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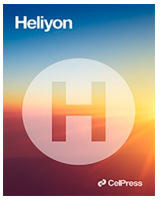

Research article

Simultaneous bioremediation of petroleum hydrocarbons and production of biofuels by the micro-green alga, cyanobacteria, and its consortium

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ABSTRACT

There are two major problems in the world, fuel deficiency and environmental pollution by fossil fuels. Microalgae are regarded as one of the most feasible feedstocks for the manufacturing of biofuels and are used in the degradation of fossil fuel spills. The present study was possessed to investigate the ability of green alga *Chlorella vulgaris*, blue-green alga *Synechococcus* sp, and its consortium to grow and degrade hydrocarbon such as kerosene (k) with different concentrations (0, 0.5, 1, and 1,5%), and also using algal biomasses to produce biofuel. The algal growth was estimated by optical density (O.D) at 600 nm, pigment contents such as Chlorophyll *a,b* carotenoid, and dry weight. The kerosene degradation was estimated by FT-IR analysis after and before the cultivation of algae and its consortium. The components of the methanol extract were determined by GC-MS spectroscopy. The results denote the best growth was determined by O.D, algae consortium with 1.5% Kerosene after ten days, meanwhile, the highest dry weight was with *C. vulgaris* after ten days of cultivation. The FT-IR demonstrated the algae and consortium possessed high efficacy to degrade kerosene. After 15 days of algae cultivation with 1% K, C. vulgaris produced the maximum amount of lipids (32%). The GC-MS profile of methanol extract of two algae and consortium demonstrated that Undecane was presented in high amounts, *C. vulgaris* (19.9%), *Synechococcus* **sp** (82.16%), algae consortium (79.51%), and also were presented moderate amounts of fatty acid methyl ester in *Synechococcus* sp. Overall, our results indicate that a consortium of algae can absorb and remove kerosene from water, and at the same time produce biofuels like biodiesel and petroleum-based fuels.

1. Introduction

One of the most important environmental problems is the contamination of water and soil by hydrocarbons derived from petroleum [1]. Large amounts of wastewater called produced water (PW) are created during the extraction of oil and gas. Produced water was discovered to have excessive lingering petroleum hydrocarbons, which significantly harmed the ecology[2]. Wastewater from the oil industry contains stubborn contaminants such as sulfur compounds, dissolved solids, and highly concentrated hydrocarbons that may constitute harm to the environment[3]. Insufficient water resources have made it harder to reduce water contamination and improve

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water quality[4]. Diesel and kerosene fuels are the maximum widely spread organic environmental pollutants[5].

All fossil fuels and minerals are depleting and nonrenewable. As a result, these resources are scarce both physically and, to a greater extent, commercially[6]. Every day, enormous amounts of petroleum products like diesel and kerosene are used as fuel to run vehicles, power industries, and heat houses[7]. Petroleum hydrocarbons, which are still a major source of energy utilized around the world, constitute a significant global environmental pollution[8]. The predicted scarcity of oil and phosphorus in the foreseeable future has induced several countries with global economies to search for fossil fuel alternatives and more efficient reservation and exploration of resources[9,10].

To clarify, biofuel production using microorganisms is expected to be solving various issues introduced by fossil fuels. Microbial Fuel Cells (MFCs), can generate renewable energy, and also remediate petroleum refinery wastewater[11,12]. Liquid fuels produced from biomass are known as biofuels, and they can be produced from fermented sugars (bioethanol) or oils (biodiesel)[13]. However, the production of biofuel can result in a conflict between food and fuel because it has a low energy content and is not compatible with the current fuel infrastructure [14]. According to Ref. [15]; Cyanobacterium appears to have few additional advantages over other microbes including fungi, yeast, and mosses because of their bigger mucilage volume with greater binding affinity, high surface area, and simple food requirements. Microalgae are highly preferred in biomass production due to their high natural accumulation of strains which reaches up to 50% of dry weight in lipids[16]. They are capable of generating various types of biofuels such as bioethanol, biodiesel, bio-oil, biomethane, bio-hydrogen, and others. Utilizing microalgae for biodiesel production has various advantages. In general, they require minimum care to grow and use contaminated water that has nutrients. They reproduce by using photosynthesis to transform solar energy into chemical energy, completing a growth cycle every few days[17]. [18]; reported microalgae may be the most promising biofuel feedstock, and it is being widely used to produce a sizeable amount of sustainable biomass that can be used as a viable agent for conversion to biodiesel. The substantial factors to consider in biodiesel production are the lipid content and fatty acid composition of every biodiesel feedstock. As a result, the microalgae species that are most convenient for biodiesel production require high lipid productivity and suitable fatty acid (FA) composition[19]. [20] assert that the production of biofuels using microalgae species that belong to the genera *Chlorella, Dunaliella, Scenedesmus, Spirulina,* and *Chlamydomonas* that contain large amounts of starch, may be considered as a valuable material for bioethanol production. An environmentally friendly sector with promising futures is the use of microalgae for the simultaneous production of biomass and wastewater purification[21,22].

Microalgae cultivation in wastewater, and saline water may be considered a suitable scientific approach to treat the imposed threats due to some favorable aspects like multi-functionality, genuine biological conversion competency, flexibility with growth system, wastewater accumulation, CO_2 sequestration, and a significant amount of carbohydrate-lipid-protein content[23].

Additionally, the simultaneous application of wastewater treatment and microalgae cultivation is a valuable method for biofuel production and pollution control[24,25]. Some types of microalgae can produce enzymes that break down dangerous organic molecules and change petroleum hydrocarbons into less toxic chemicals[26]. Naphthalene, indeno[1,2,3-*c*,*d*]pyrene, benzo(*a*)pyrene (BaP), anthracene, phenanthrene, and other PAHs have all been found to be removed by several microalgae strains [27]. [28]; noticed very slow growth of *Chlorella vulgaris* and *C. variegata* at 5% of kerosene and no growth at 10%, and death within 15 and 10 days at 10 and 20% of kerosene. *Chlorella vulgaris, Anabaena variabilis, Neochloris vigensis*, and *Desmodesmus* produced 34.28%, 37.8%, 19.29%, and 50% respectively of lipid content in synthetic wastewater[24].

