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Research Article

The Effect of the Presence or Absence of Carbon on the Accumulation and Bioremediation of Hydrocarbon Compounds in Cyanobacterium *Oscillatoria Tenuis*

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ABSTRACT

In this research, the effects of the presence and absence of carbon in the medium of cyanobacterium *Oscillatoria tenuis* on growth rate and its ability of accumulation and bioremediation of hydrocarbon compounds were investigated. Isolated cyanobacterium treated with different crude oil concentrations (control, 0.25,0.5,1 and 2)% in Chu- 10 medium .The result showed that the dry weight and chlorophyll A of the cyanobacterium was the best in the medium contain carbon than carbonless medium, the best growth was in 0.5% crude oil about 0.20mg/g. The chlorophyll content was the best in 0.25% crude oil about 16.274 µg/g in the presence of carbon. Both dry weight and chlorophyll A were increased as time increase in the presence and absence of carbon. Hydrocarbon compounds accumulation was the best in the presence of carbon than in the absence of it at the first week, the best accumulation was in 2% crude oil about 1124.728 µg/g, but these concentrations decreased as time increase untile the third week , and then rise slightly in the fourth week.

Keyword: Cyanobacteria; accumulation; bioremediation; hydrocarbon compounds

INTRODUCTION

Petroleum is a major anthropogenic contaminant in the aquatic environment and may affect the community composition of the plant, fishes, birds, mammals and phytoplankton [1, 2]. These contamination come from various sources, Oil refinery wastes release high levels of hydrocarbons, natural seepage from ground and industrial activities other than petrochemistry are also considered sources of dangerous pollutants [3, 4, 5] and petroleum derivates (especially diesel and fuel) is an important problem in the water [6], because of their carcinogenic and mutagenic properties [7]. Physical and chemical strategies are used to minimise these effects but these strategies are more expensive, and in most cases, generate further pollution [8]. Biological strategies are more economical and efficient than chemical and physical ones. In comparison to other biological methods bioremediation through microorganism is more efficient [1], because it is simple to maintain, eco-friendly, cost-effective and may leads to the complete or partial removal of the pollutants [9]. These pollutants can potentially be degraded by the great variety of soil and aquatic microorganisms. Bacteria, filamentous fungi, yeast, and cyanobacteria are known to be important hydrocarbon degraders [10, 11].

Bioremediation of oil spill cleanup is either done by bioaugmentation or biostimulation. Bioaugmentation is the addition of

microorganism capable of degrading the toxic hydrocarbons to achieve a reduction of the pollutants [1]. Some microalgae produce enzymes capable of degrading harmful organic compounds to transform the petroleum hydrocarbons into less toxic compounds [12]. These bioremediation capabilities of microalgae are useful for environmental sustainability [13, 14]. In most studies, degradation of crude oil by various species of cyanobacteria has been reported [15, 16, 17].

The aim of this study was to compare the presence and absence of carbon in the medium on the ability of cyanobacterium *Oscillatoria tenuis* on the accumulation and bioremediation of hydrocarbons compound.

MATERIALS AND METHODS

Cyanobacteria and Culture Conditions

Axenic culture of Cyanobacteria *Oscillatoria tenuis* was isolated from Shatt Al-Arab River (Basrah-Iraq), and conducted with all experiments. Chu no.10 culture medium was used as specific growing culture of which components were illustrated by [18]. The strain was grown at 27°± 2 C and ±2500 Lux as optimum physical growth conditions were provided by white fluorescent lamps under light/dark regime of 18/6 hours for the duration of the experiments. The stock cultures were continuously recultivated and introduced to the experimental systems at logarithmic phase.

Ten conical flasks (250 ml) were prepared, put in five of them 200 ml of Chu-10 medium contain carbon source. Each flask of them injected with 20 ml of the unialgal stock culture of *O.tenuis*; cultures were left two days for adaptation then added to four of them, four concentrations of crude oil (0.25, 0.5, 1, 2) % and left one as a blank.

Put in the second five conical flask, Chu-10 medium free from the carbon source, also injected with unialgal culture and added to four of them same concentration of crude oil and placed in shaker incubator for a month. Hydrocarbons biodegradation was followed by measuring the concentration of extractable hydrocarbons at the end of each week of the month.

