Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 4 [2] January 2015: 91-94

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Comparative study of Crude oil Degradation Efficiency of microbes Isolated from crude oil Contaminated site

Preeti Singh*, Dipali Parmar, Ajit Pandya
Pacific University, Udaipur, Rajasthan, India.
Email ID : preeti2811@yahoo.com

ABSTRACT
Crude oil contamination in the water and soil is one the most crucial problem to be solved at global level. Several methods including physical, chemical and biological methods are being applied to solve the problem. Use of microbes is one the most recent approach for remediation. In this study, four different bacteria were isolated from crude oil contaminated sites were analyzed for their crude oil degradation efficiency. These microbes were provided with best favorable condition for degradation of crude oil and results of degradation was analyzed using gas chromatography.
Keywords: comparative study, crude oil contamination, bioremediation

Received 20.09.2014 **Revised 21.11.2014** **Accepted 20.12.2014**

INTRODUCTION

In most of the developing countries, demand of crude oil increasing with rapid industrial development and increasing population. These supply is generally full filled by either direct pipeline or through transpiration, In any case there are chances of oil contamination. Till date several such incidence are been noted [1], [2]. Many of them were very severe and had left adverse effect on the environment. There were many methods applied to get rid of this problem. These include used of physical, chemical and biological methods. Among the various biological methods use of whole microbes was found to be one of the most potential tools for remediation of crude oil [3]–[5]. The application of microbial isolates for degradation of crude oil involves the manipulation of environmental conditions to favour microbial growth and degradation to proceed at a higher rate. Bioremediation of waste materials containing hydrocarbons and their derivatives, is depend on the ability of microorganisms to increase their population on these substrates and to degrade them to non-toxic products, such as H_2O and CO_2. Several environmental parameters affect the degradation efficiency of microbes. Among the various parameters, concentration of carbon, concentration of nitrogen, pH, temperature and oxygen availability are major factor which affects significantly.[3], [4], [6] If all the necessary conditions provided for the growth of microbes then it is possible to remediate the crude oil contamination in fastest possible rate. Remediation could be determined by number of methods including certain sophisticated equipments. Gas chromatography is one of such technique which is highly applicable for determination of crude oil degradation efficiency. It provides quick and accurate analysis which helps in determination of proper rate of degradation. In this study, gas chromatography was used to analyze the crude oil degradation efficiency of four microbes isolated from crude oil contaminated site.

MATERIALS AND METHODS

Sample Collection

Soil samples of different depth were collected from the oil field ONGC, Ankleshwar, Gujarat. The samples were collected in sterilized sample containers. The soil samples were stored at 4^0C and immediately transferred to the laboratory for further analysis.

Isolation of Microbes

Bushnell Hass medium was used to isolate crude oil degrading bacteria. In the procedure, 10 grams of sample was added in 100mL of 0.9% N-saline. It was vortexed for 10 minutes and allowed to stand for a few minutes. Supernatant was collected in other sterilized tube to be used as inoculums. These inoculums

were streaked on nutrient agar plates after serial dilution and the plates were incubated for 48 hours at 37^0C.

Enrichment of Microbes

For isolate the desired bacteria, enrichment culture technique was used. In the process, 1 mL of soil mixture was inoculated into 50 mL of Bushnell Haas broth containing 1% (v/v) crude oil as a sole carbon source. The flasks containing media and crude oil were incubated on a rotary shaker at 120 rpm at 37ºC for at least 7 days. After incubation, a loopful of culture was streaked on Bushnell Haas agar containing 1% crude oil and incubated at 37°C for 24h-48h. Selective microbial colonies were picked and sub-cultured by streaking onto Bushnell Haas Plates to obtain pure culture.

Identification of Microbes

Identification of the crude oil degrading bacteria was done by 16s r-RNA sequencing method. Obtained sequences were submitted to NCBI database.

