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Biodegradation of Crude Oil by Anabaena variabilis Isolated from Al-Dora Refinery

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Abstract:*Oil spill occurs frequently all around the world which adversely affect the environment. Thus a low cost and environmentally friendly bioremediation could be an alternative way to solve the oil pollution problem. The cyanobacteria Anabaena variabilis used in this study to investigate their ability to grow and degrade different concentrations of crude oil. It was found that the growth of cyanobacterium Anabaena variabilis in the presence of crude oil as the sole carbon source increases at the same rate as the control sample. Results obtained from FTIR analysis showed a high ratio analysis of hydrocarbon compounds through the changes that occurred in the composition of the hydrocarbon. GC-MS analysis of biodegraded crude oil was done to analyze the intermediates of the crude oil degradation. Results indicated that several intermediates are formed during the course of biodegradation of crude oil and the biodegradationpercentage was 89.00%.*

Keywords:*Crude oil, Biodegradation, Cyanobacteria, FTIR, GC-MS*

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I. INTRODUCTION

The release of oil or hydrocarbons into the environment whether accidentally or due to human activities is a major cause of water (both surface and groundwater resources) and soil pollution [1]. Every year about 35 million barrels of oil is ferried across the oceans, making the aquatic environment vulnerable to pollution from oil spills, leakages that threaten the aquatic or marine life all over [2].In large-scale accidents, oil release into the environment harms the biological system due to the biomagnification of toxic compounds with toxic elements via food webs and food chains [3, 4].

The technologies commonly used for the remediation of oil spill and contamination include mechanical and physico-chemical processes such as burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition or breakdown of contaminants .Biodegradation of pollutant to non-toxic end products by microorganisms, referred to as bioremediation which is one of the most effective methods for remediating environmental systems in both engineered and in situ remediation schemes. This approach is often cost-effective and environmentally-friendly than traditional detoxifying methods[5].Biodegradation of environmental pollutants has been characterized over years where bacteria and fungi are the most commonly studied biological agents; however, among different biological agents, microalgae and cyanobacteria are very adaptive, and can grow autotrophically, heterotrophically as well as mixotrophically[6,7]. Cyanobacteria are Gram-negative bacteria, which are the only bacteria capable of performing oxygenic photosynthesis [8].After the release of oil during the Gulf War in Kuwait, a bloom of cyanobacteria closely associated with oil was observed and the intensive growth of them is the first sign in self-cleaning activity in this oil polluted areas, this finding given the impression that cyanobacteria possess the potential to break down oil components [9].Several investigations showed that cyanobacteria degrade crude oil and other complex organic compounds such as surfactants [10, 11, 12]. For example, cyanobacterial species *Oscillatoriasalina*, *Plectonematerebrans*, *Aphanocapsa*sp., and *Synechococcus* sp., develop mats in aquatic environments, and have been successfully used in the bioremediation of oil spills in different parts of the world [11,13]. Not only oil-contaminated waters but also oil-contaminated soils be successfully remediated using a naturally occurring cyanobacterial–bacterial associations[14].

Therefore, this study was conducted to investigate the potential of cyanobacterium*Anabaena variabilis* in the biodegradation of crude oil and to evaluate oil effects on their growth.

II. MATERIALS AND METHODS

Cultures of Cyanobacteria *Anabaena variabilis* (Nostocales) were isolated from Al-Dora refinery (Baghdad-Iraq), BG-11 culture medium was used as specific growing culture of which components were illustrated by[15].The isolate were grown at 25˚C and ±2500 Lux as optimum physical growth conditions were

provided by white fluorescent lamps under light/dark regime of 16/8 hours for the duration of the experiments. The stock cultures were continuously recultivated and introduced to the experimental systems at logarithmic phase. Triplicate cultures were run for each treatment.

2.1.Cyanobacterialcultivation with crude oil

Iraqi medium crude oil (density 0.84) obtained from AL-Dora Refinery, was added to 500 mL Erlenmeyer flasks containing 200 mL, BG11 medium. An inoculum of cyanobacterial culture was added to flasks containing crude oil .The Erlenmeyer flasks were incubated at (25 °C) on constant shaking at 150 rpm.

2.2. Assessment of cyanobacterial growth

Optical density of the cyanobacetrium suspension was measured by Spectrophotometer at an absorbance of 750 nm, using culture medium (BG11) as a blank [16].

