



Biological Treatment of Crude Oil by *Synechococcus* sp.

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Abstract

Crude oil is known globally as one of the major causes of environmental pollution. This chemical compound exerts exhausting impacts on cultivable lands and water surfaces, leading to profound damage to agriculture and aquatic life. Over the years, several bioremediation approaches have evolved to manage this pollution problem properly. A phylum of microorganisms known as cyanobacteria offers an efficient alternative to established bioremediation methods. We conducted the present study to investigate the effectiveness of cyanobacteria in eliminating residual total petroleum hydrocarbon (TPH). *Synechococcus* algae treated three concentrations of crude oil (0.6, 1.8, and 3 ppm) over four time periods (on days 3, 6, 9, and 12). The gas chromatography test showed that on days 3, 6, 9, and 12 of treatment, the percentages of petroleum hydrocarbon removal were 34.66, 65.47, 83.98, and 93.12%, respectively, at a concentration of 0.6 ppm of crude oil. We recorded removal rates of 29.44, 57.42, 80.60, and 90.41% for 1.8 ppm of crude oil, and 30.67, 51.52, 76.38, and 90.74% for 3 ppm on days 3, 6, 9, and 12, respectively. Ultimately, we discovered that cyanobacteria (*Synechococcus* sp.) are effective biological pollutant removers, effectively eliminating hydrocarbon compounds from the water.

Keywords: Crude oil, Cyanobacteria, *Synechococcus*, Bioremediation.

1. Introduction

Crude oil has remained the world's primary energy source over the past half century (1). Crude oil serves as the primary component of petroleum, alongside natural gas. Hydrocarbons, which are chemical compounds composed of hydrogen and carbon in a 2:1 ratio with varying molecular mass values, form the overall backbone of crude oil, along with various amounts of nitrogen, oxygen, sulfur, phosphorus, and heavy metals (2, 3). Accidental or anthropogenic exposure to hydrocarbons in aquatic or soil environments is one of their most serious pollution sources (4). The cross-oceanic annual transport of approximately 35 million barrels of oil is one



example of the vulnerability of aquatic environments to pollution due to oil spills, leading to serious threats to forms of life in these and other types of water bodies (5). Since food webs and chains bio magnify toxic compounds and elements, large-scale oil spills cause severe damage to biological systems (6, 7). Researchers have developed mechanical and physicochemical solutions to oil contamination issues, which involve burying, evaporating, dispersing, or washing out contaminants. Nevertheless, such solutions have the disadvantages of high costs and insufficient elimination of contaminants. Bioremediation is the technology of the biological breakdown of pollutants using microorganisms. Bioremediation of ecosystems that are finely engineered and done in situ is thought to be the best way to treat hydrocarbons because it releases non-toxic materials. It is also less costly and more environmentally friendly than classical detoxification systems (8). Over time, researchers have studied and utilized various fungal and bacterial species in this context. Still, microalgae and cyanobacteria are the best because they are very flexible and can grow in a number of different ways, including autotrophic, heterotrophic, and mixotrophic (9, 10). Cyanobacteria are Gram-negative bacteria capable of performing oxygenic photosynthesis (11). Following the Gulf War in Kuwait, researchers noticed a close association between a massive crude oil spill and a cyanobacteria bloom. The intensive growth of this bloom was considered an early marker of a self-cleaning mechanism along the vast area of oil pollution. Such an observation provided supportive evidence for the ability of cyanobacteria to bioremediate chemical compounds in large crude oil spills (12). By using different types of cyanobacteria (13-15), more research showed how crude oil and other complex organic compounds (like surfactants) can be broken down. Among these, *Lyngbya*, *Oscillatoriasalina*, *Plectonematerrebrans*, *Aphanocapsa* sp., and *Synechococcus* sp. have demonstrated their ability to develop mats in aquatic environments, which has led to their successful utilization in the degradation of oil spills in various regions globally (16, 17). Naturally occurring associations of cyanobacteria with other bacteria can achieve remediation of oil-polluted water bodies and soils (18). Therefore, we conducted this study to explore the potential of the cyanobacterium *Synechococcus* sp. variables in the biodegradation of crude oil at various concentrations of petroleum hydrocarbons, and to assess the impact of oil on their growth.