Measurement of dry weight

10 ml samples were filtered on to pre-dried and weighed GF/C fibre filters every week of culture. Filters were oven dried overnight at 60℃ and reweighed using an analytical scale [19]

Measurement of chlorophyll a

The measurement of chlorophyll a was taken at the end of each week of cell cultivation. Sample (10 mL) of the culture was filtered using GF/C filter. The filtered cell was placed into 10 ml centrifuge tube, and 9 ml of 90% acetone was added. The tubes were wrapped in foil and placed in a fridge overnight to extract a chlorophyll. At next day, the samples were centrifuged at 3000 rpm for 10 min. Chlorophyll-a concentration (µg/ml) was determined using Spectrophotometer with the wavelength of 665 and 750 [19].

Measurement of hydrocarbon compounds

Ten mL samples were filtered on to pre-dried and weighed GF/C fibre filters every week of culture. Filters were oven dried and reweighed then placed in the cellulose thimble and extracted using Soxhlet intermittent extraction [20] with mixed solvents (100 ml) methanol: benzene (1:1 v/v) for 24-48 hrs. The combined extracts saponified for 2 hrs. By adding (15ml) 4M MeOH(KOH) at the same temperature and cooled to room temperature. The unsaponified matter was extracted with (50 ml) n-hexane using separator funnel. The upper unsaponified matter with hexane (hydrocarbons) was passed through open – chromatographic column separation column. The samples dried and stored until detection with the spectrofluorometer (for Total Petroleum Hydrocarbons (TPHs).

Statistical analysis

Mean comparison were conducted by one-way analysis of variance (ANOVA), followed by LSD test to determine significance. In all cases, comparisons that showed a p-value <0.05 were considered significant.

RESULTS AND DISCUSSION

Dry weigh

With Carbon

Figure (1) shows algae growth rates in(0.25, 0.5,1 , 2)% crude oil compared with blank(B) in the presence of carbon in medium, wich shows the ability of algae to continue to grow untile the end of the 4th week in the presence of crude oil in varying degrees , but less than blank where the

biggest growth rate in the 4th week about 0.27 mg/g . Among the add crude oil concentration, the best growth was in 0.5% crude oil about 0.20 mg/g at the end of the 4th week. There is a significant difference (P>0.05) between blank and add crude oil concentrations (LSD= 0.098). Another significant differences (P>0.05) between the 1st week and other three weeks (LSD= 0.032). Petroleum compounds, in general, have been shown to either inhibit or stimulate algal growth, depending on the type and level of petroleum product and the algal species concerned [21, 22].

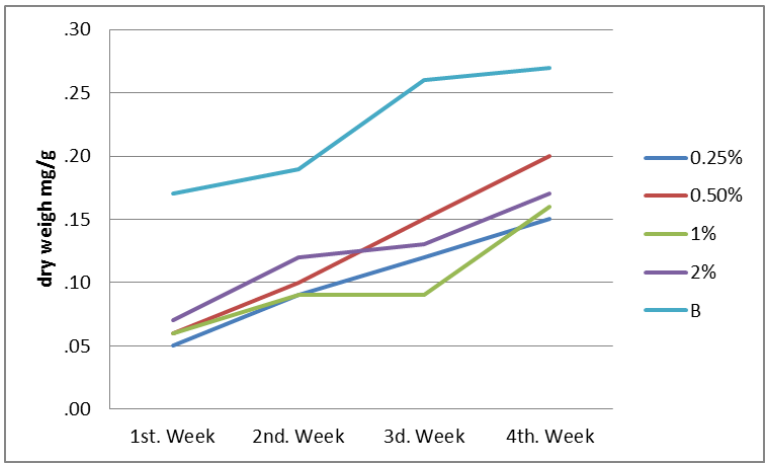

Fig.1: Dry weigh amounts in different crude oil concentration (0.25, 0.5, 1 and 2)% and Blank with carbon

Without carbon

At the absence of carbon in the medium (fig. 2), the algae continue to growth in all add crude oil concentration (0.25, 0.5, 1, 2)% but in lower levels than in the presence of carbon. The best growth was in 2% crude oil about 0.17 mg/g at 4th week. Our result is in agreement with Simona Ghita *et al.* [23] who exhibit that the filamentous cyanobacteria grown for one week in sea water, supplemented with diesel (2% v/v) a much stronger than the populations grown in the absence of diesel.