In terms of biofuel production, *Chlorella* sp. with high biomass productivity and good energy content seems to be an ideal material for biofuel production[21,29,30]. The culture conditions imposed on *Chlorella vulgaris* cells can increase the lipids content two or three times[31]. Because *C. vulgaris* has a high content of lipids, it has been used as an "algae model" by most researchers[32]. Furthermore, *Synechococcus* sp. Biomass was studied for bioethanol production[33].

The objectives of this work were to examine for the first time the potential of microalga *C. vulgaris*, and cyanobacterium *Synechococcus* sp each and in the consortium by volume (1:1) to grow and remove petroleum hydrocarbons such as kerosene under mixotrophic conditions. As well as investigate the ability *C. vulgaris*, and *Synechococcus* each or in combination to degrade kerosene petroleum hydrocarbons. Determine the amount of biomass and lipids content, after 15 days of incubation to possible uses as feedstock in biofuel production.

Table 1
BG-11 medium[34].

Constituent A	**g/L**	**Constituent B**	**g/L**
$NaNo_3$	1.5	H_3BO_3	2.86
K_2HPO_4	0.075	$MNCl_24H_2O$	1.81
$CaCl_2$ $2H_2O$	0.036	$ZnSO_4$ $7H_2O$	0.222
Na_2CO_3	0.02	$CuSO_4$ $5H_2O$	0.079
Constituent B	g/100 mL	Na_2MoO_4 $2H_2O$	0.39
$FeSO_4$	0.65	$Co(NO_3)_2$ $6H_2O$	0.0494
Disodium salt EDTA	0.93	1 mL of constituent B + 1 mL of constituent C add to 1 L of constituent A	

2. Materials and methods

2.1. Materials

The microgreen alga *Chlorella vulgaris* and the cyanobacteria *Synechococcus* sp. were obtained from the Biology Department, Faculty of Sciences and Arts (Khulais), University of Jeddah, Saudi Arabia. Kerosene was obtained from a local Saudi gas station.

2.2. Establishment of mixotrophic conditions

The algae were cultivated in batch culture, 500 mL Erlenmeyer flasks containing 120 mL BG-11 medium (Table 1) with 30 mL algal culture inoculum (v:v) (4:1), and adding Kerosene to give concentrations (0, 0.5, 1, and 1.5%). The cultures were subjected to alternate (12:12 h) light and dark periods at light intensity 2000 lux and 25–30 °C.

2.3. Assessment of algal growth

Algal growth including optical density (OD) at 600 nm was measured using a SHIMADZU UV-2600 spectrophotometer every three days and using the dry weight after 15 days of cultivation by centrifuging the culture broth at 5000 rpm for 10 min, washing twice with distilled water, and then drying the cell pellet at 65 °C till constant weight.

2.4. Pigments estimation

A known amount of culture was centrifuged for 15 min at a speed of 3000 rpm. The algal pellets were then treated with a known quantity of 99.9% methanol, maintained in the water bath for 30 min at 55 °C, and centrifuged once more. By using a SHIMADZU UV-2600 spectrophotometer, the absorbance of the combined extracts was measured at 666, 653, and 470 nm. Calculations for chlorophyll *a*, chlorophyll *b*, and carotenoids were done using the formulas created by Ref. [35].

2.5. Kerosene determination

The ability of algae and its consortium to remove Kerosene is derived by cultured filtration obtained by various treatments, the algae after 15 days of incubation are centrifuged and culture filtrates were separated, lyophilized, and determined by FT-IR analysis. The FTIR analysis was accomplished in the mid-IR region (500–4000 cm-1) (Nicolete IS10, Thermo Fisher Scientific, Waltham, MA, USA).

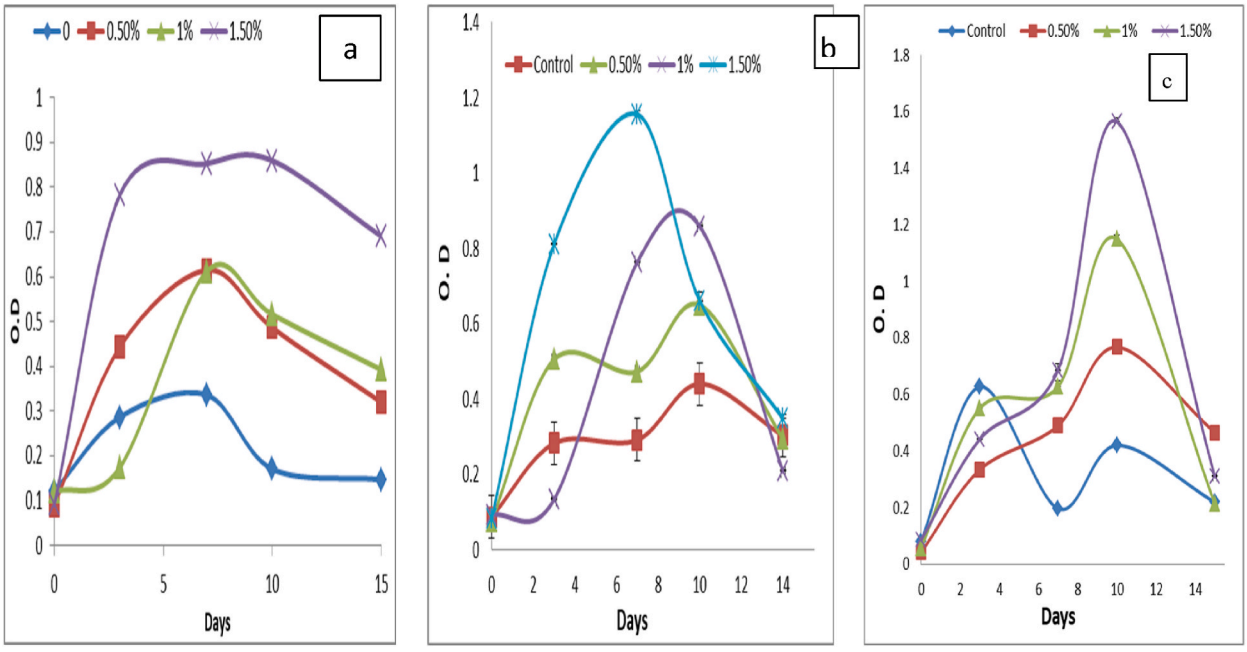

Fig. 1. Effects of different concentrations of Kerosene on *Synechococcus* sp (a), *C. vulgaris* (b), and its consortium(c) growth measured as optical density (600 nm).