2. Materials and Methods

2.1. Algae sampling and growth rate evaluation

Cultures of *Synechococcus* sp were identified and collected from the Advanced Environmental Laboratory at the College of Science for Women, University of Baghdad, Baghdad, Iraq. BG-11 culture medium, the constituents of which were described elsewhere (19), was the specific growth culture utilized. The growth of the isolate was achieved at $25 \pm 2^\circ\text{C}$ and in the cooled incubator at a constant illumination intensity of $250\text{--}268 \mu\text{E m}^{-2}$ with a 16/ 8 light-dark cycle (20). The impact of pH on growth was examined at a pH of 6.5-8 (21). The cyanobacterial suspension was prepared, and spectrophotometric measurement of the optical density of the cyanobacterium suspension was achieved at an absorbance of 750 nm, where the BG11 culture medium served as a blank.

2.2. Cyanobacteria cultivation with crude oil

Iraqi medium crude oil was collected from the Al-Dora Refinery in Baghdad for the experiment. **Table 1** shows crude oil's physical-chemical properties. To determine the ability of

Synechococcus sp to remove hydrocarbons from the aquatic environment, three different concentrations of crude oil were chosen (0.6, 1.8, and 3.0 ppm) (22). Suitable amounts of crude oil were mixed in 250 mL Erlenmeyer flasks with 100 mL BG11 medium. The cyanobacterial culture was inoculated into three different flasks with the respective concentrations of crude oil, followed by incubation ($25 \pm 2^\circ\text{C}$) with shaking at 150 rpm. After adding *Synechococcus* sp. separately, the analysis was conducted gradually (at 3, 6, 9, and 12 days) to determine the concentrations of the TPH compound.

Table 1. Physical-chemical characteristics of crude oil used in the experiment (23).

Properties and component	Value
The density of crude oil	0.84
Specific Gravity at 60/60 F	0.886
API	31.4
Water content, Vol%	0.025
Water & Sediment, vol %	Trace
Salt content, lb/1000bbl	57.3
Asphaltene content, wt %	2.1
Sulphur content, wt %	2.9
H ₂ S Dissolved, ppm	14.1
Wax content, wt%	3.8
Carbon Residue, wt%	6.2
Pour point, c	Below -25
The heat of combustion, cal/g	10800
RVP@100F, psi	9.4
Kinematic Viscosity. Cst	
@100F	8.588
@140F	5.548
Flashpoint c	Flammable
Vanadium ppm	30.75
Nickel ppm	6.5

2.3. Analysis of Petroleum Hydrocarbons

The analysis was carried out in the Ministry of Science and Technology laboratories to detect and identify total petroleum hydrocarbon (TPH) compounds using gas chromatography (GC, Shimadzu 2010, Japan). A volume of 1 μL was injected with a known concentration of a mixture of standard compounds to determine each standard compound's retention time and area, as shown in **Figure 1**. A separating flask (1000 ml) was used to mix 500 ml of the samples with 50 ml of Dichloromethan (DCM), followed by shaking and regular pressure release. After letting the sample stand for several minutes, two layers were recognized. The lower layer, i.e., the extract, was filtered with filter paper and kept in a baker. The filtrate was evaporated at room temperature to achieve a concentration of 1 ml(24), utilizing the gas chromatography protocols. The temperature values of the injector and detector Flame Ionization Detector (FID), respectively, were 280 and 330 $^\circ\text{C}$, while the column (KB-5) oven program temperature was 100–300 $^\circ\text{C}$ (10 $^\circ\text{C}/\text{min}$). The carrier gas was N₂ at 120 Kpa.

