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Algal Bioassay for Evaluating the Role of Algae in Bioremediation of Crude Oil: I-Isolated Strains

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The widespread use of petroleum and petroleum products has inevitably resulted in the discharge of oil to the environment (Laws, 2000). Concerning aquatic system, the marine environment as well as freshwater environment has received the greatest attention (NRC, 1985). Hydrocarbons include aliphatic and aromatic compounds, some of which are suspected toxic and/or carcinogenic (Amin *et al.* 1995). Microbial utilization of these compounds as sole carbon source is highly dependent on the chemical nature of the compounds within the petroleum mixture and on the environmental determinants (Atlas, 1981). The microbial degradation of crude oil has been studied in great detail (Bogan *et al.* 2001). Bioremediation has long been proposed as a treatment technology for the decontamination of PAH contaminated environments (Bogan *et al.* 2001), where it was a multistage process, with petroleum – oxidizing bacteria playing the initial and major role (Jankevicius *et al.* 1992).

The principal factors limiting biodegradation rates are the nature of the oil, the concentration and diversity of the microbial population, the availability of O_2 and the temperature (Laws, 2000). The studies that dealing with microbial degradation of hydrocarbons by algae are rather limited, even though some algae and cyanobacteria can metabolize PAH (Cerniglia and Gibson, 1979; Cerniglia *et al.* 1980 b,c, 1982 c).This paper presents the results of the use of algal bioassay to evaluate the potential role of freshwater isolated algal strains in biodegradation of crude oil.

MATERIALS AND METHODS

Two species of freshwater isolated strains, *Scenedesmus obliquus* (green algae) and *Nitzschia linearis* (diatoms) were isolated from River Nile water. The isolated strains were grown at optimum temperature (24 ± 2 °C) with continuous illumination provided by white fluorescent lamps ~ 2500 Lux. Both strains were cultured in the media of EPA (1972) with the addition of 0.05mg/l Na_2SiO_3. $5H_2O$ to the media in case of the diatoms isolated strain. The stock cultures were continuously recultivated at optimum growth conditions and introduced to the experimental systems at logarithmic phase.

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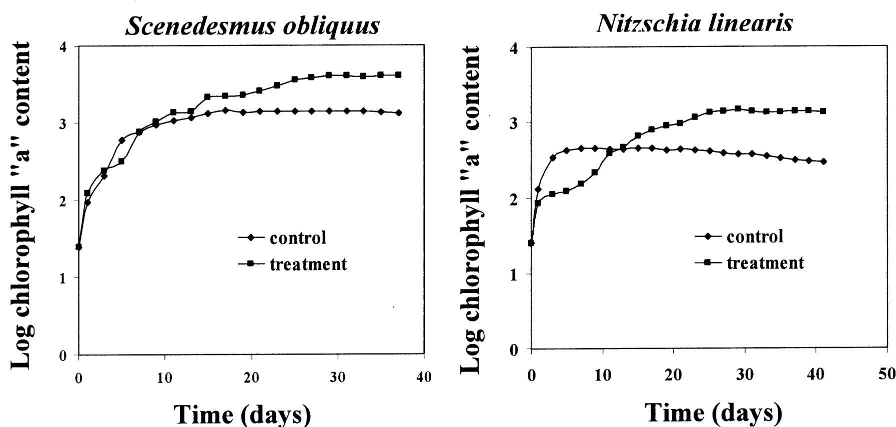


Figure 1. Growth response of isolated algal strains to crude oil.

Egyptian light crude oil (specific gravity, 0.85) was used. The oil was supplemented to the algal culture as it is without any treatment. Erlenmeyer flasks (5L) were used as incubation reactors. Each flask was filled with 3L distilled water containing algal culture specific for the isolated strains and 3 ml of the crude oil (1ml/L). Initial chlorophyll "a" concentration for all experiments ranged from 25-30 μg/l.

