52gL^{-1}$ with a reduction of surface tension from 48.02 to $26.30mNm^{-1}$.The capacity to degrade hydrocarbons was found to be 82% [17].

N-alkanes and crude oil (C13 to C18) were found to be biodegraded by *Aspergillus niger*, *Aspergillus ochraceus* and *Penicillium chrysogenum* utilizing C15, C16, C17 and C18 and was statistically insignificant for biomass and oil utilization out of ten fungal species isolated from the beaches of Oman [18].

Degradation of diesel oil present in contaminated soil by Bacillus subtilis and fungal isolates.

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Extensive studies have been done on *Bacillus sp* and it was observed that *Bacillus subtilis, Bacillus cereus, Trichoderma harzanium and Trichothercium roseum* are amongst the outstanding bacterial strains. They possess the highest capacity to consume the carbon source of hazardous hydrocarbon. Studies have been carried out in contaminated soil samples considering *Bacillus subtilis* as the control. Gravimetric study was carried on for a period of twenty-seven days to monitor the breakdown of diesel. Serial breakdown of the diesel compounds was observed on each day. Fungal isolates observed which are responsible for breakdown of diesel crude oil is shown in Table 2.

Diesel is the major constituent product of crude oil, contributing a major source in environmental pollution. In Arctic tundra soil, $7.5*10^5$ colony forming unit (CFU) was observed in control whereas 41×10^7 CFU was observed in the test sample which was polluted for a stretch of fourteen months. colony-forming unit [19]. A detailed CFU of the commonly used bacteria is provided in Table 2.

For a better understanding of the degrading effect of *Bacillus* species hydrocarbon utilization test was done by first isolating the bacteria to study their efficacy. Determination of colony numbers through counting of colony forming units, sample collection, and preparation were carried on for further diesel oil degradation studies.

Table : 1 Characteristic fungal morphology of the various fungal species that can biodegrade crude oil.

Isolated serial no.	**Morphology of the colony**	**Microscopic view**	**Microorganism**
F1	Colony is whitish green in colour with reverse side being colourless	Presence of well branched Condiophore with short branches in the apical region	*Trichoderma harzanium*
F2	Fungilose which spreaded from the centre to the exposed surface while the underlying surface was light brown colour in appearance.	Condiophore has a zigzag appearance with canidia obliquely based scares	*Trichothercium roseum*

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Table: 2 Micro-organisms (bacterial strains) producing CFU

Name of the microorganism	CFU colony forming units)
Bacillus subtilis	5.1*105
Bacillus cereus	2.8*103
Trichoderma harzanum	1.2*102
Trichothercium roseum	1.1*102

Studies have revealed that *Bacillus subtilis* shows biodegradation effectively with combination of paraffin supported fertilizer (PSF) as a Nitrogen source. Inorganic fertilizer is also reported to augment the process of biodegradation [20].

Biodegradation of plastics by microorganisms

The use of plastics is reliable and very important in our daily lives but it also has harmful side-effects to the environment. Due to availability of various types of plastic product easily, and their advantage of low weight, it is durable for long term use and it is cheaper from production point of view [21,22,23]. But the consequences is really freaking. It doesnot undergo biodegradation easily and the combustion leads to release of harmful gases to the environment [24]. There are various chemicals that are released from decomposition of the plastics waste. Plastic degradation takes a long time to degrade and complete the process [25]. Plastics not only create pollution in soil but also in marine region also. The bacteria which uses the polyethene as the sole carbon source to degrade the plastics are mainly *Kocuria palustris M16*, *Bacillus pumilus M27*, and *Bacillus subtilis H1584* [26]. The different strains of these bacteria were isolated from the pelagic waters. An overview of the biodegradation process of plastics by bacteria is shown in Figure 2.