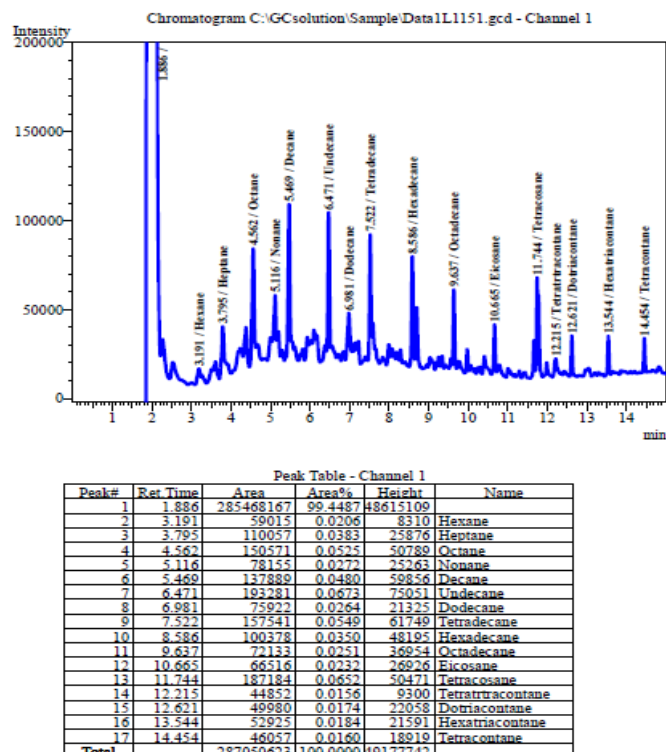


Figure 1. Chromatographic chart of standard TPH by GC

3. Results

After determining the retention time and area for each standard compound, we diluted 1L of crude oil to determine the concentration of each petroleum compound, as illustrated in **Figure 1**. **Table 2** and **Figures 2, 3** and **4** illustrate the concentrations of hydrocarbon compounds prepared after low dilution, along with their retention time values, in the crude oil prior to the addition of algae and before the biological treatment process.

Table 2. Concentrations of petroleum hydrocarbon compounds detected in each of the three crude oil concentrations before adding algae.

No	Name	Rt	Crude oil concentrations (ppm)		
			0.6	1.8	3
1	Hexane	3.1	86.5	182.6	279.8
2	Heptane	3.7	90.8	192.5	416.8
3	Octane	4.5	105.8	215.9	396.5
4	Nonane	5.1	99.8	114.5	208.9
5	Decane	5.4	70.8	159.8	366.9
6	Undecane	6.4	80.9	167.4	365.8
7	Dodecane	6.9	136.5	274.5	625.4
8	Tetradecane	7.4	90.8	190.6	386.5
9	Hexadecane	8.4	88.9	186.5	360.2
10	Octadecane	9.6	105.8	219.8	405.8
11	Eicosane	10.6	114.5	235.9	425.9
12	Tetracosane	11.7	125.9	260.5	601.5
13	Tetraetracontane	12.2	108.9	215.8	436.5
14	Dotriacontane	12.6	658.5	1356.5	2546.2
15	Hexatriacontane	13.5	854.6	1895.6	3569.5
16	Tetracontane	14.3	896.8	2145.6	3652.0

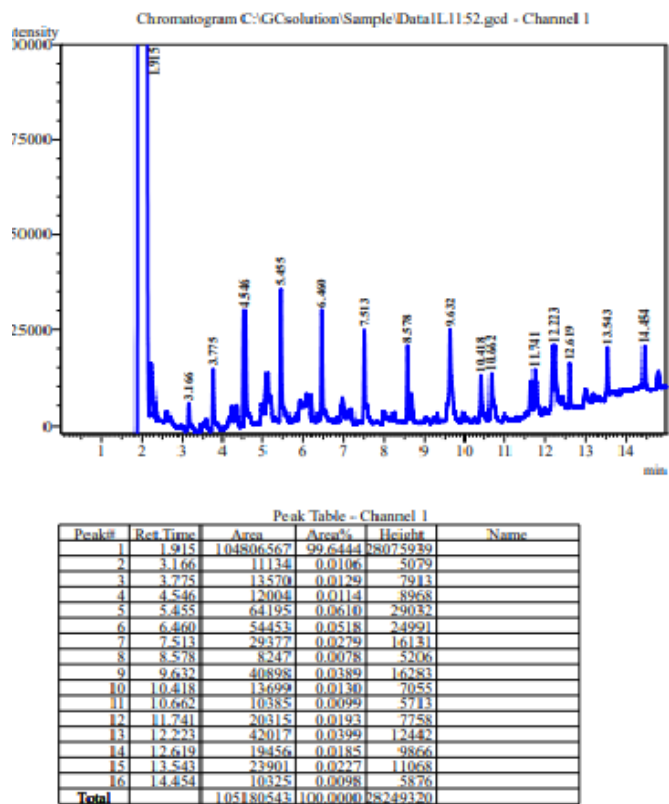


Figure 2. Chromatographic chart of control 0.6 ppm of crude oil before adding algae by GC.