Determination of Crude oil degradation

All the potential microbes were initially adapted for optimum degradation of crude oil and then inoculated with 1.0% crude oil in BH media and allowed to degrade the crude oil for 72 hrs on rotary shaker at 120 rpm at 37ºC. After incubation time, broth was collected and residual crude oil composition was determines using gas chromatography. Here crude oil was consider as control to determine the crude oil degradation efficiency.

Gas Chromatography

Determination of crude oil could be done in number of ways. Out of all, gas chromatographic analysis is one of the most efficient methods. Here, gas chromatographic analysis of crude oil and residues after degradation by microbes were done. Chromatograms obtained were analyzed and results were predicted. For the analysis, GC-2014 with FID, Shimadzu Pte Ltd. Japan was used. The following parameters were set in the instrument: column – Restek 1 capillary column (15 meters), Detector – Flame ionization detector, Injection temperature – 280ºC, detector temperature – 300ºC, gradient – 150 - 280ºC at the rate of 15ºC per minute increment, time – 11 mins. Split ratio – 1:90, make up gas – nitrogen.

RESULTS AND DISCUSSION

Four potential microbes capable of crude oil were isolated from the collected samples. Table 1 shows the characteristics of isolated microbes. From 16s rRNA sequencing, it was found that the isolated microbes are *Bacillus cereus*, *Pseudoxanthomonas mexicana, Halomonas daqingensis* and *Parapusillimonas granuli*. Sequence of these microbes were submitted to NCBI and given unique identification numbers which are KM192258, KM192259, KM192260 and KM192261 respectively.

Gas Chromatographic Analysis

Analysis of gas chromatographic results has shown that all the microbes are potential crude oil degraders. All the microbes were capable of degrading almost all the PAHs present in the crude oil. Residual analysis of crude oil in the media has shown that there were many PAHs which were degraded completely. Figure 1 to Figure 5 shows gas chromatogram of residual crude oil of each microbe after incubation along with pure crude oil.

