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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (10): 1435-1438, 2005
ISSN 1028-8880


# Biodegradation of Crude Oil by Wood-habiting *Ascomycetes* and Leaf-habiting *Deturomycetes*

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**Abstract:** Leaf-wood habitat aquatic fungi play a significant role in the degradation of oil spills in the sea. Oil hydrocarbons with high molecular weight are hydrophobic. Their ordinary degradation in aquatic mediums is not easily possible but thanks to the activity of surfactant producing fungi they have become hydrophilic agents and are degraded. Therefore, using such fungi can help us to eliminate oil pollutants. For this reason the possibility of degrading crude oil with eleven aquatic fungi species from the *Ascomycetes* and *Deutromycete*s in oil liquid medium culture under *in vitro* conditions was examined. There was high correlation between the increase of growth of mycelium fungi and the change of pH in the light and heavy crude culture medium.

**Key words:** Bio-surfactant, degrading fungi, environmental pollutants, oil hydrocarbons

## INTRODUCTION

Many researchers have examined petroleum biodegradation[1-3]. Due to its environmental nature crude oil is absorbed and degraded by animals, plants and micro-organisms (fungi, bacteria and algae). Some animals and aquatic plants are capable of accumulating crude oil in their tissues and releasing them in water[4] and Extensive studies have been conducted on enzyme mechanisms of oil degrading micro-organisms[3,5]. Biodegradation of oil hydrocarbons differs according to their chemical nature and degrading microbial species. Biodegradation of alkans is normal and simple aromatic is relatively accomplished far quicker than that of isoalkans, cycloalkans and polynuclear aromatics. Recently scientists have focused on degradation of oil by fungi[1,6]. However so far no comprehensive study has been conducted in this area. Although *Ascomycetes* and *Deutromycetes* form the majority of the fungi population. Studies on such classes of fungi have been absolutely limited to the examination of systematic, physiologic and ecological specifications[5,7-13] . However, no intermixed studies have been conducted in this area.

The present study was therefore, focused on the evaluation of the ability to degrade crude oil by various species of aquatic fungi extracted from Zayande-roud River, Isfahan, Iran and Jordan Greek River in the United States at laboratory conditions have been evaluated.

## MATERIALS AND METHODS

This study has been done in two stage (1995-2000) in Tarbiat Modaress and Tehran University.

**Fungi species:** Eleven fungi species from aquatic-habitat *Ascomycetes* and *Deutromycetes* were separated from wood and leaves floated in the water. These species include the following:

**Ascomycetes:** *Nectria haematococca* (Berk and Br), *Halosarpheia retorquens* (Shearer and Crane), *Pseduohalonectria lignicola* (Minoura and Muri), *Nectria lucidum* (Hoehn).

**Deutromycetes:** *Anguillospora* gigantea (Ronozoni), *Alternaria ramulosa* (Sacc), *Leptodothiorella bidwelli* (Viala and Ravaz), *Angiullospora longissima* (Sacc and Syd), *Tetracladium marchalianum* (*Dewlid), Monotosporella tuberculata* (Gonzol), *Seiridum* sp. (Nees) ( Table 1).

**Culture conditions:** Inoculation discs of 5 mm diameters containing fungi growing colony in a solid medium culture environments, Corn Meal Agar (CMA) were cut and transferred into Erlenmeyer flasks containing 30 mm cultivation liquid as defined by Eypss[7] and 4% (V/V) light and heavy crude oil was transferred to the mixture as well. The control culture medium containing no oil. The compound elements of the culture medium were first separately autoclaved and after cooling 0.1 g antibiotic (0.05 g streptomycin and 0.05 g penicillin) was added. The inoculated medium culture was kept for a period of one month in darkness on a shaker incubator at 42 rpm rotation speed (The same procedure was repeated three times for each species of fungi in the medium culture). The pH values of control and treated culture mediums were measured by a pH meter (Jenway model 3020-3030 in