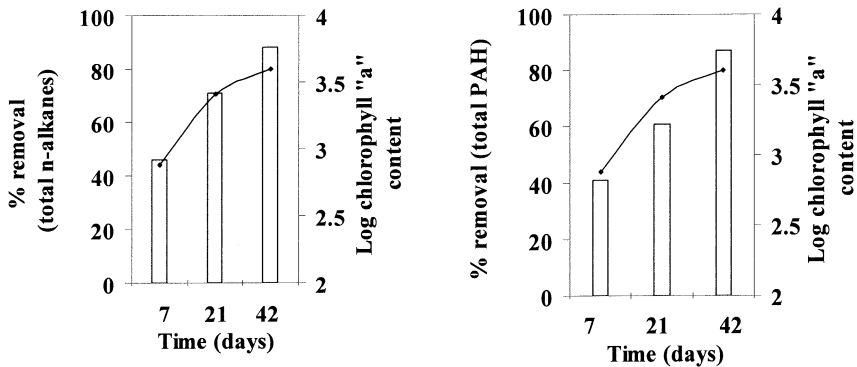
Growth of algae was determined by measurements of chlorophyll "a" content twice, according to Fitzgerald (1971) for the isolated strains. Microscopic examination was done weekly to detect the change in morphological characters of the isolated algal strains. In addition, weekly samples were withdrawn for crude oil biodegradation measurements.

Water samples were analyzed after 1st, 3rd and 6th week to determine the residual concentrations of n-alkanes (from C10 – C24) and polycyclic aromatic hydrocarbons (15 compounds). The detailed procedure for determination of petroleum hydrocarbons in water sample was described elsewhere (Gamila *et al.*, 2003). Total n-alkanes and PAHs were correlated with the growth of both kinds of algae (as chlorophyll "a" content) using the Product Moment Correlation analysis. T-test was used to differentiate between means of removal n-alkanes and PAHs by both algae species. Statistical tests for significance were performed at $P< 0.05$.

RESULTS AND DISCUSSION

Marked changes in algal biomass for the isolated strains treated with 0.1% crude oil were observed in Fig. (1). The diatom strain, *Nitzschia linearis* revealed lag period extended to 7th day with biomass less than control by 66% (biomass calculated as chlorophyll "a" content). The algae grow well after this lag period and the algal biomass production exceeds control by 228% (at 29th day). Karydis and Fogg (1980) supported the above data, which stated that *Cyclotella cryptica*

Scenedesmus obliquus



Nitzschia linearis

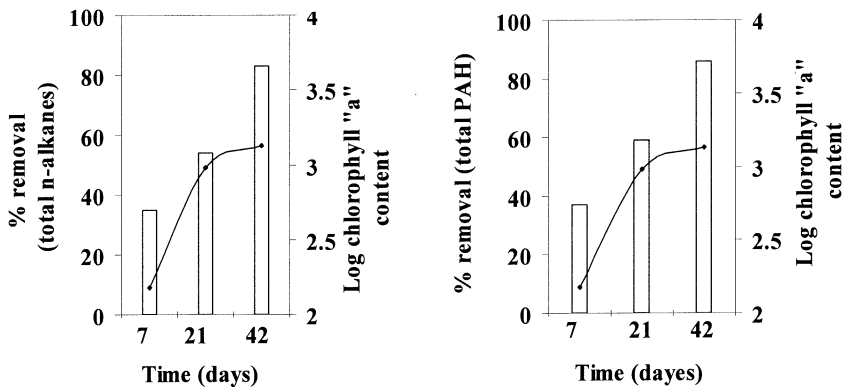


Figure 2. Percentage removal of petroleum hydrocarbons (columns) in relation to the growth of isolated algal strains (lines).

was stimulated by low and inhibited by high concentrations of North Sea crude oil and its paraffin fractions. In addition, Carman *et al.* (2000) explained that algal biomass indicated by the increase in diatom abundance was increased in diesel fuel-contaminated sediment. Conversely, Kusk (1978) contradicted our above data, where he found that Piper crude oil and naphthalene werethe most toxic to photosynthesis of diatom strain *Nitzschia palea.*

In green alga *Scenedesmus obliquus* culture treated with 0.1% crude oil (Fig. 1), the algal biomass was equivalent approximately to that of control up to the 13th day. Algal growth indicated by the increase in chlorophyll "a" reached to its maximum level (4 mg/l) in treated culture with percentage increase reached to 183% over the untreated culture (control culture). Soto *et al.* (1975, 1977, 1979) stated that the naphthalene and oil extracts have measurable adverse effects on cellular components of the green algae *Chlamydomonas angulosa.* Also, Dennington *et al.* (1975) found with *Euglena gracilis* culture after 12 days

Table 1. Degradation rate (% removal ± SD) of petroleum hydrocarbons by isolated algal strains (test of significant variation was at $p>0.05$).

