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Research article

Investigating the efficiency of biological treatment process of oil pollutants using mix of *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* algae: A case study

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Abstract: The studies on polycyclic aromatic hydrocarbons (PAHs) occurrence, distribution, health risk, and effect of them on aquatic environments are limited in worldwide. To cope with this critical challenge, the process of eliminating oil compounds and growing algae were investigated by conducting various experiments in an artificial environment of petroleum that were called M1, M2 and M3 (0.05 mg/L, 1.5 mg/L, 2.5 mg/L concentration of oil per 100 mg/L distilled water) using mix cultivating *Scenedesmus.obliquus* and *Chlamydomonas reinhardtii* algae. In this matter, the highest rate of the growth of algae was significantly reported in M2 treatment (P<0.01). Moreover, the percentage of removal of all light petroleum hydrocarbons in M1 treatment was 100% on day 14 of cultivation (P <0.05). Compared to light hydrocarbon compounds, 8 heavy combinations with 97.33% in the concentration of 0.5 g/100 mL and 85.66% in the initial concentration of 1.5 g/100 mL and 73.66% at the initial concentration of 2.5 g/100 mL of crude oil were eliminated by *S. obliquus* and *C. reinhartii* algae (P <0.01). Finally, the experimental results revealed that in terms of the potential for nutrient removal, the cultivation of mixing two algae *S. obliquus* and *Chlamydomonas reinhardtii* in wastewater, hydrocarbon compounds, and water quality and biomass production, can be distinguished as some acceptable options to exploit in the biological treatment of contaminated aquatic environments.

Keywords: Algae; *Scenedesmus obliquus* Algae; *Chlamydomonas reinhardtii*; oil wastewater; biological purification; nutrient removal

1. Introduction

The polycyclic aromatic hydrocarbons (PAHs) are a group of compounds consisting of carbon and hydrogen atoms and contain more than one fused aromatic rings. These compounds are the byproduct of natural and anthropogenic processes [1]. They can enter into the environment through various pathways, such as anthropogenic input from oil spills, urban runoff, incomplete combustion, domestic and industrial wastewater discharges, vehicle exhaust, and industrial emissions [2]. Oil leaks may be due to the release of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as leakage of refined petroleum and their products, heavier fuels used by large ships or through the drainage of oil waste into the sea [3]. The most sever oil spill occurred recently in the Gulf of Mexico in April 2010, with nearly 4.9 million barrels of crude oil entering the marine environment [4].

PAHs are ubiquitous in the environment and are commonly detected in air, soils, sediments, water bodies, and plant and animal tissues. Due to their long half-life, they are categorized among persistent organic pollutants (POPs) [5]. There are many ways to remove these pollutants from contaminated wastewater, but in recent years, biodegradation and the use of microorganisms have been considered as an appropriate and synchronous with the environment [6]. A type of biodegradation refers to the use of algae or microalgae to remove contaminants from sewage, contaminated soil and carbon dioxide from contaminated air [7]. Algae are the primary producer of biomass in aquatic ecosystems. Previous studies have shown that algae can remove organic pollutants such as phenol, pyrene and some other hydrocarbons through bio-absorption, bio-accumulation, and biodegradation. The photosynthetic effect of microalgae is 40 times more than land plants, so they have the potential to produce valuable biomass and effectively destroy nutrients for a short period of time. The biomass can be used to produce various products in downstream processes, including biofuels (bioethanol, biodiesel), livestock feed and other high-value economic products such as Astaxanthin and Carotenoid [8].

Scenedesmus is one of the most common freshwater algae genera which is utilized for waste water treatment [9]. By analyzing three species of *Scenedesmus* sp and *Chlorella* sp and *Selenastrum* sp, Haritash and Kaushik [10] concluded that the removal of aromatic compounds by algae species depends on the toxicity of the composition, and indicated that the two compounds of Florentine and Pyrenees decreased 30% by *Chlorella* sp and 73% by *Scenedesmus* sp during 7 days, respectively.

Chlamydomonas reinhardtii is a single-cell and mobile algae that lives in freshwater such as ponds and lakes. When viewed under a microscope, the egg has two flagella in the head, a cup-shaped chloroplast containing Pyrenoid and ocular spot. *Chlamydomonas* can be considered as the source of green algae [11]. Biodegradation of a contaminant by microorganisms and its decomposition rate depends on environmental conditions such as temperature, oxygen, nutrients, and the number and type of microorganisms, the natural structure and chemical composition and concentration of these pollutants [12]. In a study by Luan et al. [13], it was concluded that in an optimal laboratory condition, green alga *Chlorella* sp can remove tertiary butyl toluene from water and sediments up to 90%.