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There are many types of plastics which are present worldwide, some of them are nylon, polyesters, polylactic acid (PLA), polyvinyl alcohol (PVA), polyhydroxybutryrate (PHB) and many others [27]. To mostly degrade the plastics from the environment the most easy and efficient way are biotic and abiotic factors which helps to break down the polymers of plastics constituting

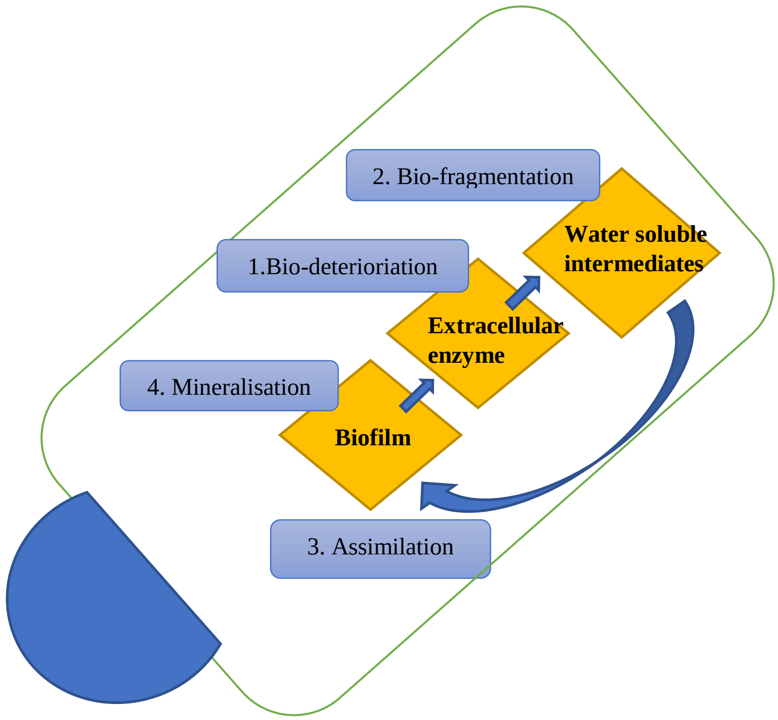

Figure 2 Steps of biodegradation of plastic by microorganisms

hydrocarbons into simpler forms [28,29]. Nowadays the various steps that are involved in biodegradations of plastics process are (bio deterioration, assimilation, bio fragmentation and mineralization) clearly depicted in Figure 2.

Bacteria and fungi family which can degrade plastics are *Bacillus sp.*, *Clostridium botulinum*, *Pseudomonas sp*, *Rhodococcus sp*, *Streptomyces*, gram-positive and gram-negative bacteria, *Staphylococcus sp*, *Diplococcus sp*, *Micrococcus sp*, *Moraxella sp* [30]. Studies showed that *Pseudomonas sp* has high probability to degrade the main plastics like PHB and PVC. There are numerous ways through which the microorganism can utilize plastic, some of the most

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convenient methods are the specific enzymes released by the microorganism. The exoenzymes secreted by the microorganism can degrade a wide range of polymers that are available in outer layer/boundary of the plastic [31]. One of the most renowned fungal isolates that can degrade plastic is *Phanerochaete chrysosporium.* This fungus has the capacity to degrade high molecular weight of polyethylene (PE) [32]. A wide range of micro-organisms playing an important role in the effective biodegradation process is tabulated in Table 3.

Table 3: Degradation of plastic by different microorganisms

Sl.No.	Plastic	Microorganism	Research Papers
1.	Polyethylene	*Rhodococcus rubber, Pseudomonas chlororaphis*	Sivan et al. (2006), Zheng et al. (2005)
2.	Polyvinyl chloride	*Pseudomonas putida*	Anthony et al (2004)
3.	Poly(3-hydroxybutyrate-co-3-mercatopropionate)	*Schlegelella thermodepolymerans*	Elbanna et al.(2004)
		Pseudomonas indica K2	
4.	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	*Clostridium botulium, Clostridium acetobutylicum*	Abou Zeid et al. (2001)
5.	Polycaprolactone	*Clostridium botulium, Clostridium acetobutylicum*	Abou Zeid et al. (2001)

In addition to degradation of plastics with the help of microorganisms, an alternative way is to recombine the chemicals with the microbes which can alleviate the process of biodegradation by plastic. The recombination of alkane hydrolase (AH) with *Pseudomonas sp* E4 expressed in *Escherichia coli* BL21 can convert 20% of polyethylene [PE] and can reduce its molecular weight to lower dry weight percentage. The petroleum-based plastics are less likely to be degraded more quickly and hence it's a time-consuming process to degrade these kind of plastics [33,34,35,36].