2.6. Lipids extraction

Using a magnetic stirrer, 25 mL of methanol was combined with a half gram of dry weight of each type of algae and algae consortium. After the solvent had evaporated, the residue was separated by filtration. Using the following equation, the isolated lipid was investigated in percentage.

Lipid % = (W1/W2)*100.

Where W1, lipids weight, W2 dry weigh.

2.7. GC-MS analysis

The injection of the methanol extract was followed by GC-MS analysis. The sample was injected into a silica capillary column HP-5MS for GC-MS analysis, with helium serving as the carrier gas. After starting the GC-MS temperature program at 60 °C for 2 min, it was heated to 280 °C at an ionizing rate of 8 °C/min. Wiley and Wiley Nist mass spectrum databases were used to assess the various peaks [36,37]; 26. Wilson & Walker, 2008).

2.8. Statistical analysis

Experiments were conducted in triplicate and results were expressed as the standard error of the mean. The analysis and graphs were carried out using Microsoft Excel (MS 2016) and One-way ANOVA, SPSS version 16, and using Duncan's multiple range tests with probability $\leq$0.05.

3. Results and discussion

3.1. Growth assessment

3.1.1. Optical density

Results in Fig. 1 demonstrate the influence of different concentrations of Kerosene (K) (0, 0.5, 1, and 1.5%) in algal growth and algae consortium with days. The results denoted that the highest growth of *Synechococcus* sp was obtained with 1.5% K (Fig. 1a). *C. vulgaris* cultivated with 1.5% Kerosene showed optical density at 1.15 nm at seven days of cultivation. The growth of *C. kessleri* was increased with days using a high concentration of crude oil 1%[38]. Kerosene (1.5%) enhanced the growth of *C.vulgaris* at 7 days, and the optical density was 1.15 nm (Fig. 1b). The maximum growth of the algal consortium was obtained on the tenth day with various concentrations (0, 0.5, 1, and 1.5%), but the best growth with obtained with 1.5% Kerosene at 10 days (Fig. 1c). The best growth of microalgae is when growing in mixotrophic conditions, which caused the promotion of biomass than that to grow under autotrophic conditions[39]. The results demonstrated that through ten days algae and consortium grow to accelerate and after that algae start to the death phase, that due to the metabolism of kerosene and produce toxic intermediate compounds that affect algae growth. The presence of hazardous chemicals caused by biodegradation, which have an impact on algal development, maybe the cause of the microalgae's promotion after ten days of cultivation in crude oil[40].

3.1.2. Dry weight

Results in Fig. 2 demonstrate the impact of kerosene concentrations on the algae growth represented by dry weight after 15 days of cultivation. The results show the best dry weight of algae and consortium with 1% k, and the best dry weight was with *C. vulgaris* followed by algae consortium and *Synechococcus* sp. The results demonstrate the different concentrations of kerosene had significant effects on the dry weight of *Synechococcus* sp. The dry weight of *C. vulgaris* cultivated under different concentrations had a significant impact among treatments, and also the dry weight of consortium under different concentrations had a significant effect. The results

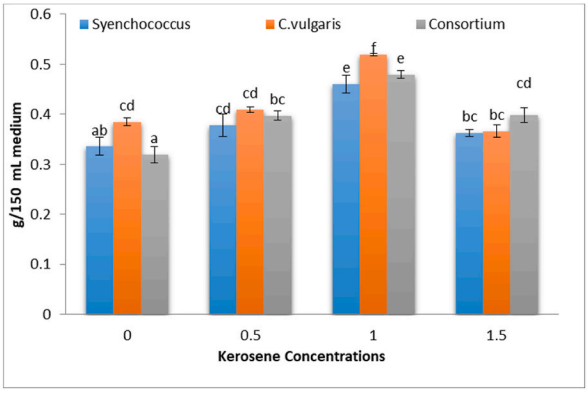

Fig. 2. Effect of different concentrations of Kerosene in dry weight of algae and its consortium.

demonstrated that the dry weight of both the two algae and its consortium grown (0% K) were different from that were grown in mixotrophic conditions (0.5, 1, and 1.5 K) [38]. reported that the dry weight of *Anabeana oryzae* and *Chlorella kessleri* grew autotrophically and were near that that grows under mixotrophic conditions. *Chlorella vulgaris* cultivated under mixotrophic possessed high dry weight than those cultivated under photoautotrophic[41,42].

3.1.3. Pigment determination

The results obtained in Table 2 refer to the influence of different concentrations of Kerosene on Chlorophyll-a and carotenoid (μg mL^{-1}) of *Synechococcus* sp. There are variations in Chl*a* and carotenoid contents in *Synechococcus* sp, the best chlorophyll-a contents (2.78 ± 0.01 μg mL^{-1}), in *Synechococcus* sp., when applied 0.5% Kerosene with 10 days of incubation. The highest concentration of carotenoid was 4.15 ± 0.3 μg mL^{-1} when the alga was treated with 0.5% Kerosene at 10 days of cultivation [43]. reported Chl*a* of *Synechococcus* sp. PCC 7002 was cultivated under mixotrophic by the addition of 3 gm glucose/L, higher than that cultivated under autotrophic conditions. The best chlorophyll contents of *Synechococcus* sp that cultivated with acetate-mixotrophic cultures[44].