Figure 1. Gas Chromatogram of Crude oil

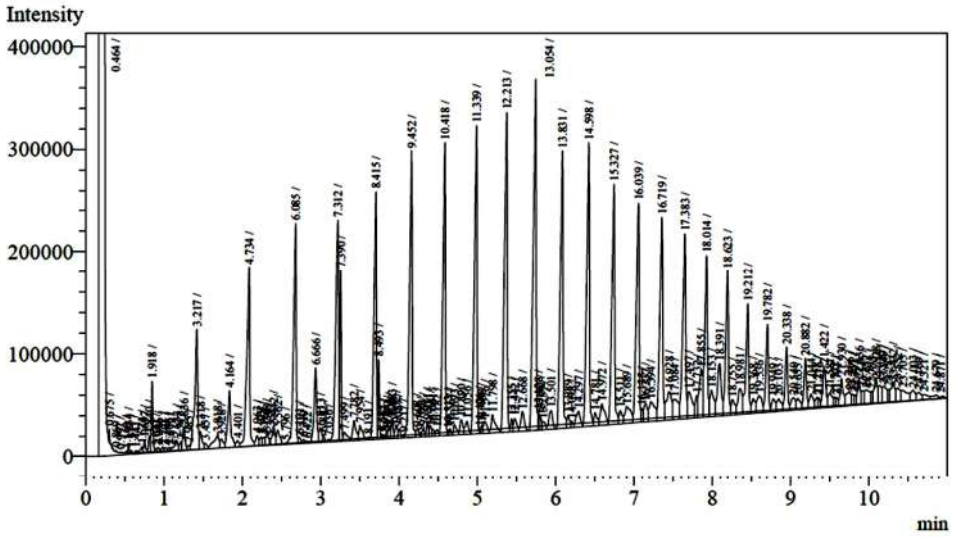


Figure 2. Gas Chromatogram of residual of *Bacillus Subtilis* broth

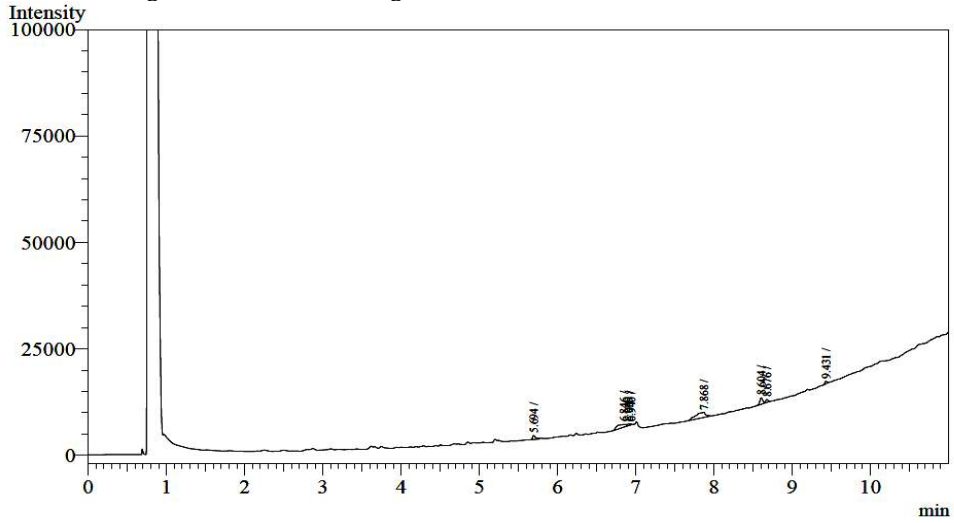


Figure 3. Gas Chromatogram of residual of *Pseudoxanthomonas mexicana* broth

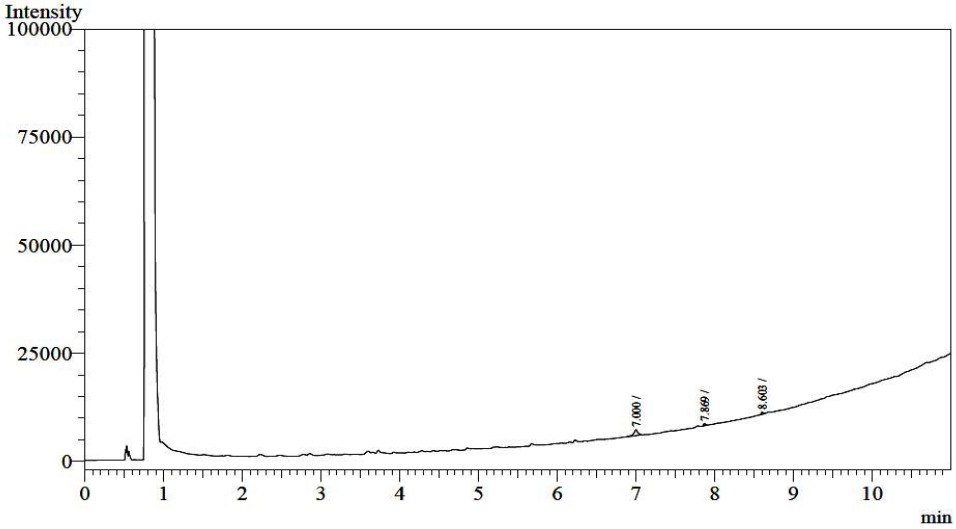


Figure 4. Gas Chromatogram of residual of *Halomonas daqingensis* broth

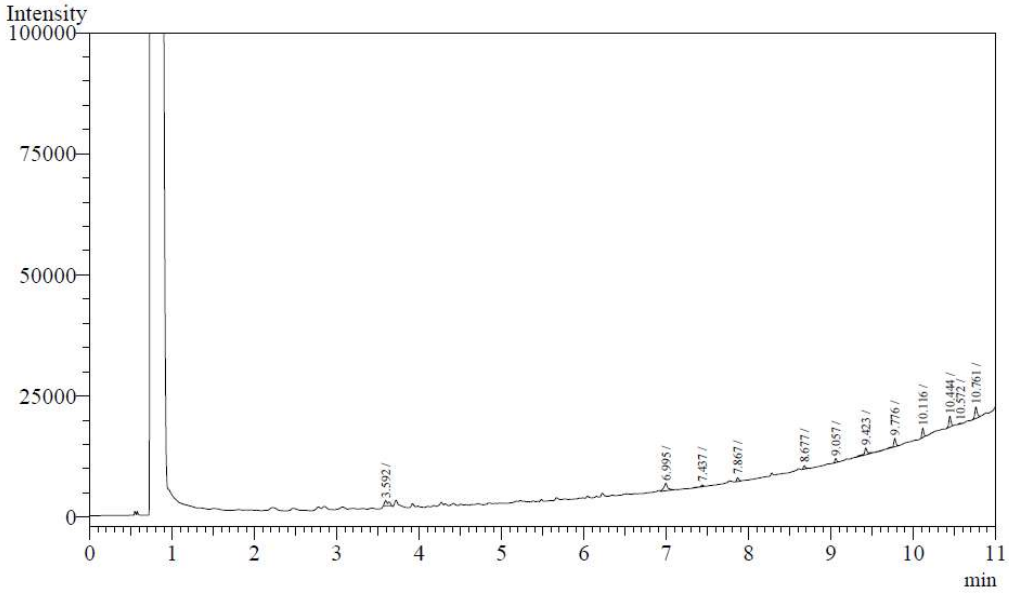
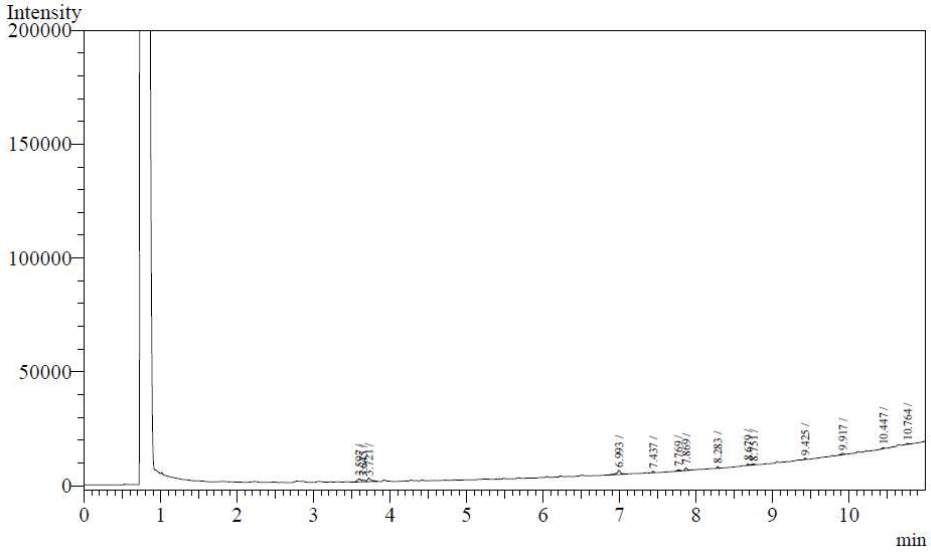


Figure 5. Gas Chromatogram of residual of *Parapusillimonas granuli* broth



As PAHs were only the carbon source available in the media, Microbes were forced to utilize them for their own growth and replication. Each PAHs were degraded gradually by the microbes. Prolong incubation has ensured the complete degradation [3], [4], [7]. Based in the results, it was found that microbes can utilize all the PAHs weather they are short chain or long chain. Many previous studies have already recommended higher incubation time with rapid aeration for better degradation of crude oil. [8], [9] The probable reason behind this may be higher activity of dehydrogenase and other hydrolytic enzymes involved in the degradation . Biosurfactant production was also observed in all the microbes which had decrease the density of media. However detail study was not done on production of biosurfactant. Several previous studies have revealed that rhamnolipid was among the most prominent surfactant. [10],[11],[12]

From the overall study it was concluded that microbes could be easily isolated and enhance to degrade the crude oil with better efficiency.