Compounds	*Senedesmus obliquus*			*Nitzschia linearis*		
n-alkanes	1st week	3rd week	6th week	1st week	3rd week	6th week
n-C10	48.7±3.2	72.5±4.6	88.8±4.2	37.8±3.1	55.5±3.9	85.8±4.4
n-C12	48.1±3.5	72.7±3.9	88.4±4.0	32.3±3.5	55.3±3.2	85.0±3.6
n-C14	46.5±3.1	72.0±3.7	90.2±4.0	37.7±3.2	56.5±4.1	86.1±3.3
n-C16	47.1±3.2	77.0±3.8	88.5±3.3	36.8±2.5	52.6±3.0	83.3±4.1
n-C18	44.3±2.7	69.4±3.2	90.1±3.5	35.3±2.9	51.3±2.7	84.1±2.7
n-C20	45.1±2.4	65.8±3.1	85.6±3.0	33.0±2.5	53.1±3.1	80.6±3.6
n-C22	43.2±2.1	67.7±2.4	86.3±2.1	34.6±1.9	52.8±2.0	79.5±2.6
n-C24	41.6±1.1	66.5±2.1	81.2±2.0	34.9±1.3	56.1±3.1	79.5±1.9
Polcyclic Aromatic Hydrocarbons (PAHs)						
Naph.	47.0±4.9	69.5±5.3	95.2±5.9	39.1±2.7	68.0±5.7	95.7±5.0
1-m.naph.	42.7±4.6	61.6±4.7	90.6±5.7	35.9±3.7	60.5±4.9	88.1±4.3
2-m.naph.	43.2±5.0	64.2±4.8	89.2±5.1	31.8±5.9	60.1±3.7	88.8±5.3
Acenaphthy.	50.7±4.7	71.2±3.2	91.3±4.3	51.3±5.2	68.0±5.1	90.4±5.7
Acenaph.	47.3±3.7	70.0±4.1	90.5±3.6	47.0±3.7	70.4±4.4	91.3±2.7
Fluorene	45.3±3.2	63.0±3.9	88.7±2.8	38.2±4.4	60.8±5.8	81.9±3.7
Phenanth.	41.8±4.3	56.2±4.4	87.3±5.3	40.0±4.2	61.2±5.4	86.9±5.1
Anth.	50.2±3.8	70.4±4.0	90.6±4.7	46.5±5.6	66.6±5.3	88.9±4.4
Fluoran.	37.7±3.8	50.6±4.6	84.1±2.6	36.5±3.7	50.1±4.4	85.0±4.7
Pyrene	32.6±4.2	55.6±3.8	82.5±5.2	22.8±3.0	50.1±2.7	77.8±5.3
B(a)anth.	36.1±3.1	58.7±3.7	82.4±4.9	28.4±5.2	53.8±4.3	91.1±4.6
Chrysene	33.4±3.0	64.3±3.2	85.7±3.4	26.5±4.3	59.8±3.7	80.0±2.7
B(b)fluoran.	16.8±2.7	39.6±2.6	75.9±3.7	11.4±3.5	41.2±3.9	70.8±3.7
B(k)fluoran.	15.2±3.1	44.3±2.8	80.5±3.2	10.5±3.6	36.1±2.7	72.7±2.6
B(a)pyrene	10.1±2.5	43.4±3.0	77.9±2.7	6.1±5.2	30.1±5.7	71.3±2.6

Naph.:Naphthalene; 1-m.naph.:1-methylnaphthalene; 2-m.naph.:2-methylnaphthalene; Acenaphthy.:Acenaphthylene; Acenaph.:Acenaphthene; Phenanth.:Phenanthrene; Anth.:Anthracene; Fluoran.:Fluoranthene; B(a)anth.:Benzo(a)anthracene; B(b)fluoran.:Benzo(b)fluoranthene; B(k)fluoran.:Benzo(k)fluoranthene.