Table 3 reports the effects of different concentrations of Kerosene on Chlorophyll-*a*, Chlorophyll-*b*, and carotenoid (μg mL^{-1}) of *C. vulgaris*, the results denote the best Chl*a* contents (2.02 μg mL^{-1}) were obtained with *Chlorella* sp at seven days of cultivation with 1.5% Kerosene. Meanwhile, the best contents of Chl *b* were 0.5% followed by 1.5% Kerosene 5.6 ± 0.3, and 5.31 ± 0.56 μg mL^{-1} at seven days of growth respectively. And also has the best carotenoid contents of *Chlorella* sp at seven days of cultivation with 1.5% Kerosene (6.33 ± 1.22 μg mL^{-1}). *Chlorella protothecoides* mixotrophic cultures produced more biomass than their autotrophic counterparts, although the latter culture accumulated more chlorophyll and carotenoids in its cells. However, the stress management technique improved carotenogenesis, enabling cellular storage of highly cited carotenoids[45]. With the large-scale growth of microalgae to create carotenoids, mixotrophy may be a promising method[46].

The impact of different concentrations of Kerosene on Chlorophyll-*a*, Chlorophyll-*b*, and carotenoid (μg mL^{-1}) of *C. vulgaris* and *Synechococcus* sp consortium is demonstrated in Table 4 . The results investigate the most Chl*a*, Chl*b*, and carotenoid content obtained with 1.5% Kerosene at ten days of cultivation, 19.47, 29, and 23.23 μg mL^{-1} respectively [38]. reported the highest amount of carotenoid content was obtained when the consortium of *C. kessleri* and *A. oryzae* was grown with 1.0% crude oil.

3.2. FT-IR spectroscopy analysis

FT-IR spectroscopy was used to compare the functional groups presented in different concentrations of Kerosene and functional groups presented in culture filtrates of algae and consortium that were grown with various concentrations of Kerosene (0, 0.5,1 and 1.5%). Table 5 Table 5 and Fig. 3 report the different bands found in different concentrations in diluted Kerosene, FT-IR proved that there are five bands in 0.5% Kerosene at Wavenumbers 3446,2375,1638,1091 and 632 cm^{-1} which represent the following active groups O–H, CO_2 asymmetric stretching, C═C double bonds, C–O single bond and [OH] bonding respectively (Fig. 3a). The number of bands present in 1% Kerosene is 7 bands such as 3448, 2372, 1637, 1468,1387, 665, and 620 cm^{-1}, these bands are slightly modified from the 0.5% Kerosene (Fig. 3b). In 1.5% Kerosene, 9 bands are present, the two different bands are 3591 and 3549 cm^{-1}, which represent O–H and H–O •H., the change of bands number and small modification may be due to the increase in mixing between BG11 medium and Kerosene (Fig. 3c).

The results obtained in Supplementary Fig. S1 and Table 6 represent the FTIR spectroscopy analysis of culture media containing different concentrations of Kerosene (0, 0.5, 1, and 1.5) after 15 days of cultivation of *C. vulgaris*, *Synechococcus,* and consortium (Supplementary Fig. S1 *C. vulgaris* (0, 0.5, 1, and 1.5) (S1a,b,c, and d), *Sneycoccocus* (0, 0.5, 1, and 1.5) (S1e,f,g, and h), consortium (0, 0.5, 1, and 1.5), (i,j,k, and m)). The results denote there were many active groups were obtained, the active group ν (NH) and ν (OH) were obtained at 3902 cm^{-1} was found in 1 and 1.5% Kerosene with *Chlorella* sp, and in control of consortium, this may be obtained when *C. vulgaris* cultivated in stress conditions. The active group at 3852 cm^{-1} was present in all treatments except in 1 and 1.5% Kerosene with the consortium. These groups naturally occur with algae metabolism and disappeared with high concentrations of Kerosene 1 and 1.5% with algae consortium. Also, 3838 cm^{-1} was not found in 1.5% k with *C. vulgaris,* 1, and 1.5% k with the consortium. Bands at 3820 and 3801 cm^{-1} represent stretching OH completely disappeared in *Synechococcus.* The peak at 3750 cm^{-1} attributed to vibrations of the free OH-group was found in control (0% K) with a small modification in *Synechococcus* (cyanobacteria). The peak at 3735 cm^{-1} was present in the culture media of algae and all treatments with slight modification after 15 days of cultivation. Band 3711 cm^{-1} assigned H-bonding, was found with 1%k with *C. vulgaris,* and consortium with treatments 0, 1and 1.5% K.

Table 2
Effect of different concentrations of Kerosene on Chlorophyll-a, and carotenoid (μg mL^{-1}) of *Synechococcus* sp.

Conc., %	Control		0.5		1.0		1.5	
Days	*Chl a*	Car	*Chl a*	Car	Chl a	Car	Chl a	Car
3	0.822 ± 0.03f	1.97 ± 0.04j	1.31 ± 0.02h	257.5 ± 1.3a	0.20 ± 0.002b	1.39 ± 0.033d	0.44 ± 0.006cd	1.92 ± 0.07f
7	1.17 ± 0.11f	0.96 ± 0.15c	0.49 ± 0.01d	2.02 ± 0.1h	0.66 ± 0.02e	2.09 ± 2.19h	0.48 ± 0.023d	0.58 ± 0.06a
10	0.449 ± 0.07cd	1.68 ± 0.04e	2.78 ± 0.01 i	4.15 ± 0.3k	0.50 ± 0.006d	1.39 ± 0.58d	0.449 ± 0.079cd	1.96 ± 0.09 **fg**
14	0.331 ± 0.01bc	0.93 ± 0.03c	0.055 ± 0.002a	0.70 ± 0.1a	0.66 ± 0.046e	1.34 ± 1.02d	0.728 ± 0.011ef	1.35 ± 0.06d

Table 3
Effect of different concentrations of Kerosene on Chlorophyll-*a*, Chlorophyll-*b*, and carotenoid (μg mL^{-1}) of *C. vulgaris*.

