ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 5, pp 886-890, May 2016

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

BIODEGRADATION OF PETROLEUM HYDROCARBON USING ASPERGILLUS SPP

Shrejal M. Bhotmange[1]., Tumane P. M[2] and *Durgesh D. Wasnik[3]

Department of Microbiology, L.I.T. Premises, R.T.M. Nagpur University, Nagpur (M.S.)-440 033

ARTICLE INFO

Article History:

Received 26th February, 2016
Received in revised form 17th March, 2016
Accepted 25th April, 2016
Published online 28th May, 2016

Key words:

Petroleum Hydrocarbons, Oil Spill, Biodegradation, A. niger

ABSTRACT

This study investigated the ability of *Aspergillus niger* to degrade petroleum products. The fungal isolate obtained in this study was *Aspergillus niger* from oil contaminated soil. Bushnell Hass Broth (BHB) was prepared for the degradation study which is supplemented with Tween 80 and resazurin. In this study, engine oil and Diesel was used for degradation. *Aspergillus niger* showed highest percentage of degradation of both engine oil and Diesel. The percentage degradation was found 75.77% and 91.75% of engine oil and diesel respectively. These results were confirmed by GC analysis. Result of GC analysis of engine oil showed that the control sample was performed showed 13 peaks whereas test sample of engine oil showed only 2 peaks with retention time of 1.288 and 1.404. On the other hand, GC analysis of Diesel, control sample was performed showed 22 peaks and test sample showed 5 peaks out of 22 with retention time of 1.293, 1.411, 1.835 and 2.616. *Aspergillus niger* degrades both the engine oil and diesel, but the highest degree of degradation was found against diesel sample (91.75%). The data obtained in the present investigation advance the knowledge of degradation of petroleum hydrocarbons by *Aspergillus niger* isolated from soil and may make these promising candidates for removal of petroleum products from contaminated environment.

INTRODUCTION

The Spilled petroleum constitutes (Hydrocarbons) are one of the main environmental pollutants. Their abundance and persistence in several polluted environmental areas have been reported (M. A. Mohammed 2004). Oil spillage may be caused by natural disasters like earthquakes in the sea surface or due to accidental leaks during exploration, refining, storage and transportation. The causes can be numerous but the consequences are the same. In case of crude oil, the different types such as heavy or light crude oil can affect the clean-up procedures. Crude oil spreads very rapidly on the sea surface and after a short period of time the thickness of the oil film can be at least 1mm. It is therefore necessary to prevent the spreading to reach the shoreline. Once it reaches the shoreline, it contaminates the soil and cause a great damage to the soil ecosystem as well (Ojo, O.A. 2005).

Some of the largest oil spills that have taken place all over the world are at Iran, Iraq, Persian Gulf, Uzbekistan, Kuwait, United States, Gulf of Mexico, Pakistan and even in India. The most recent oil spill in India is the spill of ONGC Trunk oil pipeline burst, at Uran, Mumbai on 21st January 2011. Around 40 to 45 metric tons of oil was spilled in Mumbai coast that spread around 4 sq km area. Many such disasters reported one seeping oil from a sinking ship.

Accidental release of petroleum oil into the environment leads to serious environmental pollution because it is toxic and an extremely complex mixture of hydrocarbons. Petroleum oil is a mixture of hydrocarbons including aromatic and aliphatic hydrocarbons, ranging from short-chained to long-chained hydrocarbons. Different types of hydrocarbons in petroleum oil have different degradability. The *n*-alkanes are generally considered the easily degradable fractions, but branched, cyclic, and aromatic hydrocarbons are not biodegradable, or are degraded slowly. For those reasons, most of previous studies have focused on biotransformation of polycyclic aromatic hydrocarbons and the characterization of pure isolates using single petroleum hydrocarbon or one hydrocarbon class (Yuan, S.Y 2000).

