International Biodeterioration & Biodegradation 82 (2013) 67–72

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Biodegradation of crude oil by *Scenedesmus obliquus* and *Chlorella vulgaris* growing under heterotrophic conditions

Mostafa M. El-Sheekh [a,*], Ragaa A. Hamouda [b], Adnan A. Nizam [c]

[a] Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt
[b] Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Minufyia University, Egypt
[c] Plant Biology Department, Faculty of Science, Damascus University, Syria

ARTICLE INFO

Article history:
Received 26 June 2012
Received in revised form
11 December 2012
Accepted 16 December 2012
Available online 9 April 2013

Keywords:
Biodegradation
Heterotrophic
Algae
Crude oil
Scenedesmus obliquus
Chlorella vulgaris

ABSTRACT

One of the major environmental problems nowadays is petroleum hydrocarbon contamination, particularly in the zones of petroleum production, and petrochemical industries. This study was carried out to evaluate the potential of two green algae *Scenedesmus obliquus* and *Chlorella vulgaris* to degrade crude oil. Experiments were performed by incubating algal cultures with 0.5, 1, 1.5 and 2% crude oil for incubation period of 15 days under heterotrophic conditions. It was found that *Scenedesmus obliquus* and *Chlorella vulgaris* performed the highest biodegradation rate of crude oil when 0.5 and 1% oil was applied. The highest growth of *S. obliquus* was attended with 0.5% crude oil; while it was recorded at 2% for *C. vulgaris*, under the same heterotrophic conditions. Both algae could grow and degrade oil effectively when incubated with low concentrations of oil.

1. Introduction

Soil and water hydrocarbon pollution, which represents a very serious environmental problem, has been attracting considerable public attention over the last decades. The main sources of hydrocarbon pollution are the spills and leaks of petroleum products (Potter, 1993). Bioremediation is an emerging technology that uses plants and microorganisms to clean up the environment from pollutants and is cheaper than other remediation technologies (Leahy and Colwell, 1990). Numerous microorganisms, including bacteria, fungi and yeasts have the ability to degrade hydrocarbons (Oudot et al., 1993; Chaillan et al., 2004; El-Sheekh et al., 2009). Some species of algae are capable of heterotrophic growth on organic carbon sources (Neilson and Lewin, 1974). The ability of algae to degrade organic pollutants is the reason for their growth in the presence of pollutants. Cerniglia et al. (1980) proved the ability of nine Cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms to oxidize naphthalene. Walker et al. (1975) isolated an alga, *Prototheca zopfi* which was capable of utilizing crude oil and a mixed hydrocarbon substrate exhibiting extensive degradation of n-alkanes and isoalkanes as well as aromatic hydrocarbons. Luther and Shaaban (1990) Luther (1990) confirmed the ability of *Scenedesmus obliquus*, to utilize naphthalene sulfonic acids as a source of sulfur for biomass production. Tikoo et al. (1997) observed that three species of *Chlorella* degrade pentachlorophenol. Yan and Pan (2004) reported that more than 30 azo compounds were biodegraded and decolorized by *Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Oscillateria tenuis*, in which azo dyes were decomposed into a simpler aromatic amine.

This work aims at studying the potential of microalgae *Scenedesmus obliquus* and *Chlorella vulgaris* for crude oil biodegradation, which also includes (i) Biodegradation of crude oil by the microalgae. (ii) The hydrocarbon degradation capacity under the laboratory conditions. (iii) The ability of *S. obliquus* and *C. vulgaris* to grow under the heterotrophic condition in the presence of crude oil.

2. Material and methods

2.1. Isolation of algae

The green algae *Scenedesmus obliquus* and *Chlorella vulgaris* Beijerinck were isolated from water samples collected from River

* Corresponding author.
E-mail address: mostafaelsheekh@yahoo.com (M.M. El-Sheekh).