REFERENCES

1. G. Guru, H. Gohel, S. Ghosh, and V. Braganza, "Isolation and Enrichment of Microbes for Degradation of Crude Oil," *Int. J. Eng. Sci. Innov. Technol.*, vol. 2, no. 4, pp. 144–147, 2013.
2. R. Gopinathan, M. Prakash, and R. Bharathirajan, "An experimental study for crude oil biodegradation in contaminated soil," *Int. Jounral Curr. Microbiol. Appl. Sci.*, vol. 1, no. 1, pp. 12–16, 2012.
3. A. O. Vincent, E. Felix, M. O. Weltime, O. K. Ize-iyamu, and E. E. Daniel, "Microbial Degradation and its Kinetics on Crude Oil Polluted Soil," *Res. J. Chem. Sci.*, vol. 1, no. 6, pp. 8–14, 2011.
4. T. K. Vyas and B. P. Dave, "Effect of crude oil concentrations , temperature and pH on growth and degradation of crude oil by marine bacteria," *Indian Jounral Mar. Sci.*, vol. 36, no. 1, pp. 76–85, 2007.
5. A. Pandey and R. Chandra, "Isolation of oil degrading bacteria from oil contaminated soil and expression of oil degrading genes in non oil degrading bacteria," *Jounral Drug Discov. Ther.*, vol. 1, no. 11, pp. 1–17, 2013.
6. D. Dave and A. E. Ghaly, "Remediation Technologies for Marine Oil Spills : A Critical Review and Comparative Analysis," *Am. J. Environ. Sci.*, vol. 7, no. 5, pp. 423–440, 2011.
7. S. Jegadevan and S. Mukberji, "Successful in situ oil bioremediation programmes key parameters," *Indian J. Biotechnol.*, vol. 3, pp. 495–501, 2004.
8. T. H. Adibarata and S. T. Achibana, "Microbial Degradation of Crude Oil by Fungi Pre-Grown on Wood Meal," *Interdiscplinary Stud. Environ. Chem.*, pp. 317–322, 2009.
9. T. Nikhil, V. Deepa, G. Rohan, and B. Satish, "Isolation , Characterization and Identification of Diesel Engine Oil Degrading Bacteria from Garage Soil and Comparison of their Bioremediation Potential," *Int. Res. J. Environ. Sci.*, vol. 2, no. 2, pp. 48–52, 2013.
10. P. Darvishi, D. Mowla, S. Ayatollahi, and A. Niazi, "Biodegradation of heavy crude oil in wastewater by an efficient strain , ERCPPI-1," *Desalin. Water Treat.*, vol. 28, pp. 46–54, 2011.
11. G. Giedraitytė, L. Kalėdienė, and A. Bubinas, "Correlation between biosurfactant synthesis and microbial degradation of crude oil hydrocarbons," *Ekologija*, vol. 3, pp. 38–41, 2001.
12. Z. Guo-liang, W. U. Yue-ting, Q. Xin-ping, and M. Qin, "Biodegradation of crude oil by Pseudomonas aeruginosa in the presence of rhamnolipids *," *J. Zhejiang Unviersity Sci.*, no. 8, pp. 725–730, 2005.

CITATION OF THIS ARTICLE

Preeti S, Dipali P, Ajit P. Comparative study of Crude oil Degradation Efficiency of microbes Isolated from crude oil Contaminated site. Bull. Env. Pharmacol. Life Sci., Vol 4 [2] January 2015: 91-94