Con %	Control			0.5			1.0			1.5		
Days	*Chl a*	*Chl b*	Car	Chl a	Chl b	Car	Chl a	Chl b	Car	Chl a	Chl b	Car
3	0.81 ± 0.006cd	0.76 ± 0.023abc	2.10 ± .0.25g	1.2 ± 0.13g	3.4 ± 0.04f	4.06 ± .13k	1.55 ± 0.002f	2.6 ± 0.01d	2.94 ± 0.33i	0.37 ± 0.00b	0.46 ± 0.01a	1.70 ± 0.1f
7	0.80 ± 0.098cd	1.17 ± 0.029bcd	2.13 ± 0.259h	1.52 ± 0.02h	5.6 ± 0.3g	6.91 ± 02.	1.02 ± 0.01de	2.2 ± 0.06ed	3.59 ± 0.3j	2.02 ± 0.11f	5.31 ± 0.56g	6.33 ± 1.22 m
10	1.1 ± 0.018e	1.24 ± 0.001d	1.87 ± 0.33g	1.97 ± 0.04i	6.9 ± 0.01h	4.90 ± 0.15n	0.63 ± 0.04bc	1.2 ± 0.02ed	1.31 ± 0.6d	0.56 ± 0.01bc	0.73 ± 0.01 ab	1.01 ± 0.3c
14	1.13 ± 0.01e	0.66 ± 0.01a	1.53 ± 0.32e	0.20 ± 0.05b	0.33 ± 0.06a	0.64 ± 0.11b	0.47 ± 0.04b	0.79 ± 0.02abc	1.08 ± 0.12c	0.04 ± 0.01	0.43 ± 0.01a	0.21 ± 0.05e

Table 4
Effect of different concentrations of Kerosene on Chlorophyll-*a*, Chlorophyll-*b*, and carotenoid (μg mL^{-1}) of *C. vulgaris* and *Syenchococcus* sp consortium.

Conc., %	Control			0.5			1.0			1.5		
Days	Chl a	Chl b	Car	Chl a	Chl b	Car	Chl a	Chl b	Car	Chl a	Chl b	Car
3	**0.51** ± 0.01b	**0.52** ± 0.00a	**1.76** ± 0.1a	**0.46** ± 0.00b	**0.74** ± 0.01a	**1.89** ± 0.06a	**1.59** ± 0.01e	**2.5** ± 0.02d	**1.65** ± 0.08a	**0.42** ± 0.015b	**0.59** ± 0.22a	**1.82** ± 0.2. a
7	0.44 ± 0.07b	0.55 ± 0.07a	1.68 ± 0.04a	0.16 ± 0.00a	0.51 ± 0.01a	1.28 ± 0.10a	0.95 ± 0.04c	1.3 ± 0.04bc	0.91 ± 0.06a	2.8 ± 0.086f	8.95 ± 0.11f	11.34 ± 0.9b
10	0.48 ± 0.01b	1.72 ± 0.07c	2.21 ± 0.11a	1.13 ± 0.03d	2.38 ± 0.05d	2.19 ± 0.2a	5.2 ± 0.13h	11.9 ± 0.05g	6.99 ± 0.2a	19.47 ± 0.053i	29 ± 0.46h	23.23 ± 22a
14	0.47 ± 0.02b	1.21 ± 0.02b	2.21 ± 0.2a	0.53 ± 0.01b	0.74 ± 0.02a	1.50 ± 0.08a	0.44 ± 0.02b	0.37 ± 0.02a	0.30 ± 0.03a	3.96 ± 0.017g	7.1 ± 0.021e	0.5 ± 0.08a

Band at 3675 cm^{-1} was present only in consortium control. Peak 3648 cm^{-1} was found in control and all treatments except 0.5% k with *C. vulgaris*. Band 3347 cm^{-1} represents OH symmetric and asymmetric stretching are present in control and all treatments with small modification except 1.5% K with *C. vulgaris.* Bands 2131 and 2119 cm^{-1} revealed to CO, approximately present in all treatments and control. The band at 1771 cm^{-1} related to COOH protonated groups was present only with 1% K with the consortium. Band 1733 cm^{-1} is present in all treatments and control of consortium, meanwhile absent with *Syenchococcus* (cyanobacteria). Bands at 1716 revealed C–H bending, which was present only with *C. vulgaris* and algae consortium with 1% K, which may be related to algae metabolism with Kerosene. The band at 1646 cm^{-1} represented OH groups was presented in all treatments and control with some modification. The band at 1640 cm^{-1} was detected with *C. vulgaris.,* control, 0.5% K with *Synechococcus* and 1 and 1.5% K with algae consortium. Bands 1558 cm^{-1} presented in all treatments except 0.5% K with *C. vulgaris and Synechococcus*. Bands at 1540 cm^{-1} were presented in all treatments except with 0.5% K with *Synechococcus* and algae consortium. The band at 1507 cm^{-1} was presented in all treatments and control. Bands at 1489 cm^{-1} revealed to NH, were presented in all control, and all treatments of consortium. The band at 1472 cm^{-1} did not present only with 0.5% k and *Synechococcus.* The band at 1457 cm^{-1} revealed to C–H was present in all treatments except 0.5% K with algae consortium. Bands at 1418 cm^{-1} revealed Lipids, α-methylene CH_2 scissoring band detected in the control and all treatments of algae consortium, and not detected with control and treatments of *Synechococcus.* The results obtained in Table 4 denote the bands obtained in different concentrations of kerosene at 3591, 3549, 3446, 3303, 2375, 2091, 1638, 1468, 1387, 1091, 665, and 632 cm^{-1} that were completely absent in treatments with *C. vulgaris, Synechococcus* and its consortium, that denote to complete degradation of kerosene. *Rhodococcus*, *Bacillus*, and *Aerobacter* species used kerosene as the sole carbon source of energy[47]. Achromobacter, Alcaligenes, and Cupriavidus decomposed 1% kerosene, which showed that aromatic fractions deteriorated more quickly than aliphatic fractions[48]. Through its mixotrophy, *C. vulgaris*. might effectively use petroleum hydrocarbons as a source of carbon [49]. By using the hydrocarbon present in oil field formation water as a source of carbon, *C. vulgaris* BS1 may cultivate[50].