0964-8305/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2012.12.015

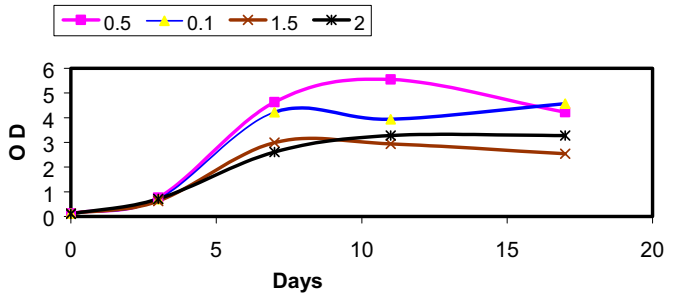

Fig. 1. Effect of different concentrations of crude oil on growth of *Scenedesmus obliquus* measured as optical density (560 nm).

Nile near Tanta city, Egypt. The algae were purified through serial dilution followed by plating. Each algal colony was isolated and inoculated into liquid medium at 25 °C ± 1 Stanier et al. (1971). The purity of cultures was ensured by repeated plating and regular observation under the microscope.

2.2. Algae cultivation with crude oil

Crude oil was added to 250 ml Erlenmeyer flasks containing 100 ml BG11 medium with appropriate amount of algal culture to give a total crude oil concentrations (0.5, 1, 1.5 and 2%). The Erlenmeyer flasks were incubated at 25 ± 1 °C at a constant shaking rate of 80 rpm under dark condition.

2.3. Assessment of algal growth

The biomass of algae was determined by measuring the optical density of the algal suspension at 560 nm (Wetherell, 1961) using Unico UV-2000 spectrophotometer.

2.4. Pigments estimation

A known volume of culture was centrifuged at speed of (8000 rpm) for 10 min, after that the algal pellets were treated with known volume of ethyl alcohol and kept in water bath for 30 min at 55 °C, and then centrifuged again. The colour of pellets must be white to ensure maximum extraction of pigments. If it was not the extraction must be repeated. Absorbance of the pooled extracts was registered on Unico UV-2000 spectrophotometer at 650, 665 and 452 nm. Calculations were made according to the formulae devised by Senger (1970) for chlorophyll *a*, chlorophyll *b* and carotenoids.

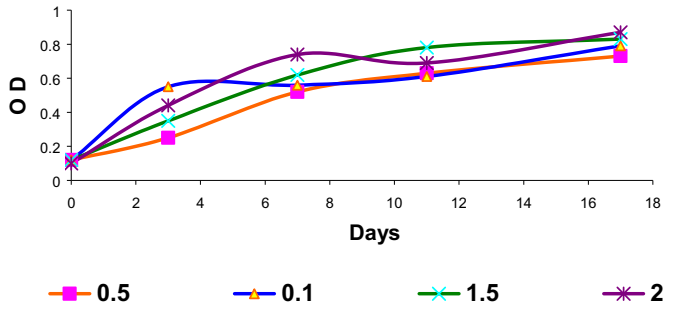

Fig. 2. Effect of different concentrations of crude oil on growth of *Chlorella vulgaries* measured as optical density (560 nm).

Table 1
Effect of different concentrations of crude oil on Chlorophyll-*a*,*b* and Carotenoids content of *Chlorella vulgaris* (μg/ml). Values are mean of 3 replicates ± standard error of the mean.