Table 1: Morphologic and ecologic characteristics of degrading crude oil aquatic wood-habitat *Ascomycetes* and leaf-habitat *Deutromycetes*

| Fungi species | Separated from | Habitat | Morphologic characteristics |
|---|---|---|---|
| *Halosarpheia retorquens* (shearer and Crane) cs-549 | Zayande-roud, Isfahan and Jordan Greek | Wood-habitat species in river and swamp in Illinois | Pale ash up to dark green mycelium in old embodied with aerial mycelium that gives a beautiful image under the microscope in sexual reproduction produces asc. Then the asc content is discharged and the ascopores forms develop |
| *Nectria haematococca* (Berk and Br)-J.135 Anamorph: *Fusarium* | Jordan Greek River, US | Produced from rapid growing colonies on healthy wood and living in soil and every other place It decays and corrupts the root. | White mycelium and containing aerial branched mycelium, separated, in non-gender form possessing long microconidia, narrower at the end and a little sinking, long barrel shaped and pit-like edge |
| *Nectria lucidum* Anamorph: *Cylindrocarpon* | Jordan Greek River, US | From rapid growing colonies on healthy wood. It is normally the weak pathogen of trees in tropical regions | Branched mycelium, yellow to light brown, in non-gender (cylindrocarpon) form possessing macroconedia, long barrel shaped, nearly even at the whole length, nematid-like, with 8 to 10 compartments longitudinal wall |
| *Pseudohalonectria lignicola* | Jordan Greek River, US | From rapid colony birth available in decayed wood in rivers and fresh water | Light yellow to dark brown colony, very tiny branched mycelium, possessing longitudinal wall, producing asc-stroma during Sexual reproduction. Formation of anterior cruiser structure (cane handle) be seen in the formation of ascocarp |
| *Alternaria ramulosa* (5acc) | Zayande-roud River, Isfahan | After blossoming it fall into the water | Dark to black soft wool or silk colony, separate conidiophore or in small groups, thick, sheared by short branches at the end, dark brown, conidias in one cluster at 30x60 or 25 - 15 dimensions |
| *Anguillospora gigantea* (Ranzoni ) | Jordan Greek River | Running waters | Crimson to red mycelium, multicellular, Sigmond conedia or hair condedio, length 600 to 720, which is released after wall collapse |
| *Anguillospora longissima* (Sacc and Syd) Ingold (9) | Zayande-roud River | Running, static, temporary running waters and little polluted waters | Light green mulicellular mycelium surrounded by young dark rows, Sigmond conidia or hair thin conidia, length: 300 to 400 microns that is released independently with the breakage of cell |
| *Montosporella tuberculata* | Jordan Green River | Running waters | Chrome to dark colony, row and conidophore (alerophore) containing walls with 3 to 4 microns thickness, conidophore at various lengths from 10 to 90 microns, round to oval conidia |
| *Tetracladium marchalianum* | Jordan Greek and Zayande-roud rivers | Running and static and polluted waters | Yellow colony with low growing aerial mycelium, branched mycelium, conidium (alerospore), containing 10 oblique branches, 20 to 40 microns in length and 2 to 3 microns in width. The two ends are nearly global in form |
| *Leptodothiorella bidwelli* (Viala and Ravaz) Anamorph: (*Guignardia bidwelli*) | Zayande-roud river | River water | Sunken mycelium during culture, divided with side walls, brown to light gray, conidiamata, global in shape, dark brown, separate or gathered, with thick walls bending towards thin conidia generating branch and narrower wall. Conidia dimensions: 2 to 4x6 to 9 microns |
| Seiridium sp. | Zayande-roud river | River water | Sunken branched mycelium containing longitudinal shining condiphore wall 10 to 30 microns spatial walls. Conidia shining, edged, nearly like scythe, with 6 to 40 spatial walls |

England) once every 15 days. Then the fungi mycelium was separated using Whatman sifting paper. It was washed three times by distilled water and was heated for 4 h at 70°C in the oven until a constant dry weight was achieved.