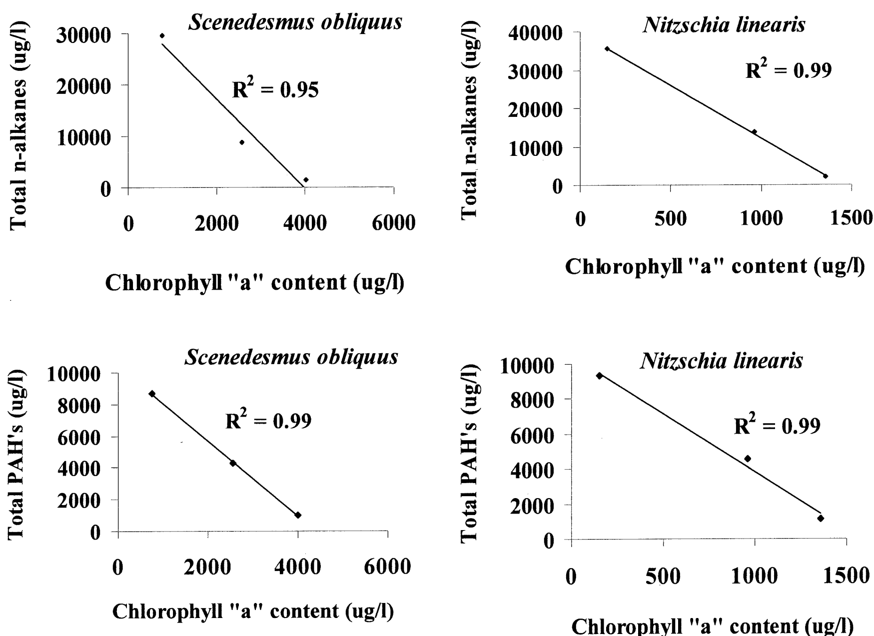


Figure 3. Correlation between petroleum hydrocarbons concentration and algal growth (significant negative correlation, $P< 0.05$).

treatment with oil concentrations ranging from 0.1% to 10% (V/V) did not inhibit or stimulate its growth. In addition, Gaur and Kumer (1981) observed that *Oocystis* sp., *Chorella vulgaris* and *Selenastrum capricornutum* were inhibited by all of the crude oil studied up to concentration of 30 μl/10 ml.

In both strains, the standing biomass reached to its maximum value at 29th day upon the treatment. In control culture of both strains the biomass reached to its maximum value at 15th day and 17th day for *Nitzschia linearis* and *Scenedesmus obliquus*, respectively, which may be indicated that the treatment of algal culture with crude oil led to prolongation the growth phase as well as high algal biomass production.

The effects of crude oil on the morphological characters of the green algae *Scenedesmus obliquus* and the diatom strain *Nitzschia linearis* have been observed. The most pronounced feature is that the algal cells in both strains were aggregated in clusters containing oil drops between their cells, forming an abnormal shape comparing with control culture. This is consistent with the study of Soto *et al.* 1979, who observed that aqueous crude oil extract had caused abnormalities in the cells of *Chlamydomonas angulosa.*

This investigation aimed to study the potential efficiency of isolated algal strains in biodegradation of crude oil. The other variables that could influence petroleum hydrocarbons biodegradation such as nutrients, pH or dissolved oxygen did not

monitored. Table (1) and Figure (2) showed the potential efficiency of test organisms in bioremedation of PAHs. The main result obtained was that the biodegradation rates depend on the type of algal strain used and on the time of exposure to crude oil. On the other hand, high bioremedation efficiency was achieved by *Scenedesmus obliquus* followed by *Nitzschia linearis*.

For all n-alkanes and PAHs (Table 1), *Scenedesmus* revealed high percentage removal which increased with the exposure time. The percentage removal of n-alkanes by *Scendesmus* ranged between 46% (after 1st week) to 88% (after 6th week), while it ranged between 41% to 87% for PAHs, through the same time of exposure (Fig.2). *Nitzschia linearis* showed less efficiency than *Scenedesmus* in removal of petroleum hydrocarbons. The percentage removal ranged between 35% to 83% for n-alkanes and between 37% to 86% for PAHs compound during the 6 weeks of experiment.

Generally, no significant variation exists between the biodegradation of n-alkanes and PAHs by both algal strains ($P > 0.05$), but n-alkanes were mostly degraded to a greater extent than PAHs by *Scenedesmus*. Conversely, *Nitzschia* have efficiency to biodegrade PAHs slightly higher than n-alkanes (Table 1 & Fig.2). Negative strong correlations were found between the algal growth of both strains and the total concentrations of petroleum hydrocarbons (Fig. 3). These correlations were statistically significant ($P < 0.05$), and it may be indicate that both strains of algae have the same capability for biodegradation the petroleum hydrocarbons.

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