Conclusions

Oil spillage and use of plastic by human activity and its disposal on aquatic eco-system has become life threatening for the marine environment. Different microorganisms specially *Bacillus sp* and fungal isolates such as *Trichoderma sp* play an effective role on the biodegradation process.

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Although the process by microorganisms is quite beneficial to the environment, the major bottleneck is that plastic is composed of complex hydrocarbons, so the effectiveness is quite sluggish. Moreover, urbanization has lead to diminished soil quality, so the microflora is reduced in nature.

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References:

[1] C. Mostazo, M.R.Cozzo, . Biotreatment of hydrocarbons from petroleum tank bottom sludges in soil slurries. Biotechnology Letters.18:1241-1246. Francis, M., 1989. Land farming of oily wastes In: Cready, M. [Eds] Proceeding of the fifth Annual General Meeting of BIOMINET. LGP Camnet Special Publication. Pp. 119-127, 1996

[2] M.G. Carles, M.L Larsen, L. G. Holland, Spilled oil: static mixtures or dynamic weathering and bioavailabilty, Plos One, pp.1-22, 2015.

[3] R.M. Atlas, 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. vol, 45, pp.180 209, 1981.

[4] R.M. Atlas, 1984.Use of microbial diversity measurements to assess environmental stress. In: M.J. Klugand C.A. Reddy (eds.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C. pp. 540 -545,1984.

[5] A. Korda, P. Santas, A. Tenente, R. Santas. Petroleum hydrocarbon bioremediation. Appl. Microbial. Biotechnol. vol,48, pp.677-689, 1997

[6] A. Korda, P. Santas, A. Tenente, R. Santas. Petroleum hydrocarbon bioremediation. Appl. Microbial. Biotechnol. vol,48, pp.677-689, 1997

[7] O. Obire, O. Nwaubeta. Biodegradation of refined petroleum hydrocarbons in Soil, J App Sci Environ. Management. vol, 5(1), pp. 43-46, 2001.

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

[8] K. Ding, Y. Luo, T. Sun, P. Li. Boiremediation of soil contaminated with petroleum using forced-aeration composting, vol,3, pp.1224-1234, 2002.

[9] E. Kerry. Bioremediation of experimental petroleum spill in the vestfold Hills, Antarctica. Polar – Biology. vol, 13:3, pp.163-170, 1993.

[10] J. J Kilbane, R. Ranganathan, M Monicom, L. Ribuero S.Kaysus 2000 Selective removal of nitrogen from quinoline and petroleum by *Pseudomonas ayucida* IGTN 9M.App.Environ. microbiol. vol,66, pp.688-693, 2000.

[11] J. J. Cooney, S. A. Silver, and E. A. Beck, "Factors influencing hydrocarbon degradation in three freshwater lakes," Microbial Ecology, vol. 11, no. 2, pp. 127–137, 1985

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

[13] A. Eishafie, A.Y. Alkindi, S. Eishafie, A.Y. Alkindi, C. Al-Busaidi, C. Bakheit, S. N. Albahry Biodegradation of crude oil and n-alkanes by fungi isolated from Oman, Mar Pollut Bull, vol, 54, pp. 1692-1706, Nov,2007.

[14] G. A. Sergy, C.C. Guenette, E. H. Owens, R.C. Prince, K. Lee, In-situ treatment of oiled sediment shorelines. Spill Sci. Technol. Bull, vol,8(3): 2pp.37-244, 2003.

[15] B. Bharathi, E. Gayathiri, S. Natarajan, S. Selvadhas, R. Kalaikandhan, "Biodegradation of crude oil by bacteria isolated from crude oil contaminated soil – a review", International Journal of Development Research, vol,7, (12), pp.17392-17397, 2017.