**Statistical analysis:** Analysis of variance (ANOVA method)[16] was employed to compare average dry weight and two estimates were made:

- Variance within every samples($S^2P$).
- Variance between all samples ($S^2\bar{x}$). The following formula was used in order to determine the minimum amount of difference or make the difference meaningless (LSD):

$$LSD = t(a, r(n-1))\sqrt{\frac{2}{n}S^2p}$$

$$a = 0.05$$

## RESULTS AND DISCUSSION

All the species under study were capable of growing in oil medium culture. Reduction of pH and increase of dry weight of mycelium in the oil treatments was extensive compared with the untreated medium (Table 2 and 3). From the growth of *Seridium* sp. was reduced in oily mediums. The majority of changes in pH occurred in light crude oil culture mediums with the exception of *Pseudohalonectria lignicola* and *Leptodothiorella bidwelli* during the first 15 days of culture (Table 2). In the heavy crude oil too with the exception of *Tetracladium marchalianum*, *Montosporella tuberculata* and *Anguillospora longisssma*. pH decline was witnessed after 30 days. A positive integration existed between increasing of mycelium and pH reduction in the culture medium and such integration was 62 and 75% higher that the untreated changes in light and heavy crude oil treatment. Changes in pH and dry weight mycelium

Table 2: Changes of pH in three liquid medium cultures during 15 and 30 days cultures of fungi species at 27°C in dark condition

| | Control | | Light oil | | Heavy oil | |
|---|---|---|---|---|---|---|
| Fungi species | ΔpH 15th | ΔpH 30th | ΔpH 15th | ΔpH 30th | ΔpH 15th | ΔpH 30th |
| *Asconmycetes*: | | | | | | |
| *Halosarpheiaretovqueus* | 0.4 | 0.8 | 0.8 | 0.1 | 0.3 | 0.3 |
| *Nectria haematococca* | 0.85 | 0.15 | 0.95 | 0.75 | 0.7 | 0.6 |
| *Nectria lucidum* | 0.5 | 0.5 | 0.75 | 0.6 | 0.55 | 0.65 |
| *Pseudohalonectria* lignicola | ``0.4 | 0.2 | 0.45 | 0.75 | 0.6 | 0.45 |
| *Deutromycetes*: | | | | | | |
| *Iepto dithiorella* | 0.45 | 0.95 | 0.6 | 0.9 | 0.5 | 1.2 |
| *Seiridium* sp. | 0.4 | 0.9 | 0.45 | 0.45 | 0.4 | 0.5 |
| *Alternaria ramelosa* | 0.4 | 0.8 | 0.9 | 0.5 | 1 | 0.1 |
| *Anguillospora gigantea* | 0.25 | 0.50 | 1.05 | 0.6 | 0.4 | 0.8 |
| *Anguillospora longissima* | 0.65 | 0.15 | 1.05 | 0.3 | 0.75 | 0.25 |
| *Montosporella tuberculata* | 0.4 | 0.2 | 0.8 | 0.2 | 0.7 | 0.3 |
| *Tetracladium marchalianum* | 0.4 | 0.2 | 0.6 | 0.5 | 0.65 | 0.40 |

ΔpH 15th = pH 15t h _ pH 1th, ΔpH 30th =pH 30th_ pH 15t h

Table 3: Change of pH and dry weight of mucelium in three liquid medium culture during 30 days of culture fungi species in 27°C in dark conditions