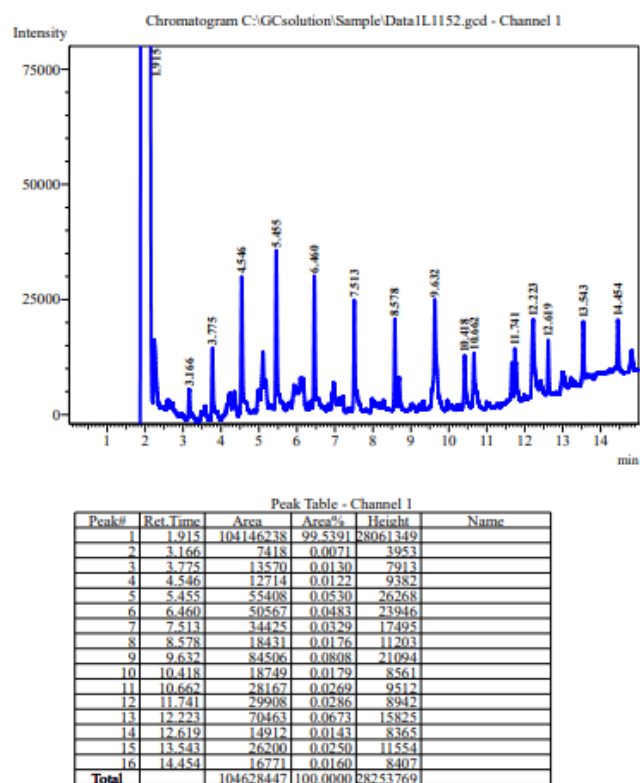


Figure 3. Chromatographic chart of control 1.8 ppm of crude oil before adding algae by GC.

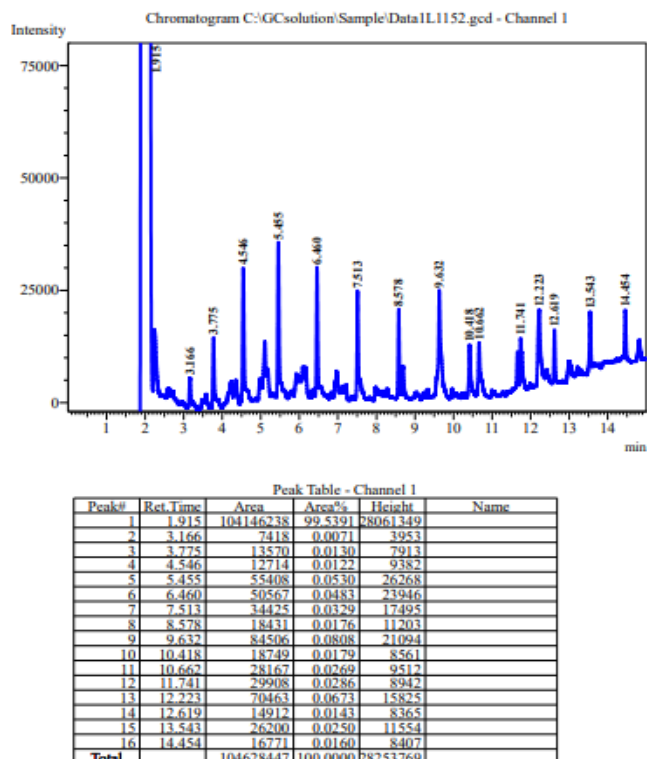


Figure 4. Chromatographic chart of control 3 ppm of crude oil before adding algae by GC.

The results in **Table 3** show the lowest, highest, and average concentrations of hydrocarbon compounds found during the analysis. The average concentrations of total hydrocarbons were 1858.910, 4251.395, and 8188.275 in the concentrations of 0.6, 1.8, and 3 ppm of crude oil, respectively.

Table 3. Statistical description of TPH concentration in three different concentrations of crude oil (0.6, 1.8 and 3 ppm).

Statistical description	TPH Con. Ppm		
	0.6	1.8	3
Mean	1858.910	4251.395	8188.275
Std. Deviation	1294.808	2744.444	4947.670
Minimum	255.400	768.450	1392.450
Maximum	3715.800	8014.000	15044.200

The concentrations of TPH compound residues each day. Before and after treatment, we determined the cell density of the algal culture. The results demonstrated a decrease in the concentrations of TPH residues across the three prepared concentrations, as depicted in **Figures 5 and 6**, over the course of the experimental days. The tested cyanobacteria used these hydrocarbons as their sole carbon source, leading to this decrease, as evidenced by the algal culture's increased cell density after incubation.