Clean-up and recovery of hydrocarbons from an oil spill is difficult and the strategies for cleaning up an oil spill are greatly affected by a variety of factors such as the type of oil spilled, the temperature of the water body, and the types of shorelines and beaches involved. A number of approaches and technologies have been developed for spreading of oil spills in marine shorelines and freshwater environments. Many mechanical and chemical methods can be applied to clean-up the spills (Zhu, X *et. al.*2001).

However, there are few studies investigating the biodegradation of complex mixtures of hydrocarbons by indigenous microorganisms, although some bacterial and fungal strains are identified to utilize a wide range of petroleum hydrocarbons.

The use of filamentous fungi to remediate contaminated soils may offer advantages over bacteria for the following reasons: extension of hyphae can explore through hydrocarbon-contaminated soil aggregates and they have the ability to grow under harsh environmental conditions: low moisture and

pH levels, and nutrient deficiency. The previous studies have focused on few fungal species like white-rot fungi, although this species does not dominate contaminated and natural soil. It would be interesting to be able to find new indigenous fungal isolates that have the capability to degrade a wide range of hydrocarbons.

Recently, there were a few studies being conducted to isolate and characterize the hydrocarbon degrading fungi. Certain microbes show increase in population due to use of petroleum hydrocarbons as nutrients (Westlake *et al.,* 1974). Such species are commonly being used for remediation of contaminated site.

These organisms are directly involved in biogeochemical cycles of the degradation of many carbon sources, including petroleum hydrocarbons (Santos *et al.,* 2011). The inputs of various condition related to indigenous microbial communities at contaminated sites are also required for their use in bioremediation approaches (Desai *et al.,* 2010). The present study was conducted using *Aspergillus niger* to study their role in petroleum product degradation.

MATERIALS AND METHODS

Collection of Soil Sample

Soil samples were collected from four different oil contaminated sites of Nagpur city. Soil samples were collected from specific locations within the workshop that had heavy spillage of used engine oil and diesel. The soil was characterized by hardened surfaces and blackish in colour. Soil was collected randomly 5-10 cm beneath the surface using spatula and were packed in sterile polybags and taken to laboratories within 6 hours for further screening.

Isolation of Fungi from Soil Sample

Serial Dilution Technique

A serial dilution is a series of sequential dilutions used to reduce a dense culture of cells to a more usable concentration. Each dilution will reduce the concentration of bacteria by a specific amount. First tube filled with 10ml distilled water and rests of 7 test tubes are filled with 9ml distilled water and all were sterilized by autoclaving. To the first tube, 1gm of soil sample was added aseptically, shaken it well. After uniform shaking, 1ml from first tube was added to second tube and so on till the last tube and 1ml from last tube was discarded. The content of the tube numbers 4, 5, 6 were then transferred to respective sterilized petriplates containing PDA and incubate at 25^0C for 7 days.

Identification of Fungal Isolate

Morphological identification was done by microscopic observations. This includes Lacto Phenol Cotton Blue Staining.

Biodegradation Studies

100 ml Bushnell Hass Broth (BHB) was prepared in different flasks each as per composition. Tween 80 and resazurin were added 1ml each to the broth in both flasks. Resazurin acts an as indicator which irreversibly reduced to the pink colour when acidic content produced after degradation of oil. Broth flasks were autoclaved, cooled to room temperature and inoculated. 2ml sample of engine oil and diesel was added to two different flasks. 2ml of spores suspension of *Aspergillus niger* was inoculated in flasks containing BHB.

Control flask was prepared which was not inoculated with *Aspergillus niger*. Flasks were incubated at room temperature for 28 days at 25^0C. On the other hand, Potato dextrose broth (PDB) also prepared, supplemented with respective oil, autoclaved, cooled and inoculated with spores of *Aspergillus niger* for comparative study.