| | Control | | Light oil | | Heavy oil | |
|---|---|---|---|---|---|---|
| Fungi species | ΔpH↓ | Δwt.↑ | ΔpH↓ | Δwt.↑ | ΔpH↓ | Δwt.↑ |
| *Ascomycetes:* | | | | | | |
| *Halosarpheia retorquens* | 1.2 | 0.215 | 0.90 | 0.261 | 0.60 | 0.231 |
| *Nectria haematococca* | 1.0 | 0.364 | 1.70 | 0.536 | 1.30 | 0.35 |
| *Nectria lucidum* | 1.0 | 0.200 | 1.35 | 0.316 | 1.20 | 0.298 |
| *Pseudohalonectria lignicola* | 0.6 | 0.126 | 1.20 | 0.212 | 1.05 | 0.299 |
| *Deutromycetes:* | | | | | | |
| *Leptodothiorella bidwelli* | 1.40 | 0.210 | 1.50 | 0.439 | 1.7 | 0.340 |
| *Seiridium* sp. | 1.30 | 0.283 | 0.90 | 0.245 | 0.9 | 0.195 |
| *Alternaria ramulosa* | 1.20 | 0.268 | 1.40 | 0.334 | 1.1 | 0.237 |
| *Anguillospora gigantea* | 0.75 | 0.158 | 1.65 | 0.314 | 1.2 | 0.282 |
| *Anguillospora longissima* | 0.80 | 0.156 | 1.35 | 0.254 | 1.0 | 0.289 |
| *Montosporella tuberculata* | 0.60 | 0.124 | 1.00 | 0.182 | 1.0 | 0.268 |
| *Tetracladium marchalianum* | 0.60 | 0.184 | 1.10 | 0.298 | 1.05 | 0.261 |

ΔpH = pH 30th _ pH 1th, Δw.t = wt 30th_ wt 1th

in oil treatment are statistically meaningful. The control samples significantly different from the treated ones, however the difference between light and heavy oil crude treatment was not significant.

The chemical structure of the majority of carbon sources needed for fungi is sophisticated and in fact some changes happen in the physiology of the metabolism of the fungus[15]

Fungi species under study are from *Ascomycetes* and Aquatic *Deutromycetes* living on leaves and wood floated in water[6,9,16] and their existing cellulose is being used[10,13]. Moreover, some fungi might degrade lignin to some extent[11]. In limited lignin degrading phenyl oxidizes and their related organic compounds are also involved. Oil degrading fungi such as *Pseudohalonectria lignicola* use similar mechanism to degrade monocyclic aromatic hydrocarbons. At higher concentration they are toxic for cytoplasm membranes however in lower concentration phenyl oxides degrade them. Aromatic organic compounds with five rings or more are seldom used by fungi[16]. *Pseudohalonectria lignicola, Anguillospora gigantea, Halosparpheia retorquens* and *Nectria haemaatococca* that contain phenyl oxides enzymes can degrade phenyl organic compounds. Some of these fungi, which contain oxidatohydrolitic enzymes, degrade the cellulose too. Of course the cellulose system in the majority of the exo cellobiohydrolose fungi is not complete which explain why crystal cellulose is not degraded by all fungi species.

In the present study the degrading power of *N. lucidium* is less than *N. haematococca*. Similar condition was reported by Zareh Myvan and Shearer[16].

A large part of the components that form wood and leaf is made of carbon similar to oil hydrocarbons. Therefore, the oil degrading power of *Ascomycetes* and aquatic *Deutromycetes* species under study and increase of biomass in oil culture mediums is not unlikely (Table 2 and 3). Oil hydrocarbon degradation is accompanied by a decline of pH (or production of acid) in the culture medium due to the presence of enzymes in the fungus (Table 2 and 3). Organic acids such as pyruic acid, sucsinc acid and cytric acid are reported to contain

complex nutrition substra[15]. Most of the changes in pH is said to be the result of pyruic acid activity.

Organic aquatic fungi are reported to be abundant in fresh and brine water because such fungi can degrade oil hydrocarbon and play a useful ecological role under oil pollutant habitat conditions. Although the degradation of oil by the fungi under study has been confirmed in the laboratory condition, a study in real-life conditions and determining the nature of hydrocarbons generated by oil degradation calls for more research.

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