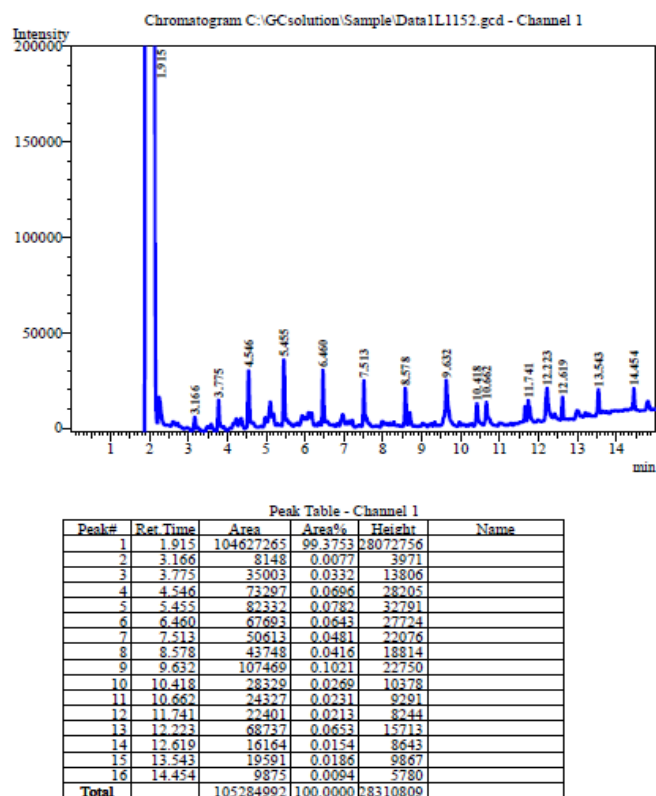


Figure 5. Separation patterns of hydrocarbons using gas chromatography throughout the experimental days.

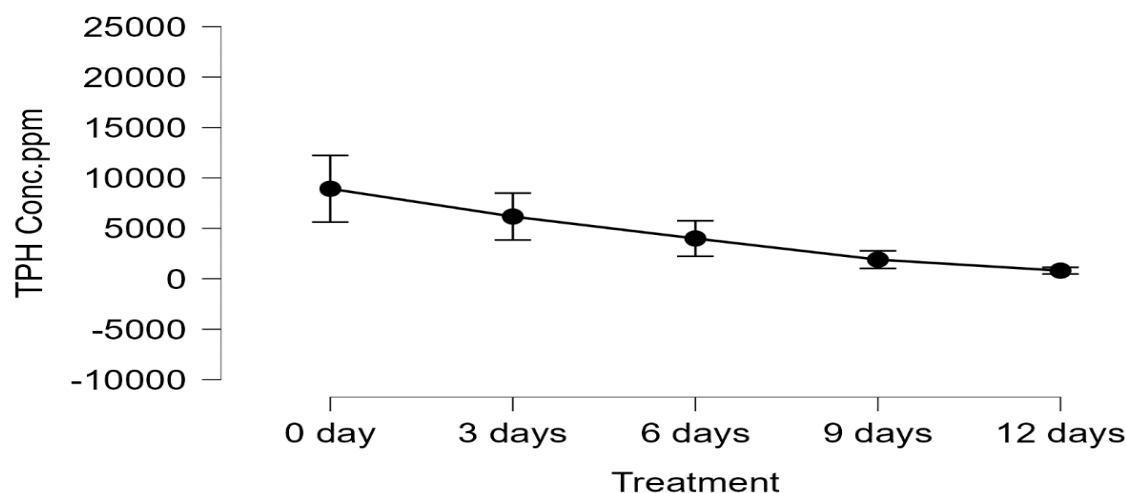


Figure 6. The effects of *Synechococcus* sp. alga on the removal of TPH.