3.3. Lipid content percentage

The effect of Kerosene on the lipids percentage of algae and consortium dry weight obtain in Figure (4). The results demonstrate that *C. vulgaris* was grown in 1% kerosene and had the best lipids content 32%. In all treatments, *C.vulgaris* possessed the highest lipid contents. There were significant effects of different concentrations of kerosene on lipid contents in *Syenchococcus* and algae consortium. *Chlorella sorokiniana* accumulated up to 16.4% lipids when wasted under mixotrophic conditions[63]. High lipid content of *C. vulgaris* (37.6%wt) when grown in a shaker under mixotrophic conditions after 6 days of cultivation[64]. Bioelectro-stimulants evidenced efficient degradation of hydrocarbons in contaminated soils than control operation[65]. Soil microenvironment in

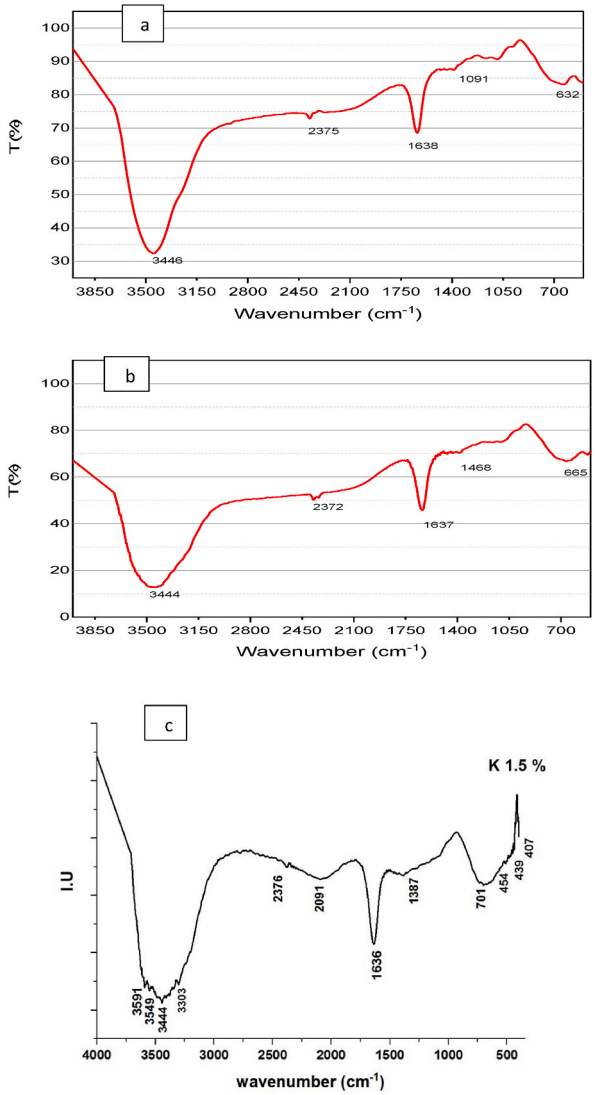

Fig. 3. FT-IR spectroscopy analysis of Kerosene 0.5,(a), 1 (b) and 1.5(c) %.

Table 5
FT-IR spectroscopy analysis of different concentrations of Kerosene.

Wavenumber cm^{-1}	0.5	1	1.5	Active groups	References
3591	ND	ND	D	O–H	[51]
3549	ND	ND	D	H–O •H	[52]
3446	D	+2	−2	Stretching O–H symmetric	[53]
3303	ND	ND	D	free/unbound O–H	[54]
2375	D	−3	+1	CO_2 asymmetric stretching	[55]
2091	ND	ND	D	C–N stretch	[56]
1638	D	−1	−2	C═C double bonds	[57]
1468	ND	D	ND	CH_2 bending	[58]
1387	ND	D	D	C–C	[59]
1091	D	ND	ND	C–O single bond	[60]
665	ND	D	+36	CO_2	[61]
632	D	−12	ND	[OH] bonding	[62]

correlation with the based bioelectrochemical system(BES) forms complex processes, providing suitable conditions for the effective treatment of petroleum refinery wastewater (PRW) [66].

The chemical formula of kerosene is $C_{12}H_{26}$–$C_{15}H_{32}$, and kerosene consists of n−n−alkanes, alkyl benzenes, and naphthalenes. The

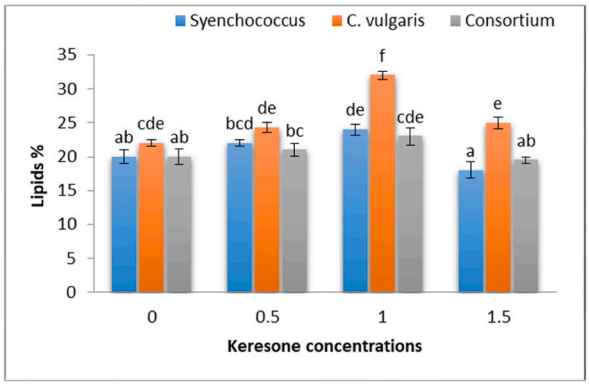

Fig. 4. Effect of Kerosene concentration (0,0.5,1 and 1.5%) on lipids percentage of *C vulgaris*, *Syenchococcus,* and consortium after 15 days of growth.

structure of kerosene is present in Fig. 5.