Extraction Procedure

After 28 days, extraction procedure was performed by taking 1:1 concentration of 50ml of n-hexane and 50ml of broth containing degraded engine oil and diesel separately in a separating funnel. Funnel was shaken vigorously and then allowed to stand for some time in order to separate the layers in the solution.

The upper organic layers collected in tared beaker and evaporate n-hexane. The gravimetric estimation of residual oil left after biodegradation was made by weighting the quantity of degraded oil in tared beaker. The percentage degradation of Engine oil & Diesel was then calculated as described by Adekunle *et al* 2007. Some quantity of sample was taken in eppendroff tube and was sent for Gas Chromatography Analysis.

Degradation Percentage Analysis

Formula used

$$\text{Percentage of oil degradation} = \frac{\text{Initial Concentration of oil - Final Concentration of oil}}{\text{Initial Concentration of oil}} \times 100$$

GC Analysis

The reaction samples were analyzed by gas chromatography (model GC-2010 plus, Shimadzu Corp., Tokyo, Japan) using a capillary column, MXT-Biodiesel TG (Restek, USA; 15 m × 0.32 mm × 1µm film thickness of diphenyl dimethyl polysiloxane) and a flame ionization detector. Nitrogen was used as a carrier gas at a flow rate of 2.75 mL min^{-1}. Column oven temperature was initially maintained at 100^oC for 3 minutes, then increases to 250^oC at the rate of 30^oCmin^{-1} and held here for 3 minutes. The injector and detector temperature were maintained at 270^oC. A sample volume of 1µL engine oil and diesel each in hexane was injected using a split mode, with the split ratio of 1:50.

RESULT AND DISCUSSION

Aspergillus spp. was isolated from different soil samples and it was found to be effectively utilizing the oil samples as a sole carbon source. *Aspergillus* was confirmed on the basis of microscopic observations.

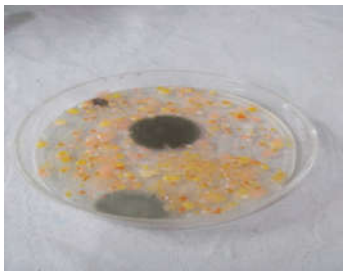

Figure1 *Aspergillus spp.* was isolated from Soil Sample Contaminated with Oil

Degradation Percentage Analysis

GC Analysis

GC analysis was performed to determine the degradation result of diesel and engine oil separately.

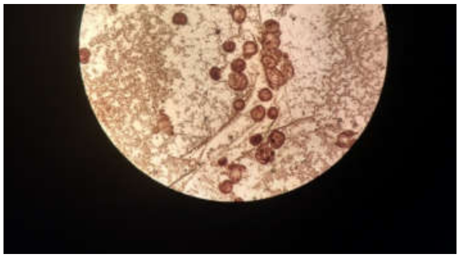

Figure 2 Morphological Observation of *Aspergillus Spp*

Biodegradation of Oil Sample

(a) Control flask **(b)** Engine oil in BHB

Figure 3 Control and Sample Flasks Showing Respective Results of Engine Oil

(a) Control flask **(b)** Diesel in BHB

Figure 4 Control and Sample Flasks Showing Respective Results of Diesel

Table No 1 Percentage of degradation of Engine Oil and Diesel

Sr. No.	Oil Sample	Initial oil Concentration (gms)	Final oil Concentration (gms)	Percentage of Degradation
1	Engine Oil	1.4943	0.3621	75.77%
2	Diesel	1.1023	0.0909	91.75%

Engine Oil Analysis

GC analysis of control sample (Fig.5) was performed where 13 peaks were obtained. Results of test sample performed showed only 2 peaks with retention time of 1.288 and 1.404 (Fig.6).