3.1. The removal efficiency of TPH in the concentration of 0.6 ppm of crude oil

Table 4 shows the initial and resulting concentrations of hydrocarbon compounds in the concentration of 0.6 ppm of crude oil and the percentage of their removal by the algae *Synechococcus* sp. over different incubation times. It was found that some compounds, such as Dotriacontane and Hexatriacontane, had lower removal rates (87.79% and 89.44%, respectively). Other compounds such as Decane, Tetracontane, Undecane, Dodecane, Nonane, Hexane, Hexadecane, Heptanen, Tetradecane, Octane, Octadecane, Tetrattracontane, Eicosane had higher removal rates (91.17%, 93.22%, 93.69%, 94.57%, 94.73%, 99.42%, 99.43%, 99.44%, 99.44%, 99.52%, 99.52%, 99.54%, and 99.56%, respectively). In comparison, the highest

removal rate was exerted against Tetracosane (99.60%). The results show that the total removal rate of the compounds on day 3 was 34.66 %, on day 6 was 65.37 %, and on day 9 was 83.88 %, while the highest removal rate was 93% on day 12 of treatment.

Table 4. Removal rates of petroleum hydrocarbon compounds of the crude oil concentration of 0.6 ppm using *Synechococcus* sp. as detected through GC analysis.

No	Name	Initial con	3day	6day	9day	12day	*Total removal
1	Hexane	86.5	32.5 (62.42%)	UDL (99.42%)	UDL	UDL	(99.42%)
2	Heptane	90.8	50.6 (44.27%)	UDL (99.44%)	UDL	UDL	(99.44%)
3	Octane	105.8	62.8 (40.64%)	18.2 (82.79%)	UDL (99.52%)	UDL	(99.52%)
4	Nonane	99.8	55.8 (44.08%)	20.5 (79.45%)	12.5 (87.47%)	5.25 (94.73%)	(94.73%)
5	Decane	70.8	32.5 (54.09%)	16.5 (76.69%)	10.2 (85.59%)	6.25 (91.17%)	(91.17%)
6	Undecane	80.9	39.8 (50.80%)	20.5 (74.66%)	12.6 (84.42%)	5.1 (93.69%)	(93.69%)
7	Dodecane	136.5	90.8 (33.47%)	33.6 (75.38%)	13.6 (90.03%)	7.4 (94.57%)	(94.57%)
8	Tetradecane	90.8	52.9 (41.74%)	22.5 (75.22%)	10.8 (88.10%)	UDL (99.44%)	(99.44%)
9	Hexadecane	88.9	33.9 (61.86%)	UDL (99.43%)	UDL	UDL	(99.43%)
10	Octadecane	105.8	62.5 (40.92%)	UDL (99.52%)	UDL	UDL	(99.52%)
11	Eicosane	114.5	71.5 (37.55%)	UDL (99.56%)	UDL	UDL	99.56%
12	Tetracosane	125.9	86.5 (31.29%)	UDL (99.60%)	UDL	UDL	(99.60%)
13	Tetratetracontane	108.9	44.5 (59.13%)	UDL (99.54%)	UDL	UDL	(99.54%)
14	Dotriacontane	658.5	452.6 (31.26%)	214.5 (67.42%)	150.2 (77.19%)	80.4 (87.79%)	(87.79%)
15	Hexatriacontane	854.6	635.9 (25.59%)	458.9 (46.30%)	174.5 (79.58%)	90.2 (89.44%)	(89.44%)
16	Tetracontane	896.8	622.5 (30.58%)	477.8 (46.72%)	210.5 (76.52%)	60.8 (93.22%)	(93.22%)
**Total Removal %			34.66 %	65.37 %	83.88 %	93 %	

*. Total Removal %: Removal percentage of each hydrocarbon compound during experimental days.

**, Total Removal %: Removal percentage of all hydrocarbon compounds during experimental days.

3.2. The removal efficiency of TPH in the concentration of 1.8 ppm of crude oil

Table 5 displays the initial and resulting concentrations of hydrocarbon compounds in the crude oil at a concentration of 1.8 ppm, along with the percentage of these compounds removed by the algae *Synechococcus* sp. The results of the removal rate of each hydrocarbon compound on day 12 of treatment showed that some compounds, such as Tetracontane, Dotriacontane, and Hexatriacontane had the lowest values (88.11 %, 88.28 %, and 88.68 %, respectively). Other compounds such as Tetratetracontane, Decane, Nonane, Eicosane, Tetracosane, Octadecane,

Undecane, Tetradecane, Dodecane, Hexane, Hexadecane, and Heptane showed rates of 90.50 %, 90.73 %, 91.04 %, 91.30 %, 91.78 %, 93.08 %, 93.18 %, 93.44 %, 95.04 %, 99.72 %, 99.73 %, and 99.74 %, respectively. The highest removal rate was exerted by Octane (99.76 %). The results also show that the total removal rate of compounds on day 3 was 29.44 %, on day 6 was 57.42 %, and on day 9 was 80.58 %, whereas the highest removal rate was 90.38 % on day 12 of treatment.