The results in Table 7 demonstrate the GC-MS analysis of methanol extract *C. vulgaris*, *Syenchococcus* sp, and its consortium developed with 1% kerosene, after 15 days of cultivation. The GC-MS analysis demonstrated there were 5 compounds were found, Cyclotetrasiloxane, octamethyl 51.98%,1-Hexanol, 2-ethyl14.07%, Undecane 19.9%, 2,5-Dihydroxybenzaldehyde10.85%, and Cyclododecane 3.20%. These compounds may be related to absorbed *C. vulgaris* to kerosene. The GC-MS analysis of *Syenchococcus* sp reported there were 9 compounds, Undecane 82.16%, Octanoic acid methyl ester 10.64%, Pentadecanoic acid, 14-methyl-, ester 0.88%, Pentadecanoic acid, 13-methyl-, ester 1.77%, Hexadecanoic acid, methyl ester 0.50%, Cyclotrisiloxane, hexamethyl (1.25%), Perhydro-htx-2-one, 2-pentyl-, acetate ester(1.35%),5-(4-Nitrophenyl)-1,3,4-oxadiazole 2(5H)-one (0.35%), and 2-(1,3-Benzodioxol-5-ylmethyl)-1H- isoindole-1,3(2H)-dione (1.11%). The GC-MS analysis of methanol extract of algae consortium (*C. vulgaris* + *Syenchococcus* sp) possessed 5, compound Cyclotetrasiloxane, octamethyl (15.69%), Undecane (79.51%),5,5″-Diethynyl-2,2′:6′,2″-terpyrid (2.19%),2-(1,3-Benzodioxol-5-ylmethyl)-1H- isoindole-1,3(2H)-dione(1.43%), and 1,1,1,3,5,5,5-Heptamethyltrisiloxa ne (1.17%). Dodecamethylcyclohexasiloxane is one of the most lipophilic was found in *C. vulgaris* biomass when grown in wastewater effluents, that due to microalgae adsorption from wastewater effluents[67]. Decamethyl tetrasiloxane was presented in *C. vulgaris* biomass before and after the degradation of Disp. Orange 2RL[68]). Volatiles compounds such as1-Hexanol are produced by microalga *Phormidium autumnale,* when grown in heterotrophic conditions[69]. The results demonstrated that the presence of a high amount of Undecane in *C.vulgaris* (19.90), *Syenchococcus* sp (82.16), and consortium (79.51%), maybe the absorption of low molecular weight of hydrocarbon (alkene) kerosene contents. The main compounds of microalgae *Tetradesmus obliquus* cultivated for 15 days using biodigester effluent as nutrients were undecane (8.1% w/w) and pentadecane (10.62% w/w) [70]. The fatty acid methyl ester octanoic acid has been discovered in biodiesels produced by transesterifying a mixture of beef tallow, soybean oil, and babassu oil[71]. The main Fatty acid methyl esters found in coconut oil kerosene-like fuel are octanoic acid methyl ester[72]. 2,4-dihydroxybenzaldehyde is a natural phenol compound [73]. The usage of cyclododecane in various reaction-type power plants is extremely appropriate. Because this particular compound has a very high heat of combustion both in terms of weight and volume, it can be used to good effect in turbine and jet propulsion engines, depending on the need for fuels with extremely high energy contents. Additionally, this fuel has a "very high luminometer number," making it even more advantageous for usage in reaction-style power plants[74]. Pandecanoic acid is converted into the methyl ester and identified as a trace element in biodiesels produced through the transesterification of mixtures of soybean oil, babassu oil, and beef tallow[71], there was a higher amount of Cyclotetrasiloxane, octamethyl in methanol extract of *C. vulgaris* that cultivated with 1% kerosene. Siloxanes may have been adsorbed from wastewaters and removed from the biomass samples, where they may have been used as fuel additives, cleaning/washing agents, adhesives, paints, lacquers, and varnishes, fillers, reprographic agents, process regulators, anti-set-off, and anti-adhesive agents, among other things[75]. Microgreen alga *Scenedesmus*

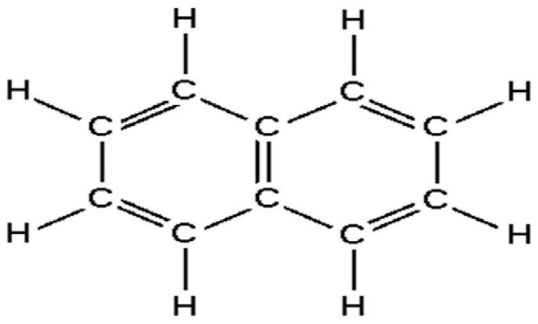

Fig. 5. Structure of kerosene.

Table 6
FT-IR spectroscopy analysis of culture filtrates of algae and its consortium, grown with different concentrations of Kerosene (0,0.5, 1, and 1.5) after 15 days.

Wavenumber cm^{-1}	K%	*C.vulgaris*	*Sneycoccocus*	Consortium	Active groups	References
3902	0	ND**	ND	D[a]	ν (NH) and ν (OH)	[78]
	0.5	ND	ND	ND		
	1	D	ND	ND		
	1.5	D	ND	ND		
3852	0	D	+1	+1	O–H stretching	[79]
	0.5	D	+1	+1		
	1	+1	D	ND		
	1.5	D	D	ND		
3838	0	D	D	D	stretching OH -	[80]
	0.5	D	D	D		
	1	D	D	ND		
	1.5	ND	D	ND		
3820	0	D	ND	D	stretching OH -	[80]
	0.5	ND	ND	D		
	1	D	ND	ND		
	1.5	D	ND	ND		
3801	0	D	ND	D	stretching OH -	[80]
	0.5	ND	ND	D		
	1	ND	ND	D		
	1.5	ND	ND	D		
3750	0	D	−2	D	vibrations of the free OH-group	[81]
	0.5	ND	ND	D		
	1	D	ND	ND		
	1.5	D	ND	D		
3735	0	D	D	D	stretching O single bond H bonds	[82]
	0.5	D	D	−1		
	1	+1	D	−5		
	1.5	D	D	D		
3711	0	ND	ND	−1	H-bonding	[83]
	0.5	ND	ND	−1		
	1	D	ND	ND		
	1.5	ND	ND	−1		
3675	0	ND	ND	D	nitrile H-bonded	[84]
	0.5	ND	ND	ND		
	1	ND	ND	ND		
	1.5	ND	ND	ND		
3648	0	D	D	D	O–H stretching	[85]
	0.5	ND	D	D		
	1	D	D	D		
	1.5	D	D	D		
3347	0	D	−1	+7	OH symmetric and asymmetric stretching	[86]
	0.5	−1	−6	+15		
	1	+8	−6	D		
	1.5	ND	+7	D		
2131	0	D	ND	ND	CO	[87]
	0.5	ND	ND	+4		
	1	+7	−1	ND		
	1.5	D	ND	D		
2119	0	ND	+1	D	CO	[88]
	0.5	D	ND	ND		
	1	ND	ND	D		
	1.5	ND	D	ND		
1771	0	ND	ND	ND	COOH protonated groups	[89]
	0.5	ND	ND	ND		
	1	ND	ND	D		
	1.5	ND	ND	ND		
1733	0	D	ND	D	C═O stretching	[90]
	0.5	ND	ND	D		
	1	D	ND	D		
	1.5	ND	ND	D		
1716	0	ND	ND	ND	C–H bending	[91]
	0.5	ND	ND	ND		
	1	D	ND	D		
	1.5	ND	ND	ND		
1646	0	D	+7	+7	OH groups	[92]
	0.5	+6	+7	+7		
	1	+7	+7	+6		