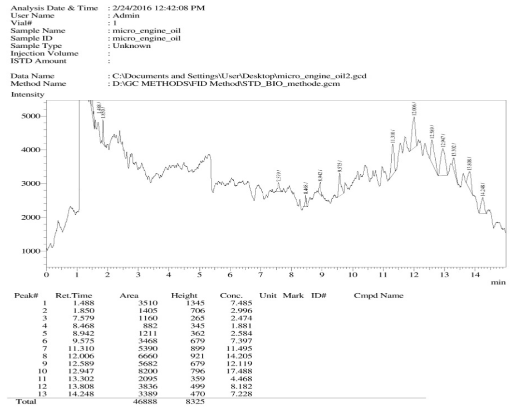

Figure No 5 GC analysis of control engine oil

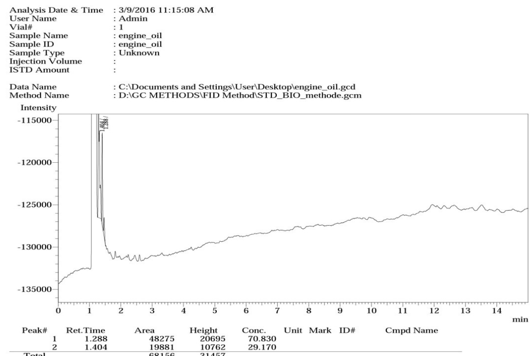

Figure No 6 GC analysis of engine oil

Diesel Analysis

GC analysis of control sample (Fig.7) was performed where 22 peaks were obtained. Results of test sample performed and showed 5 peaks out of 22 with retention time of 1.293, 1.411, 1.835 and 2.616 (Fig.8).

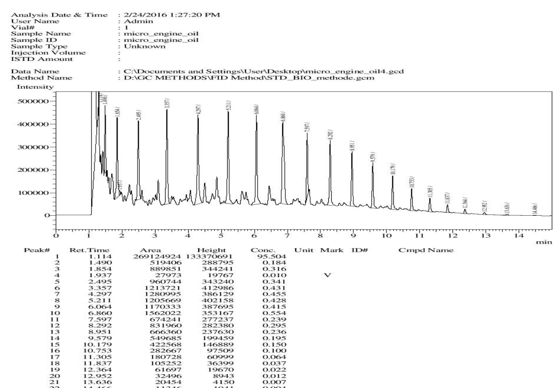

Figure No 7 GC analysis of Control Diesel oil

Fungi play a central role in the biodegradation or decomposition of organic compounds and are producers of an array of extracellular enzymes. In particular, filamentous fungi have been implicated in the biodegradation of a wide range of aromatic hydrocarbons and thus they could contribute significantly to bioremediation efforts (Hughes K.A. *et. al*., 2007). Petroleum contamination is a global problem and in Polar Regions these spills result in extensive damage to ecosystems as cold region ecosystem recovery is a

much slower process than that of warmer regions (Aislabie, J. *et. al*., 2001).

