ISSN 0976 – 3333

Available Online at www.ijpba.info

**International Journal of Pharmaceutical & Biological Archives 2011; 2(5):1426-1429**

***ORIGINAL RESEARCH ARTICLE***

# Biodegradation of Cyclohexanol by Microorganisms Isolated From Oil Spilled Soil around Namakkal

**B. R. Kartheek*[1], R. Maheswaran[2], G. Kumar[1*], G.Sharmila Banu[3]**

[1]*Faculty of Medicine, Masterskill University College of Health Sciences, Pasir Gudang Campus, Jalan Lembah, Bandar Seri Alam- 81750 Masai, Johar, Malaysia*
[2]*Department of Microbiology, MVJ Rajalakshmi College, Marathahalli, Bangalore, Karnataka-560037, India,*
[3] *Department of Zoology, NKR Government Arts College for Women, Namakkal, Tamilnadu 637 001, India*

Received 29 Jun 2011; Revised 07 Oct 2011; Accepted 16 Oct 2011

## ABSTRACT

The hydrocarbon degrading organisms were isolated from the oil spilled area in and around Namakkal and identified bacterial and fungal species such as *Pseudomonas, Bacillus, Micrococcus, Aspergillus, Rhizopus, Mucor and pencillium.* The ability to degrade cyclohexanol in pure and mixed cultures was tested in the laboratory condition by using turbidometry growth curves and the degraded hydrocarbons using thin layer chromatography. Of all the isolates *Pseudomonas* sp. showed higher degrading ability (68.8%) than others (*Bacillus* sp.54.64%, *Micrococcus* sp. 44.1%) equaling to mixed cultures (81.1%)

**Key words**: Oil degradation, Cyclohexanol, Bacteria, Oil spill

## INTRODUCTION

The growth of petroleum industries in our country especially increasing number of oils well has made oil pollution a serious environmental problem. The removal of oil that has been accidentally spilled the environment is great concern to the petroleum industry. The crude oil comes from a variety of sources and its chemical composition is extremely variable, consisting of a large number of normal and branched chain aliphatic hydrocarbons, alicyclic, aromatic and heterocyclic compounds [1]. Bacteria, fungi and algae and other microbes are capable of degrading almost any pollutant in the environment. The break down of petroleum hydrocarbons are through enzyme mediated reaction. The hydrocarbon oxidizing bacteria are primary agents in the natural decomposition of oil [2]. These bacteria use hydrocarbons as an electron donor oxidizing it to carbon dioxide. Virtually all kinds of hydrocarbons are susceptible to microbial oxidation. Microbes are believed to bring about the degradation of mineral oils polluting the marine environments. The subject of oil pollution is probably one of the most emotive subjects in any discussion on environmental pollution [3]. One major type is generally considered to play a much more important role in soil than in water.

The commonest oil degraders are *Pseudomonas* sp, *Acinetobacter* sp, *Aeromona* sp, *Flavobacterium* sp, *Corynebacterium* sp and *Bacillus* sp; predominant is *Pseudomonas* sp reported by several authors [4, 5, 6]. Strains of *Acinetobacter, Rhodococcus* and *Alkaligens* degrade several alkanes. *Pseudomonas putida* can degrade effectively benzene and alkylbenzenes with acid chain shorter than four carbon atoms [7, 8]. It also degrades naphthalene and several alkanes phenanthrene and naphthalene was degraded by *Sphingomonas* sp. The study was undertaken to investigate the occurrence of cyclohexanol degrading bacteria and fungi in the oil spilled soil sediments collected from the vicinity of the petrol stations in and around Namakkal and the analysis of the degradation of cyclohexanol.

## MATERIALS AND METHODS

### Collection of the samples

All cultures used in this study were obtained from the soil which was contaminated with crude oil over a period of time. Soil sample was obtained from a diesel fuel spill near the Andavar petrol bunk, Namakkal. It was collected in a sterilized screw cap bottle or sterile polythene bags. The entire sample was stored at refrigeration temperature before the experimental work.