2.3. Biodegradation activity of crude oil

Degradation of oil in cultures was tested by FTIR and gas chromatographic methods .For FTIR The crude oil in samples were extracted by adding Solvent of hexane: acetone (1:3) to separate hydrocarbons from the liquid media, the upper organic layer were mixed with pure KBr in the ratio of about 1:100, pellets were fixed in the sample holder, and the analyses were carried out using Fourier transform infrared (FTIR) in the mid IR region of 400–4000 cm-1 with 16-scan speed[17], for GC-MS analysis oil residues were extracted thoroughly with n-hexane, dried to concentrate, solubilized in a small volume of n-hexane then subjected to chromatographic analysis [18, 19].The percentage of hydrocarbon biodegradation was estimated according to the below equation[20]:

$$\%\ \mathbf{ofhydrocarbonsbiodegradation} = \frac{\mathbf{Totalpeakareaofcontrolsample - Totalpeakareaoftestsample}}{\mathbf{Totalpeakareaofcontrolsample}} \times \mathbf{100}$$

III. RESULTS AND DISCUSSION

3.1. Estimation of cyanbacterial growth

Growth was detected by measuring Optical Density, highest values of OD recorded for 1% crude oil as (0.409) at 14th day of incubation. The results showed an increase in the growth from the beginning of experiment (zero time) at (0.5, 1) % crude oil and control, 1% of crude oil was the optimum concentration for their maximum growth figure (1).

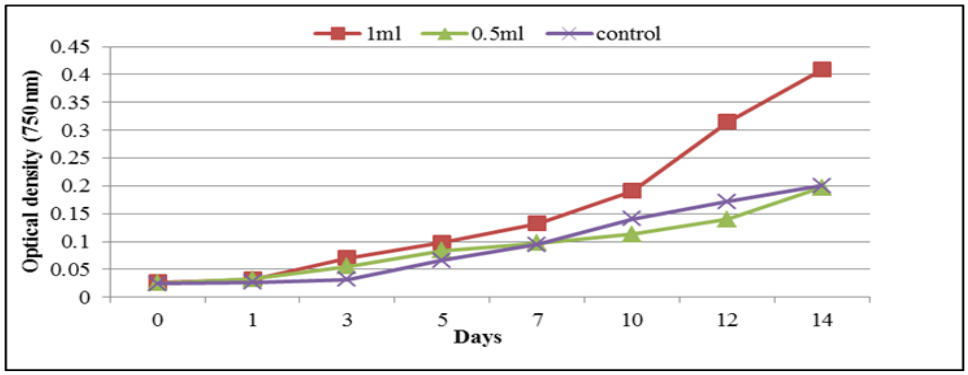

Figure 1: optical density for *A.variabilis* at 0.5 % and 1% crude oil.

The cyanobacterial strain utilized crude oil hydrocarbons as sole source of carbon and energy, which was evident from the increase in cell density of culture after incubation. The statistical analysis show no significant difference (P<0.05) between (0.5, 1) % crude oil concentration and control with the high growth observed for 1% crude oil Table (1).

Table 1: Mean ± SD of growth measurement during crude oil biodegradation by *A. variabilis*.

Crude oil concentration %	Optical density (750) nm
Control	0.094 ± 0.0027 a
0.5	0.093 ± 0.0031 a
1	0.159 ± 0.0038 a
LSD	0.082 NS

The increase in the growth of the *A. variabilis*after days of treatment with the crude oil could be explained by the presence of compounds resulted from the biodegradation of low levels compounds of crude oil and the ability of the cyanobacteria to use these degraded compounds as mitogenic source for their growth[16].Similar to our finding [21] study the biodegradation of crude oil by *Oscillatoriaagardhii* and *Anabaena spharica* the results show that both cyanobacterial strains revealed a high algal biomass in comparison with that obtained by the control culture.

3.2. Biodegradation activity of crude oil

Biodegradation of crude oil was done to identify the ability of each single and mixed cyanobacterial isolates using FTIR and GC-mass analysis.

In this study, using the analysis of the hydrocarbons by FTIR technique employed to determine process of biodegradation through absorbed the changes in structure of hydrocarbon before and after treatment by control and after 14th days of incubation with 1 ml of crude oil. The control samples showed the absorption bands places of aliphatic (CH2) rocking and aromatic C-H bending at (675-900 cm-1), (C-H)bending (910-990 cm-1) of alkenes ,(C–C) and (C–H) stretching (3000-3100 cm−1) of aliphatic and aromatic compounds, and (C–H) deformation (1378-1500 cm−1) of Aliphatic CH2 and CH3 groups and absorption band of (C=C) for Aromatic ring figure (2-A).