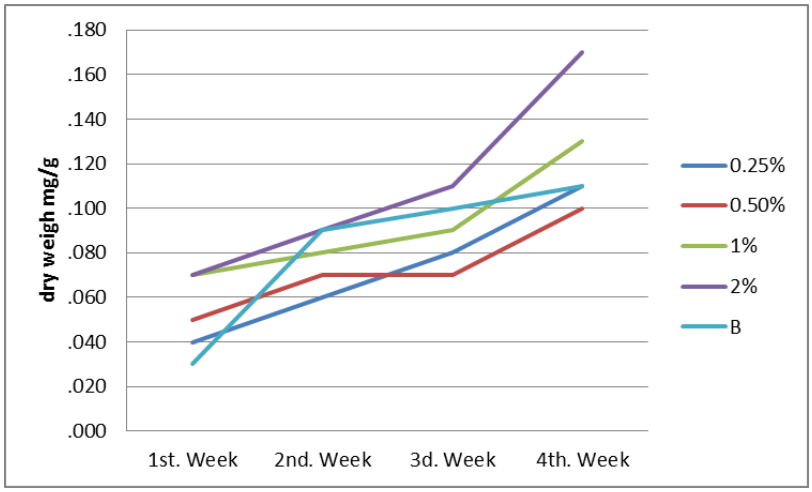

Fig.2: Dry weigh amounts in different crude oil concentration (0.25, 0.5, 1 and 2)% and Blank without carbon

Chlorophyll a

With carbon

Chlorophyll a content in different crude oil treatments is shown in (Table 1).There was an increase in this content to increase the period for each crude oil treatment , especially in 0.25% crude oil concentration which appears the high concentrations in all weeks , the highest concentration was in the 4th week about 16.274 µg/g. There are significant differences between blank culture and adding crude oil

concentrations, inhibition of chlorophyll a biosynthesis was not occurred [24].

Table 1: Chlorophyll (a) µg/g content in different crude oil concentrations in *O.tennuis* grown in the presence of carbon

Conc.	1st. Week	2nd. Week	3d. Week	4th. Week	Mean	SD
B	3.499	5.540	7.984	19.604	9.157	7.202
0.25%	3.693	4.155	8.310	16.274	8.108	5.826
0.5%	3.231	3.273	5.540	15.855	6.975	6.018
1%	1.694	2.216	4.924	15.513	6.087	6.442
2%	.395	2.014	4.155	14.399	5.241	6.297
mean	2.502	3.440	6.183	16.329	7.113	
SD	1.417	1.456	1.863	1.959	5.842	
LSD(concentration)(P<0.05)				1.734		
LSD (period) (P<0.05)				2.743		

Without carbon

In the absence of carbon, we notice a decrease in chlorophyll a content rather than in the presence of carbon (Table 2).The highest content was in 0.25% crude oil treatment in all weeks. Chlorophyll a content is decreased to increase the added crude oil concentration, at the end of the period of the experiment , we find the highest content in 0.25 % crude oil about 16.206 µg/g and less content was in 2% crude oil about 13.389% µg/g at the same time. The amount of accessory photosynthetic pigment depended on crude oil concentrations [25].

Table 2: Chlorophyll (a) µg/g content in different crude oil concentrations in *O.tennuis* grown in the absence of carbon

Conc.	1st. Week	2nd. Week	3d. Week	4th. Week	Mean	SD
B	0.307	1.187	5.540	12.146	4.795	5.408
0.25%	1.917	3.139	4.986	16.206	6.560	6.552
0.5%	0.923	3.047	4.617	15.023	5.903	6.266
1%	0.461	2.770	4.001	14.312	5.386	6.129
2%	0.415	0.325	2.770	13.389	4.225	6.213
mean	0.805	2.094	4.383	14.215	5.374	
SD	0.665	1.266	1.061	1.548	5.507	
LSD(concentration)(P<0.05)				1.677		
LSD (period) (P<0.05)				1.289		

Total hydrocarbons

With carbon

Table 3 elucidate total hydrocarbon concentrations in *O.tennuis* in the presence of carbon which we observed high concentrations of total hydrocarbon compounds, which increased with increasing add the concentration in the first week. The lowest value in concentrate 0.25% crude oil about 632.972 µg/g, while the highest value in the concentrate 2% crude oil about 1124.728 µg/g, Cerniglia *et al.* [26] and Raghu Kumar *et al* [10] found that filamentous cyanobacteria remove efficiently alkanes and polycyclic aromatic hydrocarbons (PAHs).