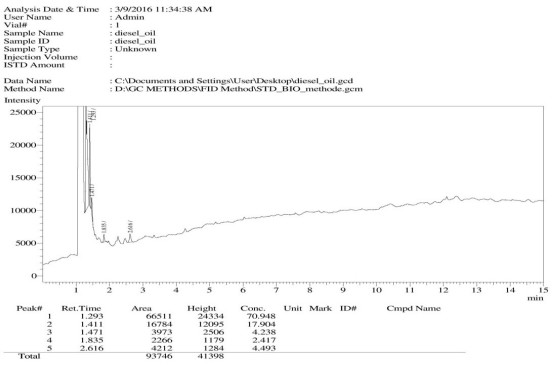

Figure No 8 GC analysis of Diesel oil

Okerentugba and Ezeronye demonstrated that Penicillium spp., Aspergillus spp. and Rhizopus spp. were capable of degrading hydrocarbons especially when single cultures were used (Okerentugba P.O *et al* 2003). Oboh *et al*., have reported the abilities of bacterial species such as *Pseudomonas*, *Bacillus*, *Alcaligenes, Citrobacter* and fungi such as *Aspergillus*, *Penicillum*, *Rhizopus and Rhodotorula* which can grow on crude petroleum as the sole carbon and energy source when screened for hydrocarbon utilization. In their study, it was observed that there was growth and extension of the hyphae forming mycelium in the medium (Oboh, O. B. *et al* 2006). Uzoamaka *et al*., reported that some eight isolates of fungi showing potentials for hydrocarbon biodegradation including *Aspergillus versicolor, A. niger*, *A. flavus, Synce phalastrum spp*., *Trichoderma spp*., *Neurospora sitophila*, *Rhizopus arrhizus and Mucor spp*, (Uzoamaka *et al* ., 2009).

In the study by Enabulele. *et al*., the fungal isolates *Aspergillus* spp. showed efficiency to degrade the hydrocarbon. In a taxonomic study of fungi, hydrocarbon assimilation is most common in the orders Mucorales and Monilales, as well as in the genera *Aspergillus* and *Penicillium* which come under the Order Eurotiales (Enabulele. *et al*., 2013).

During the degradation of hydrocarbon, pH was found to decrease gradually from the day of incubation. The optimum pH for biodegradation of hydrocarbons is around 6-8. Biodegradation of crude petroleum in an acid soil could be Doubled by limiting to pH 7.4 (Mentzer, E. *et al*., 1996). In the present work, the Decrease in pH may be due to the release of organic acids in the medium which changes the color of Resazurin from blue to pink. Petroleum contaminated soil contains various hazardous materials such as aromatic hydrocarbons and polycyclic aromatic hydrocarbons; they are potentially toxic, mutagenic, and carcinogenic (Jelena, S.M. *et al*., 2008).

Microorganisms promoting fouling of oilcan live in a wide range of pH from 4 up to 9, however, they tend to prefer a neutral pH (Boszczyk-Maleszak, H. *et al*., 2006). Ekpenyong *et al*. reported that in studies involving mixed microbial consortium, the expression was not as much as was observed in the yeast or mould consortial studies, but decreased from 7-6 gradually suggesting possible neutralizing effect by basic intermediate products mostly from organisms that utilize oxidative biodegradation pathways (Ekpenyong, M.Z. *et al* 2007).

In the present study, *Aspergillus spp.* was capable enough to degrade engine oil and diesel. The fungal isolates used the sample oil and inhibited the growth in the media provided which seems to be useful and profitable for the future need. *Aspergillus* being an infectious fungus could break the bond between the compounds present in the oil samples and converted it into the simpler products which are not harmful for the environment. The percentage degradation was found 75.77% and 91.75% of engine oil and diesel respectively.

Liberation of carbon dioxide during the degradation of hydrocarbons can be used as an indication for the activity of fungi in the growth medium. The maximum release of CO_2 was found during degradation of hydrocarbons at 7.5% for *Aspergillus* sp. (Balba *et al.*1998) stated that mineralization studies involving measurements of total CO_2 production can provide excellent information on the biodegradability potential of hydrocarbons in contaminated soils.

The approach, considered to be a preliminary step in the feasibility study, provided rapid, relatively unequivocal time-course data suitable for testing different biological treatment options, like the effect of nutrient supplementation, microbial inoculation, etc. The test can be useful for confirming active hydrocarbon degradation during full scale bioremediation.

CONCLUSION

The result of present study shows that *Aspergillus niger* isolated from the soil sample have high tendency to degrade engine oil and diesel. This *Aspergillus niger* can be exploited in the biodegradation of crude petroleum oil spill and bioremediation of the environment.

References

Adekunle A.A, Adebambo O.A, 2007. Petroleum Hydrocarbon Utilization by Fungi Isolated From Detarium Senegalense (J. F Gmelin) Seeds. *Journal of American Science*, 3(1): 69-76.