(*continued on next page*)

Table 6 (*continued*)

Wavenumber cm^{-1}	K%	*C.vulgaris*	*Sneycoccocus*	Consortium	Active groups	References
	1.5	D	+6	+6		
1640	0	D	ND	ND	C═O groups in amides	[93]
	0.5	ND	D	ND		
	1	ND	ND	−4		
	1.5	ND	ND	−4		
1558	0	D	D	D	Amide II	[94]
	0.5	ND	ND	D		
	1	D	D	D		
	1.5	D	D	D		
1540	0	D	D	D	Amide II	[95]
	0.5	D	ND	ND		
	1	D	D	D		
	1.5	D	D	D		
1521	0	D	D	D	C–C aromatic compounds	[96]
	0.5	ND	ND	D		
	1	D	D	D		
	1.5	D	D	D		
1507	0	D	D	D	Skeletal vibration of aromatic rings	[97]
	0.5	D	D	D		
	1	D	D	D		
	1.5	D	D	D		
1489	0	D	D	D	NH	[98]
	0.5	ND	ND	D		
	1	D	ND	D		
	1.5	ND	ND	D		
1472	0	D	D	D	C═C stretch	[99]
	0.5	D	ND	D		
	1	D	D	D		
	1.5	D	D	D		
1457	0	D	D	D	C–H	[100]
	0.5	D	D	ND		
	1	D	D	D		
	1.5	D	D	D		
1418	0	D	ND	D	Lipids, α-methylene CH_2 scissoring band	[101]
	0.5	ND	ND	D		
	1	D	ND	D		
	1.5	ND	ND	D		

[a] D – Detected, ND**- Not Detected.

Table 7
GC-MS analysis of dry-weight algae and consortium methanol extracts after 15 days of cultivation.

Rt	Compounds Chemical formula		*C. vulgaris*	*Syenchococcus* sp	Consortium
6.980	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si$	51.98	Nd[a]	15.69
7.612	1-Hexanol, 2-ethyl	$C_8H_{18}O$	14.07	Nd	Nd
8.912	Undecane	$C_{11}H_{24}$	19.90	82.16	79.51
9.394	Octanoic acid methyl ester	$C_9H_{18}O_2$	Nd	10.64	Nd
9.896	2,5-Dihydroxybenzaldehyde	$C_7H_6O_2$	10.85	Nd	Nd
15.530	Cyclododecane	$C_{12}H_{24}$	3.20	Nd	Nd
23.613	Pentadecanoic acid, 14-methyl-, ester	$C_{17}H_{34}O$	Nd	0.88	Nd
23.691	Pentadecanoic acid, 13-methyl-, ester	$C_{16}H_{32}O$	Nd	1.77	Nd
23.743	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Nd	0.50	Nd
28.454	5,5″-Diethynyl-2,2′:6′,2″-terpyrid	$C_{19}H_{11}N_3$	Nd	Nd	2.19
28.521	2-(1,3-Benzodioxol-5-ylmethyl)-1H- isoindole-1,3(2H)-dione	$C_{16}H_{11}NO_4$	Nd	Nd	1.43
28.549	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	Nd	1.25	Nd
28.577	1,1,1,3,5,5,5-Heptamethyltrisiloxa ne	$C_7H_{22}O_2Si_3$	Nd	Nd	1.17
28.604	Perhydro-htx-2-one, 2-pentyl-, acetate ester	$C_7H_{14}O_2$	Nd	1.35	Nd
28.633	5-(4-Nitrophenyl)-1,3,4-oxadiazole 2(5H)-one	$C_9H_7N_3O_3$	Nd	0.35	Nd
28.916	2-(1,3-Benzodioxol-5-ylmethyl)-1H- isoindole-1,3(2H)-dione	$C_{16}H_{11}NO_4$	Nd	1.11	Nd

[a] Nd – Not detected.

obliquus can grow under mixotrophic and heterotrophic conditions using azo dye as a carbon source to produce a high lipid content and also the highest dye removal percentage [76,77]. (see).

4. Conclusion

This study insight into the cultivation of green alga *C. vulgars,* cyanobacterium *Synechococcus,* and its consortium in different concentrations of kerosene, and also determined the biomasses and lipid content. The mixotrophic *C. vulgaris, cyanobacteria Synechococcus,* and its consortium can remove Kerosene from media. *C. vulgaris* grown under mixotrophic conditions possessed the highest amount of dry weight and lipide content. The maximum compound presented in the methanol extract of *Synechococcus* and consortium of *C. vulgars,* and *Synechococcus* was Undecane. Cyclotetrasiloxane, octamethyl was the furthermost compound found in the methanol extract of *C. vulgaris*. The *C. vulgars, Synechococcus,* and its consortium can absorb, and complete the removal of alkene from surrounding media. It is possible to use algae and consortium grown under mixotrophic as feedstock to produce biofuel.

Author contribution statement

Ragaa Hamouda: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Abrar Alhumairi: Performed the experiments; Contributed reagents, materials, analysis tools or data.
Amna Saddiq: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16656.

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