Table 3: Total hydrocarbons µg/g content in different crude oil concentrations in *O.tennuis* grown in the presence of carbon

Conc.	1st. Week	2nd. Week	3d. Week	4th. Week	Mean	SD
0.25%	632.972	427.143	245.907	406.080	428.026	158.800
0.5%	906.782	716.005	242.924	131.211	499.231	371.583
1%	1033.475	347.935	197.665	321.934	475.252	377.882
2%	1124.728	282.429	183.377	389.802	495.084	428.143
mean	924.489	443.378	217.468	312.257	474.398	
SD	213.908	191.140	31.681	126.078	314.214	
LSD(concentration)(P<0.05)				NS		
LSD (period))(P<0.05)				481.111		

NS (No significant)

Total hydrocarbon concentrations in algae decreased with increasing the period until the third week of experience for all add crude oil concentrations, some algae like *Prototheca zopfi* was capable of utilising crude oil and mixed hydrocarbons substrate and exhibited extensive degradation of n- alkanes and iso-alkanes as well as aromatic hydrocarbons [27]. However, it rebound again in the fourth week, a period in which the algae reach to a stationary phase and I think that at this phase algae starts producing hydrocarbon compounds. Cells in the early stationary phase could produce more hydrocarbon compounds [28]. As confirmed Kojima and Zhang [29] that the maximum hydrocarbons productivity in algae during exponential and stationary phases of growth.

Without carbon

Total hydrocarbons in the absence of carbon illustrated in the table (4) which shows the ability of the alga to accumulate hydrocarbon compounds which decrease as an increase in the period where there are significant differences among weeks (P<0.05).In the 0.25% crude oil treatment, the alga accumulates at 1st week about 533.136 µg/g and these value decrease until 187.951 µg/g at 4th week; this may be related to that some cyanobacteria which have the ability to fix nitrogen could be contributing to degrade oil hydrocarbons [23]. In 0.5, 1 and 2% crude oil treatment, the values begin to decrease until 3rd week then return to rise slightly in the 4th week. This may be due to the ability of the alga on the production of hydrocarbon compounds in this period of the life cycle (stationary phase), the algae production rate of hydrocarbons varies during the growth cycle of the alga *Botryococcus braunii* and the maximum production rate recorded during the early stationary phase [30, 31].

Table 4: Total hydrocarbons µg/g content in different crude oil concentrations in *O.tennuis* grown in the absence of carbon

Conc.	1st. Week	2nd. Week	3d. Week	4th. Week	Mean	SD
0.25%	533.136	332.655	220.134	187.951	318.469	155.979
0.5%	671.840	247.665	202.392	379.432	375.332	211.456
1%	722.430	195.842	188.617	476.421	395.828	255.666
2%	587.647	497.260	200.771	313.872	399.888	174.911
mean	628.763	318.356	202.979	339.419	372.379	Total
SD	84.586	131.932	12.984	121.059	184.732	
LSD(concentration)				NS		
LSD (period)				289.344		

NS (No significant)

If we note the hydrocarbon compounds concentrations in the alga in the presence and absence of carbon, we find it to be higher in the presence of carbon than in the absence of carbon and this leads us to believe that the medium containing carbon stimulate alga to produce more hydrocarbon compounds than in the medium does not contain it . This result agreement with Han *et al.* [32] result which says that the adding organic carbon into heterotrophic microalgal cultures can increase both lipid content and microalgal biomass productivity.

CONCLUSION

This study showed that growth rate and chlorophyll A content of cyanobacterium *Oscillatoria tennuis* was the best in the presence of carbon than in the absence of carbon and they were increasing as the period increased in all treatments.

The accumulation of hydrocarbon compounds in the cyanobacterium was the best in the presence of carbon than in the absence of it and the concentration hydrocarbon compounds decrease with increase period untile the third week of the experiment which shows the ability of the algae on degradation of hydrocarbon compounds and these compounds increase in the fourth week which shows the ability of cyanobacterium to produce hydrocarbons compound.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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