Table 5. Removal rates of petroleum hydrocarbon compounds of crude oil with a concentration of 1.8 ppm using *Synechococcus* sp., as detected through GC analysis.

No	Name	Initial con	3day	6day	9day	12day	*Total Removal
1	Hexane	182.6	85.6 (53.12%)	20.5 (88.77%)	UDL (99.72%)	UDL	99.72 %
2	Heptane	192.5	104.9 (45.50%)	55.8 (71.01%)	UDL (99.74%)	UDL	99.74 %
3	Octane	215.9	120.6 (44.14%)	60.8 (71.83%)	UDL (99.76%)	UDL	99.76 %
4	Nonane	114.5	92.5 (19.21%)	44.5 (61.13%)	22.5 (80.34%)	10.25 (91.04%)	91.04 %
5	Decane	159.8	96.5 (39.61%)	39.8 (75.09%)	21.5 (86.54%)	14.8 (90.73%)	90.73 %
6	Undecane	167.4	87.4 (47.78%)	50.4 (69.89%)	29.8 (82.19%)	11.4 (93.18%)	93.18 %
7	Dodecane	274.5	120.6 (56.06%)	74.5 (72.85%)	30.5 (88.88%)	13.6 (95.04%)	95.04 %
8	Tetradecane	190.6	110.5 (42.02%)	60.9 (68.04%)	22.8 (88.03%)	12.5 (93.44%)	93.44 %
9	Hexadecane	186.5	101.5 (45.57%)	55.8 (70.08%)	UDL (99.73%)	UDL	99.73 %
10	Octadecane	219.8	123.6 (43.76%)	96.5 (56.09%)	30.5 (86.12%)	15.2 (93.08%)	93.08 %
11	Eicosane	235.9	152.9 (35.18%)	97.4 (58.71%)	50.6 (78.55%)	20.5 (91.30%)	91.30 %
12	Tetracosane	260.5	196.5 (24.56%)	135.6 (47.94%)	50.9 (80.46%)	21.4 (91.78%)	91.78 %
13	Tetratetracontane	215.8	185.4 (14.08%)	112.5 (47.86%)	60.8 (71.82%)	20.5 (90.50%)	90.50 %
14	Dotriacontane	1356.5	956.2 (29.50%)	680.5 (49.83%)	360.5 (73.42%)	158.9 (88.28%)	88.28 %
15	Hexatriacontane	1895.6	1262.5 (33.39%)	956.8 (49.52%)	368.5 (49.52%)	214.5 (88.68%)	88.68 %
16	Tetracontane	2145.6	1856.9 (13.45%)	869.8 (59.46%)	505.1 (76.45%)	254.9 (88.11%)	88.11 %
17	**Total Removal%		29.44 %	57.42 %	80.58 %	90.38 %	

*. Total Removal %: Removal percentage of each hydrocarbon compound during experimental days.

**. Total Removal %: Removal percentage of all hydrocarbon compounds during experimental days.

3.3. The removal efficiency of TPH in the concentration of 3 ppm of crude oil

Table 6 shows the initial and resulting concentrations of hydrocarbon compounds in the concentration of 3 ppm of crude oil and the percentage of their removal by the algae *Synechococcus* sp. After 12 days of the experiment, it was found that Tetratetracontane had the lowest value (79.35%). Other compounds such as Tetracosane, Hexatriacontane, Dotriacontane,

Tetradecane, Octadecane, Nonane had values of 84.95 %, 85.39 %, 85.82 %, 88.58 %, 89.13 %, 89.22 %, respectively, while Hexadecane, Eicosane, Tetratetracontane, Dodecane, Decane, Tetracontane, Undecane, Hexane, and Octane had values of 91.53 %, 91.57 %, 93.36 %, 94.38 %, 94.58, 98.06 %, %, 99.82 %, 99.87 %, respectively. The highest removal rate was recorded at Heptane (99.88 %). The table shows that the total removal rate of compounds on day 3 was 30.67 %, on day 6 was 51.52 %, and on day 9 was 76.37 %, whereas the highest removal rate was 90.73 % on day 12 of incubation.

Table 6. Total removal rates of petroleum hydrocarbon compounds of crude oil of concentration of 3 ppm using *Synechococcus* sp., as detected through GC analysis.