For *A. variabilis* treated culture the results appeared that the spectra reflected more pronounced alterations than control Figures (2-B) after 14th days of incubation with 1% crude oil, the charts was showed an obvious change in specific regions area, and also there was changes in bands shape, peak position, peak intensity and bands width. The absorption bands were disappeared with the range of wave number (910-990 cm−1), that refer to the (C-H) bending and (3010-3100 cm−1) (C-H) stretching of aromatic hydrocarbons which indicate the ability of this cyanobacterium for aromatic hydrocarbons degradation[22, 23, 24]Also, a new bands show between (2000 – 3600 cm-1) which related to amines and carboxylic acids compounds as a result of biodegradation process. It is clear that from the previous explanation the difference in absorption bands and its appearance/disappearance exhibited a good indication of crude oil biodegradation [25].

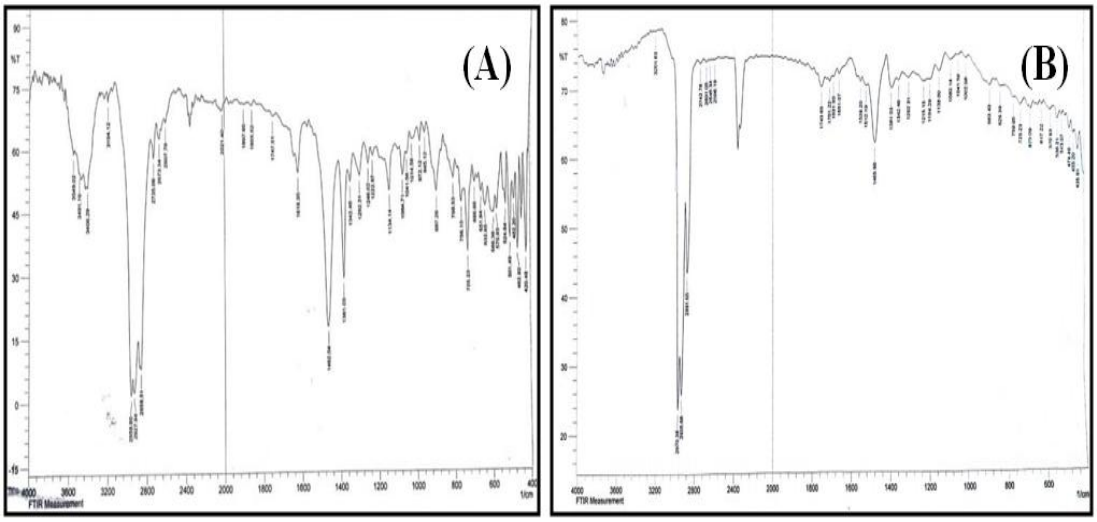

Figure 2: FTIR spectra for 1% crude oil biodegradation, A-control, B-treated culture.

GC-MS analysis was done for medium crude oil extracted from control (crude oil without cyanobacterial inoculum) and *A.variabilis*treated culture after 14 day of incubation in liquid BG11 medium at 25 °C, pH 7 at 150 rpm to identify the mass spectrum. The number of hydrocarbon fractions and hydrocarbon compounds were identified from their mass spectra and retention time.

Table (3) represent GC-MS analysis for 1 % crude oil degradation by *A.variabilis* after 14 days of incubation at 25 °C ,pH 7 and 150 rpm, results indicated that there was changes in number and length of the peaks were significantly reduced in the chromatogram of the biodegraded crude oil compared with control. New peaks were also visible indicating the production of new intermediates during the biodegradation process also there is reduction in peak number to the half due to complete biodegradation of some hydrocarbons especially the saturated hydrocarbons[16] and some peaks was disappeared completely. The percentage of crude oil biodegradation by *A.variabilis* (related to sum of peaks area) was 89.00%.

Table 2: List of compounds detected through GC/MS analysis of control after 14th days of incubation with 1% crude oil

Peak No.	Retention time	Peak Area	Compound name
1	3.158	1861738	Decane
2	3.569	1145846	Hexan,2,2,3,2-tetramethyl
3	3.859	206181	Benzaldehyde,2-hydroxy
4	4.212	96749	Heptane,5-ethyl-2-methyl
5	4.457	263312	1-decane,3,4-dimethyl
6	4.533	149485	Hexadecane,1-chloro
7	6.714	74377	Undecane
8	7.411	2327	Nonan,5-(2 –methyl propyl)
9	7.814	343314	Undecane
10	8.113	524444	Pentadecane
11	8.615	667428	Hexadecane
12	10.591	3709812	Tetradecane
13	10.753	1374957	Heptadecane
14	11.042	1137300	Hexadecane,7-methyl
15	11.283	1118920	3-tetradecane
16	12.3	1525375	Eicosane
17	13.56	2190933	Tetradecane
18	14.876	1982230	Hexadecanoic acid,ethyle ester
19	16.31	1822690	Pentadecane,8-hexyl
20	17.634	2070107	Heptadecane
21	18.412	3814935	Heptadecane
22	19.514	3813149	Tetracosane
23	20.061	795578	Phenenthrine
24	20.721	1374957	Tricontane
Total	32066144		