Balba, M.T. Al-Avvadhi, N. Daher, R.A. 1998. Bioremediation of Oil - Contaminated Soil. Microbiological Methods for Feasibility Assessment and Field Evaluation. *Journal of Microbial Methods*; 32:155-164.

Boszczyk- Maleszak, H. Zabost, A. Wolicka, D. Kacieszczenko J. 2006. Effectiveness of biodegradation of petroleum products by mixed bacterial populations in liquid medium at different pH values. Polish *Journal of Microbiology;* 55(1):69–73.

Desai C, Pathak H, Madamwar D, 2010. Advances in molecular and "omics" technologies to gauge microbial communities and bioremediation atxenobiotic/ anthropogen contaminated sites. Biore source Technol*,* 101(6): 1558–1569.

Ekpenyong, M.Z. Antai, S.P. Essien, J.P. 2007. Quantitative and Qualitative Assessment of Hydrocarbon-Degrading Bacterial and Fungi in Qua Iboe Estuary, nigeria. Research *Journal of Microbiology*; 2(5):415-425.

Enabulele, O.I. Obayagbona O.N. 2013. Biodegradation Potentials of Mycoflora Isolated from Auto Mobile Workshop Soils on Flow Station Crude Oil Sludge. International Research *Journal of Biological Science*; 2(5):9-18.M

Jelena, S.M. Beskoski, V.P. Ilic, M.V. Ali S.A.M, Gojgiccvijovic, G.D. Vrvic M.M. 2008. Bioremediation of soil heavily contaminated with crude oil andits products: composition of the microbial consortium. *Journal of Serb. Chemical Society*; 74(4):455–460.

Mentzer, E. Ebere, D. 1996. Remediation of Hydrocarbon Contaminated sites. A Paper Presented at 8th Biennial International Seminar on the Petroleum Industry and nigerian Environment, November.

Mohammed M. A. 2004. Treatment techniques of oil-contaminated soil and water aquifers. International Conference on Water Resources & Arid Environment, 1-11.

Oboh, O. B., Ilori, M.O., Akinyemi, J.O. Adebusoye S.A. 2006. Hydrocarbon degrading potential of Bacteria isolated from a nigerian Bitumen (Tarsand) Deposit. Nature Science; 4(3):51-57.

Ojo, O.a. (2005). Petroleum – hydrocarbon utilization by nature bacterial population from a Wasrewater canal Southwest nigeria. African *Journal of Biotechnology* 5(4): 333 – 337.

Okerentugba P.O, Ezeronye O.U, 2003. Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluents in nigeria. African *Journal of Biotechnology*. 2(9): 288-292.

Santos H.F, Camero A, Paes J.E.S, Rosado S, Peixoto R.S, 2011. Bioremediation of Mangroves Impacted by Petroleum. Water Air Soil poll, 216: 329-350.

Uzoamaka, G.O. Floretta, T. M.O. Florence M.O. 2009. Hydrocarbon degradation potentials of indigenous fungal isolates from petroleum contaminated soils. *Journal of Physical Natural Science*; 3(1):1-6.

Westlake, D.W, Jobson, A. Phillippe, R. Cook, F.D. 1974. Biodegradability and crude oil composition. Can *J Microbiol*, 20(7): 915-928.

Yuan, S.Y, Wei, S.H and Chang, B.V. (2000). Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. Chemosphere 41(9): 1463 – 1468.

Zhu, X. Venosa, A.D, Suidan, M.T and Kenneth, L., 2001. Guidelines for the biore mediation of marine shorelines. U.S. Environ mental Protection, 1-163.

Hughes, K.A., Bridge, P. Clark, M.S. 2007. Tolerance of Antarctic soil fungi to hydrocarbons. *Science of Total Environment*; 1:372(2-3):539-48.

Aislabie, J. Fraser, R. Duncan, S. Farrell, R.E. 2001. Effects of oil spills on microbial heterotrophs in Antarctic soils. *Polar Biol*; 24:308–313.

****