***Corresponding Author:** Dr.B.R. Kartheek**, Email:** drkartheek@yahoo.com, **Phone No:** 07-386 0541/0542 ext: 193

**Enumeration of cyclohexanol degrading microorganisms**

Ten grams of the oil contaminated soil sample was enriched in 100ml of the mineral salt medium with 1 ml of cyclohexanol. The shake flask culture was incubated at room culture was incubated at room temperature in a mechanical shaker for seven days. In this enrichment technique several cyclohexanol degrading organism were isolated in the preliminary screening procedure. Viability was checked by pour plate method in nutrient agar and chloramphenical incorporated potato dextrose agar. The individual bacterial colonies were sub cultured on nutrient agar and the fungal isolated were sub cultured on potato dextrose agar for further identification. Isolated fungal and bacterial colonies were identified by using standard procedure.

**Bacterial cultures:** The sub cultured organisms on the nutrient agar slants were further subjected to identification by biochemical tests.

**Fungal cultures:** Identification was done based on conidia spore, septate and hyphae using lacto phenol cotton blue mount [9].

**Measuring bacterial growth on cyclohexanol by turbidimetrically**

The mineral salt medium was prepared. To the 100ml of mineral salt medium 10ml of cyclohexanol was added. Aseptically transferred the un-inoculated mineral salt medium to one of the cuvettes which were the control to standardize colorimeter. Aseptically inoculated a flask of mineral salt medium with a loop full of the test organisms; swrilled to mix the contents. pH of the medium was measured by pH meter. Transfer the inoculated mineral salt medium from the flask to the medium from the flask to the second cuvette and measured the absorbance on the colorimeter at 600nm before incubation. Different growth phases were observed and growth curve was plotted. After 5 days of growth pH was measured and the degraded oil was separated in the separating funnel using diethyl ether and used thin layer chromatographic analysis for identifying the organic compound.

**RESULTS AND DISCUSSION**

Hydrocarbon oxidizing bacteria were primary agents in the natural decomposition of oil. These bacteria use hydrocarbon as an electron donor oxidizing it into carbondioxide. *Pseudomonas* sp, *Bacillus* sp, and *Micrococcus* sp were the major groups of bacteria involved in oil degradation [10]. In fungal isolates of oil polluted soil. It was clear that *Aspergillus* sp, *Rhizopus* sp, *Mucor* sp, *Pencillum* sp, etc; are the common micro flora in the oil contaminated soil.

Presence of growth in the oil layer and turbidity in the liquid phase after inoculation confirm the degradation of particular hydrocarbon (cyclohexanol). This view was also supported by Austin *et al.* [10]. Optical density was taken periodically for 4 days. Which confirm further growth [11].

(**Table 1**) shows *Pseudomonas* sp. which exhibits a faster growth than other pure and mixed cultures after 24 h and the *Micrococcus* sp, exhibited less degradative effect on cyclohexanol [10]. Micro organisms were grown on hexadecane at initial pH 7.0, 7.5 and so on, showed there was a decrease in pH with increase of growth (final pH 5.1, 4.9, etc). This is in conformation with results of this pH concominant with growth of the organisms (**Table 2**).

Cyclohexanol was degraded partially by pure and mixed cultures [12]. pH value lowered from 7.0 to 2, 2.4, 2.2, etc., by the different microorganisms due to degradation of hydrocarbons to simple acids (Table 2). (**Fig.1**) shows kinetics of different bacterial isolates substituted on cyclohexanol, where pseudomonas sp. Showed peak values. The growth was high at logarithmic phase by all pure cultures and as well as in mixed culture (**Fig 2**).