Table 3: List of compounds detected through GC/MS analysis of 1% crude oil treated with*A.variabilis*after 14 days of incubation

Peak No.	Retention time	Peak Area	Compound name
1	2.024	66492	Methylcyclopentane
2	2.586	52661	Heptane
3	8.306	101205	Decane
4	10.161	188461	2-methyldecane
5	11.847	242306	Dodecane
6	13.396	163329	Nonane, 2-methyl
7	14.834	169001	Hexadecen-1-ol
8	16.185	105964	Tridecane, 6-propyl
9	17.461	76280	1-Undecene, 4-methyl
10	18.666	77002	Dodecane, 2,6,11-trimethyl
11	19.814	53971	Octane, 3,4,5,6-tetramethyl
12	20.903	35339	6-propyltridecane
13	21.643	315304	Dodecane, 2,6,11-trimethyl
14	21.944	42561	Cis-9-Octadecenal
15	23.563	1609615	Octadecanoic acid
16	23.688	95121	Oxirane, tridecyl
17	26.342	58262	1,2-cyclododecanediol
18	28.131	74018	Hexatriacontane
Total	3526892		

A. variabilis visually (from the remaining of crude oil in each flask) appeared to have active biodegradability for crude oil figure (3).

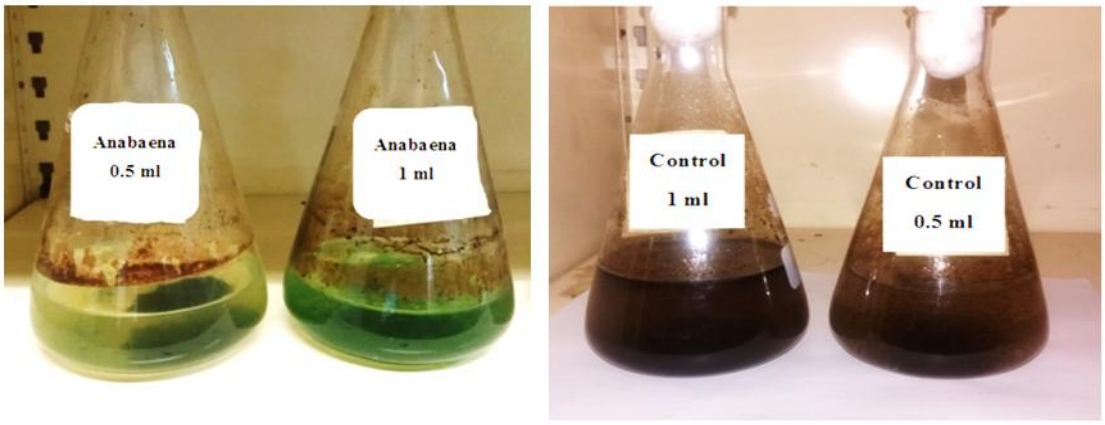

Figure 3: Morphological changes of crude oil degraded by *A. variabilis* that incubated at 25°C on liquid BG11 media.

The capability of cyanobacteria to degrade compounds including petroleum hydrocarbons has been reported by other researchers and evidence abound on the fact that microbial communities dominated by cyanobacteria are actively involved in oil degradation[26, 27, 28].The growth of cyanobacteria after oil spills into the Arabian Gulf forming heavy thick mats gave the impression that cyanobacteria possess the potential to degrade oil components[29, 9]. It has been reported that some microalgae and cyanobacteria can adapt oil pollution rather quickly by single gene mutations, and can also proliferate under low oil concentration as a result of physiological adaptation[30]. Different biodegradation trends were also reported by other researchers [31, 32]using the cyanobacteria *Fischerellaambigua* and *Nostocpiscinale*, respectively, under heterotrophic conditions.
The overall results indicate that A. variabilisused in this study were able to degrade crude oil efficiently.

IV. CONCLUSION

The result established the fact that the cyanobacterium isolated from Al-Dora refinery is capable of utilizing crude oil as a source of carbon and energy. Isolated strain investigated for biodegradation in the present study are highly recommended for beneficial bioremediation applications of petroleum hydrocarbons in the studied area; they are resident flora in this region and have already been adapted to the site conditions .Cyanobacteria are photosynthetic and are believed to acquire carbon as a source of energy from atmospheric CO2 and have been seen to degrade hydrocarbons.

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