	Crude oil conc.	3th day	7th day	11th day	15th day
Chlorophyll-*a*	0.5	1.24 ± 0.0	3. 30 ± 0.06	4.55 ± 0.23	4.22 ± 0.20
(μg ml^{-1})	1.0	1.43 ± 0.30	2.69 ± 0.02	5.65 ± 0.31	5.17 ± 0.26
	1.5	1.50 ± 0.34	3.41 ± 0.01	5.36 ± 0.18	6.13 ± 1.29
	2.0	1.61 ± 0.04	3.22 ± 0.34	4.82 ± 0.52	4.42 ± 0.34
Chlorophyll-*b*	0.5	2.04 ± 0.0	1.75 ± 0.05	4.79 ± 0.86	4.31 ± 0.27
(μg ml^{-1})	1.0	2.21 ± 0.03	1.78 ± 0.17	5.53 ± 0.23	5.14 ± 0.23
	1.5	2.07 ± 0.84	2.62 ± 0.26	4.94 ± 0.45	6.22 ± 1.24
	2.0	2.35 ± 0.53	2.21 ± 0.31	4.56 ± 0.46	4.21 ± 0.43
Carotenoids	0.5	1.61 ± 0.0	1.28 ± 0.38	0.52 ± 0.23	0.11 ± 0.07
(μg ml^{-1})	1.0	0.70 ± 0.19	1.13 ± 0.06	0.20 ± 0.01	0 ± 00
	1.5	0.87 ± 0.08	1.34 ± 0.09	0.59 ± 0.03	0.24 ± 0.24
	2.0	1.07 ± 0.51	1.24 ± 0.11	0.34 ± 0.08	0.30 ± 0.07

2.5. Biodegradation activity of the algae for crude oil

Biodegradation of crude oil was analysed by using GC–MS HP 6890 gas carrier helium (1 ml/min). Capillary Column. 30 m × 0.25 rnm ID × 0.25 um film and the temperature programming was 70–290 °C, 5/15 min.

2.6. Statistical analysis

Experiments were conducted in triplicate. Results were expressed as ± standard error of the mean.

3. Results and discussion

3.1. Estimation of algal growth

Results in Figs. 1 and 2 show the heterotrophic algal growth using crude oil as sole carbon source. The growth of *S. obliquus* increased in the presence of high concentrations of crude oil as compared with *C. vulgaris*. The highest growth of *S. obliquus* was attended at 0.5% crude oil, while it was recorded at 2% crude oil for *C. vulgaris*. This result is in agreement with Gamila and Ibrahim (2004) who indicated that the treatment of algal culture of (*S. obliquus*, *Nitzschia linearis*) with crude oil led to prolongation of the growth phase as well as high algal biomass production. Kong

Table 2
Effect of different concentrations of crude oil on Chlorophyll-*a*,*b* and carotenoids content of *Scenedesmus obliquus* (μg/ml). Values are mean of 3 replicates ± standard error of the mean.

	Crude oil conc.	3th day	7th day	11th day	15th day
Chlorophyll-*a*	0.5	4.97 ± 0.10	7.82 ± 1.47	4.01 ± 0.82	6.26 ± 0.23
(μg ml^{-1})	1.0	4.93 ± 0.59	5.95 ± 0.44	5.60 ± 0.28	4.56 ± 0.36
	1.5	4.23 ± 0.37	3.32 ± 0.08	5.65 ± 0.08	6.32 ± 1.58
	2.0	3.23 ± 0.77	5.69 ± 0.68	3.54 ± 0.62	5.83 ± 0.34
Chlorophyll-*b*	0.5	8.02 ± 0.02	6.93 ± 1.38	0.65 ± 0.06	6.70 ± 0.49
(μg ml^{-1})	1.0	7.69 ± 0.49	6.934 ± 0.10	0.28 ± 0.077	4.57 ± 0.25
	1.5	6.04 ± 0.70	6.93 ± 2.33	0.08 ± 0.08	6.30 ± 2.29
	2.0	3.91 ± 1.00	6.93 ± 1.45	0.51 ± 0.21	6.19 ± 0.61
Carotenoids	0.5	0.0	0.0	3.06 ± 0.45	0.0
(μg ml^{-1})	1.0	0.0	0.67 ± 0.22	4.1 ± 0.18	0.2 ± 0.12
	1.5	0.05 ± 0.05	0.0	4.05 ± 0.08	0.03 ± 0.03
	2.0	1.01 ± 0.45	0.0	2.55 ± 0.40	0.0