The total amount of cyclohexanol decreased exponentially decreased exponentially with maximum decreases occurred at the logarithmic phase with time [13]. From the raised optical density values and lowered pH values, it showed rapid growth of microorganisms on cyclohexanol. Degradation occurred after 24h and 48h of incubation. The growth rate decreased as shown by decreased in optical density [14] (Table 1; Figs.1 & 2). From the liquid phase remaining degraded oil was separated from the medium that was taken for thin layer chromatographic studies [3, 15, 16]. From chromatogram Rf values of control cyclohexanol for different organism were calculated (**Table 3**)

*Pseudomonas* sp. Showed higher degradation than other cultures. Mixed cultures of all the bacterial isolates showed a higher degradation rate next to *Pseudomonas* sp than *Bacillus* sp and *Micrococcus* sp.





© 2010, IJPBA. All Rights Reserved.

**Table 1: Growth of different bacterial isolates on cyclohexanol as a substrate**

| Bacterial isolates | Time (h) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | 108 |
| Mixed culture | 0.007 | 0.018 | 0.022 | 0.029 | 0.019 | 0.016 |
| *Bacillus* sp | 0.006 | 0.010 | 0.016 | 0.024 | 0.018 | 0.012 |
| *Pseudomonas* sp. | 0.005 | 0.015 | 0.020 | 0.031 | 0.027 | 0.016 |
| *Micrococcus* sp. | 0.005 | 0.012 | 0.014 | 0.017 | 0.016 | 0.013 |

**Table 2: Effect of pH on different bacterial isolates**

| Bacterial Initial | pH | Final pH |
|---|---|---|
| *Pseudomonas* sp | 7.0 | 2 |
| *Bacillus* sp. | 7.0 | 2.4 |
| *Micrococcus* sp. | 7.0 | 2.2 |
| Mixed culture | 7.0 | 2 |

**Table 3: $R_f$ values of control cyclohexanol**

| Bacterial isolated | $R_f$ values for degraded cyclohexanol |
|---|---|
| *Pseudomonas* sp | 0.4265 |
| *Bacillus* sp. | 0.3676 |
| *Micrococcus* sp. | 0.3036 |
| Mixed culture | 0.5455 |

$R_f$ values for cyclohexanol (control) = 0.6727

**Fig 1: Growth curve of pure cultures**

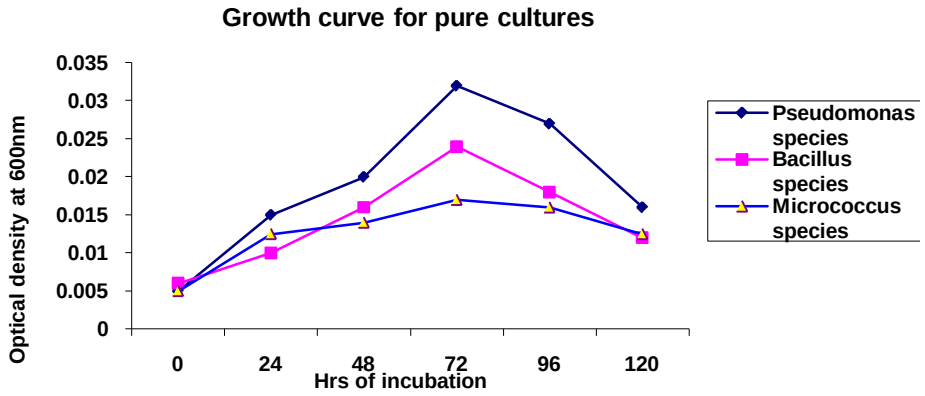
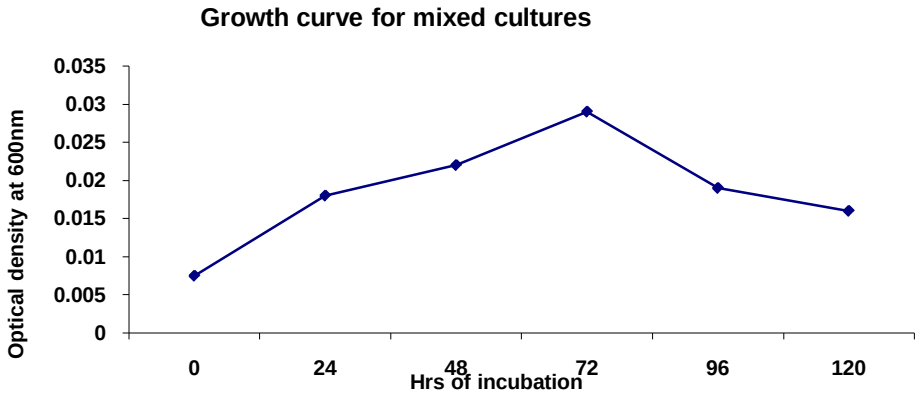