[15] J. H. Exner, 1994. Introduction In: Flathman, P.E., Jerger, D.E., Exner, J.H. (Eds.). Bioremediation: Filed Experience. Lewis Publishers, Boca Rat'on FL, USA. Ferrari, M.D., Neirotti, E., Albornoz, 1994.

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

[17] M. Bao, Y.Pi, L. Wang, P. Sun, Y. Li, L Cao "Lipopeptide biosurfactant production bacteria Acinetobacter sp D3-2 and its biodegradation of crude oil", Environ. Sci. process Impacts., pp.1-7, 2014.

[18] A. Eishafie, A.Y. Alkindi, S. Eishafie, A.Y. Alkindi, C. Al-Busaidi, C.Bakheit, S. N. Albahry Biodegradation of crude oil and n-alkanes by fungi isolated from Oman, Mar Pollut Bull, vol, 54, pp. 1692-1706, Nov,2007.

[19] R. M. Atlas, R. Bartha. Biodegradation and mineralization of petroleum in seawater at low temperature. Canadian Journal of Microbiology, vol,18, pp. 1851-1855, 1972

[20] M.G. Carles, M. L. Larsen, L. G. Holland, Spilled oil: static mixtures or dynamic weathering and bioavailabilty, Plos One, pp.1-22, 2015.

[21] A. L. Andrady, M. A. Neal. Applications and societal benefits of plastics. Philos T R Soc B vol, 364, pp. 1977– 1984, 2009.

[22] A.L. Andrady, (2015d) Plastic products. In Plastics and Environmental Sustainability. Hoboken, New Jersey: John Wiley & Sons, pp. 83–119, 2015.

[23] R. C. Thompson, S.H. Swan, C.J. Moore, F.S. F.S. om Saal Our plastic age. Phil Trans R Soc B vol, 364, pp. 1973–1976, 2009.

[24] B. Gewert, M.M. Plassmann, M. MacLeod. Pathways for degradation of plastic polymers floating in the marine environment. Environ Sci Proc Imp, vol, 17, pp. 1513– 1521, 2015.

[25] E. J. North, R.U Halden. Plastics and environmental health: the road ahead. Rev Environ Health, vol, 28 (1),2013.

[26] K. Harshavardhan, B, Jha. Biodegradation of low density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. vol, 77, pp. 100-106, Dec15, 2013.

[27] M. Shimao. Biodegradation of plastics. Current Opinion in Biotechnology, vol, 12, pp. 242–247, 2001.

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

[28] B. Ipekoglu, H. Böke, O. Cizer. 2007. Assessment of material use in relation to climate in historical buildings. Building and Environment, vol. 42, pp. 970-978, 2007.

[29] C. Helbling, M. Abanilla, L Lee, V.M. Karbhari. 2006. Issues of variability and durability under synergistic exposure conditions related to advanced polymer composites in civil infrastructure. Composites Part A: Applied Science and Manufacturing, vol, 37pp. 1102-1110, 2006.

[30] K. Kathiresan. Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. Revista de Biologia Tropical,vol, 51, pp.629-634,2003.

[31] A. Lugauskas, L. Levinskait, D. Peciulyte. Micromycetes as deterioration agents of polymeric materials. International Biodeterioration and Biodegradation, vol, 52, pp. 233-242, 2003.

[32] Y. Iiyoshi, Y. Tsutsumi, T. Nishida Polyethylene degradation by lignin-degrading fungi and manganese peroxidase. J Wood Sci, vol, 44, pp.222, 1998.

[33] R.-J. Mueller. Biological degradation of synthetic polyesters–enzymes as potential catalysts for polyester recycling. Process Biochem vol,41, pp. 2124–2128, 2006.

[34] Y. Zheng, E.K.Yanful, A.S. Bassi.A review of plastic waste biodegradation. Crit Rev Biotechnol vol, 25, pp. 243–250, 2005.

[35] Y. Tokiwa B. Calabia, C. Ugwu, S. Aiba, Biodegradability of plastics. Int J Mol Sci vol, 10, pp. 3722, 2009.

[36] R.-J. Mueller. Biological degradation of synthetic polyesters–enzymes as potential catalysts for polyester recycling. Process Biochem, vol,41, pp. 2124–2128.