NO	Name	Initial con	3 days	6 days	9 days	12 days	*Total Removal
1	Hexane	279.8	155.9 (44.28%)	96.8 (65.40%)	UDL (99.82%)	UDL	99.82 %
2	Heptane	416.8	259.8 (37.66%)	174.5 (58.13%)	UDL (99.88%)	UDL	99.88 %
3	Octane	396.5	284.5 (28.24%)	168.9 (57.40%)	UDL (99.87%)	UDL	99.87 %
4	Nonane	208.9	164.8 (21.11%)	96.8 (53.66%)	48.9 (76.59%)	22.5 (89.22%)	89.22 %
5	Decane	366.9	145.8 (60.26%)	99.8 (72.79%)	44.5 (87.87%)	20.6 (94.38%)	94.38 %
6	Undecane	365.8	235.9 (35.51%)	130.8 (64.24%)	40.5 (88.92%)	19.8 (94.58%)	94.58 %
7	Dodecane	625.4	425.8 (31.91%)	208.9 (66.59%)	90.8 (85.48%)	41.5 (93.36%)	93.36 %
8	Tetradecane	386.5	152.6 (60.51%)	105.9 (72.60%)	81.4 (78.93%)	44.1 (88.58%)	88.58 %
9	Hexadecane	360.2	195.8 (45.64%)	126.5 (64.88%)	50.2 (86.06%)	30.5 (91.53%)	91.53 %
10	Octadecane	405.8	245.9 (39.40%)	186.8 (53.96%)	69.8 (82.79%)	44.1 (89.13%)	89.13 %
11	Eicosane	425.9	269.8 (36.65%)	166.2 (60.97%)	77.8 (81.73%)	35.9 (91.57%)	91.57 %
12	Tetracosane	601.5	358.9 (40.33%)	324.8 (46.001%)	153.6 (74.46%)	90.5 (84.95%)	84.95 %
13	Tetratetracontane	436.5	320.5 (26.57%)	198.7 (54.47%)	144.5 (66.89%)	90.1 (79.35%)	79.35 %
14	Dotriacontane	2546.2	1985.6 (22.01%)	1365.8 (46.35%)	895.5 (64.82%)	360.8 (85.82%)	85.82 %
15	Hexatriacontane	3569.5	2658.9 (25.51%)	1856.5 (47.98%)	952.2 (73.32%)	521.4 (85.39%)	85.39 %
16	Tetracontane	3652.0	2568.6 (29.66%)	1985.2 (45.64%)	902.8 (75.27%)	70.65 (98.06%)	98.06 %
17	**Total Removal%		30.67 %	51.52 %	76.37 %	90.73 %	

*. Total Removal %: Removal percentage of each hydrocarbon compound during experimental days.

**. Total Removal %: Removal percentage of all hydrocarbon compounds during experimental days.

4. Discussion

Bioremediation using cyanobacteria is characterized by sustainability and being friendly to the environment, which is widely favorable in the middle of the global trend toward cleaner energy sources. The utilization of fungal and bacterial species in the biological treatment of

hydrocarbons has been an attractive research topic for several decades. But better benefits, like being able to treat a wider range of hydrocarbons and releasing fewer greenhouse gases, have made cyanobacteria, in which blue-green algae power the remediation system, more popular. Cyanobacteria are better at collecting oil and making it available to the environment more quickly than other bacteria, and they don't need as much food to grow (25, 26). In the present study, we utilized a local isolate of *Synechococcus* sp., which showed a good ability for petroleum hydrocarbon degradation, which could be due to the activities of certain enzymes produced by these microorganisms (27, 28). The results revealed that *Synechococcus* sp. provided 99% of total petroleum hydrocarbon degradation within twelve days, indicating its potential advantages as a bioremediating agent. Although not fully explored and exploited, this approach of bioremediation has the potential to go in harmony with existing green energy practices, particularly in the context of growing global concerns related to climate change and the continuous search for green energy options (29).

5. Conclusion

This investigation revealed that the utilized cyanobacterial species could be an excellent bioremediation agent that can be utilized to clean environments contaminated with crude oil. High rates of degradation of various hydrocarbons were recorded. The availability, sustainability, and environmentally-friendly features of this treatment source, combined with the efficient enzymatic mechanism of hydrocarbon degradation, make it an excellent tool for achieving global goals of sustainable development.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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