**Fig 2: Growth curve of mixed cultures**




## REFERENCE


1. Gutrick GL, Rosenberg E. Soil Science. *Anna Revs Microbiol*. 1977, 31: 379.
2. Madigan Michael T, John M Jashinko, Jack Parker S. Biology of microorganism. 8th ed. Upper Saddle River; Prentice Hall. 1999, 582-583.
3. Raymond RL, Hudson, JO, Jamison VW. Oil degradation in soil. *J. Applied. Environ. Microbiol.* 1976, 31: 522-535.
4. Noordman W, Ji W, Brusseau M, Janssen D. Effects of Rhamnolipids biosurfactants-on removal of phenantrene from soil. *Environ. Sci. Technol.* 1998, 32: 1806-1812.
5. Anh CH. The characteristics of crude oil biodegradation insand columns under tidal cycles. M.S. Thesis, University of Cincinati, OH, USA, 1999.
6. Morán A, Olivera N, Commendatore M, Esteves J, Siñeriz F. Enhancement of hydrocarbon waste biodegradation by addition of a biosurfactant from Bacillus subtilis. *Biodegrad*. 2000, 11: 65-71.
7. Solanas AM, Parés, R, Bayona JM, Albaigés J. Degradation of aromatic petroleum hydrocarbons by pure microbial cultures. *Chemosphere* 1984, 13: 593-601.
8. Pérez RM, Abalos A, Abad G, Marañón AM. Naphthalene biodegradation for *Pseudomonas aeruginosa* AT18", Rev.






© 2010, IJPBA. All Rights Reserved.

Tecnologia Química. 2003, XXIII (3):20-26.
9. Jagadish Chander. A text book of medical mycology. Ch-23 (149-154, Ch-24), 1996, 158-162.
10. Austin B, Calomiris JJ, Walker JD, Colwell RR. Numerical taxonomy and ecology of petroleum degrading bacteria. *Appl. Environ. Microbiol*. 1977, 34: 60-68.
11. Vivekanandhan G, Vasudevan N, Lakshmanaperumalswamy P. Degradation of crude oil by a bacterial consortium. *Pollution* 1999, 18 (3): 245-249.
12. Dart RK, Stretton RT. Microbiological aspects of pollution control. 2nd edn. 1980, 301-315
13. William H. Stahl. The microbial degradation of plasticizers. *J.Appl.Microbiol.* 1992, 18: 30-35.
14. Walker JD, Colwell RR, Petrakin L. Biodegradation of chespeak bay sediment bacteria. *Canadian J.Microbiol*. 1976, 22: 423-428.
15. Patil KC, Matsumura F, Batch GM. Degradation of Endrin, Aldrin and DDT by soil microorganisms. *J.Applied Microbiol.* 1970, 19: 879-881.
16. Dagher F. Comparative study of five polycyclic aromatic hydrocarbon degrading bacteria strains isolated from contaminated soils. *Canadian J.Microbiol*. 1997, 43 (4): 368-377.
17. Phillip M. Fedorak, Donald Westlake WS. Microbial degradation of alkyl carbozoles in Norman cells crude oil. *J.Applied. Environ. Microbiol.* 2006, 47: 859-862.





© 2010, IJPBA. All Rights Reserved.