Some species of algae can decompose different types of hydrocarbons and oxidize them into less harmful components. This refers to their potential for reforming crude oil [14]. However, further studies on the role of different algae systems are needed to better understand the effect of algae on

the destruction of crude oil hydrocarbons. Therefore, in the present work, we plan to investigate the effectiveness of mixing two algae *S. obliquus* and *C. reinhartii* in the treatment of oil wastewater, which is considered as a culture medium, separately and in combination.

2. Materials and methods

2.1. Algae cultivation

Strains of algae *S.obliquus* was separated from the algae collection of Gorgan Natural Resources and Agriculture University Specify that this University is in Iran. we used BG11 medium which is a typically used mineral medium for microalgae cultivation designed by Rippka et al. [15]. Algal cultivation in 500 mL of Erlenmeyer flasks containing 450 mL of BG11 culture medium at 25 ℃ under constant pressure was performed in a continuous incubator at 150 rpm. Light intensity was supplied by white and bright fluorescent lamps in 4000 lux with a 12-hour light cycle. To purify the algae colony, the algal cells used in this experiment were collected by centrifugation from a culture medium in a growth stage, washed three times thoroughly with Q-Milli water, and put in the distilled water to use for further experiments.

2.2. Preparing co-culture and Algae cultivation with crude oil

For preparing co culture of two species, the same number of species, 12,000 algae cells equivalent to 10 percent per 100 mL/l of distilled water were added to culture media. This number of algae according to the same rule to the substance concentration were done [14].

Table 1. Result of crude oil treats.

Type of Algae	M1		M2		M3	
Type of carbons	F	DF	F	DF	F	DF
Naphtalene	244**	5	1361**	5	6700**	5
Acenaphtylen	361**	5	227**	5	3721**	5
Acenaphten	1831.5**	5	2469**	5	1322**	5
Florene	800**	5	3593**	5	5436**	5
Phenanthrene	2500**	5	1252**	5	35656**	5
Anthracene	841**	5	844**	5	3324**	5
Fluorantene	300**	5	804**	5	1600**	5
Pyrene	800**	5	645911**	5	213698**	5
Decan	13411**	5	551509**	5	2305439**	5
ondecan	20641**	5	569315**	5	3456442**	5
Dodecan	10668**	5	549171**	5	1944643**	5
Tridecan	9669**	5	612017**	5	2251468**	5
Nanodecan	25987**	5	204871**	5	2456443**	5
Eicosane	5317**	5	173271**	5	436023**	5
Tricosane	8463**	5	73177**	5	314976**	5
Tetracosane	5913**	5	70819**	5	413437**	5

Notes: ** indicates a significant difference between the values at 0.01; * Indicates a significant difference between the values at 0.05; Note: F stands for ratio of two variances and DF stands for degree of freedom.

Crude oil with various percentages (0.5, 1.5 and 2.5%) was added for dilute to 100 mL Erlenmeyer flasks containing same amount of two algae to prepare an oil culture medium. The Erlenmeyer flasks were incubated at 25 ℃ with a constant vibrational speed of 80rpm in dark conditions. Then, in the same conditions as the algae culture medium, the results of the analysis of the control treatments can be seen in Table 1 [14].

2.3. Measuring phosphates, nitrates and nitrite

Nitrite, nitrate and phosphate nutrients were measured using the Vogtec company kits (made in UK) and a 2800HACH DR photometer, which was performed every other day [16].

2.4. Measuring TOC, COD, BOD

For analysis of BOD_5, standard solutions of potassium hydrogen phthalate were prepared. To determine BOD_5 according to the ALPHA 5210B method, a glass of 300 mL of impenetrable air was filled with seeded water. 0.05 mL of the culture medium's water sample was transferred to the bottle and then the bottle filled with the same dilute aeration water. The 5BOD calculated from the difference between initial and final dissolved oxygen concentrations [17]. After 5 days of incubation at 20 ± 1 ℃, the initial oxygen concentration (doi)and on the fifth day ($DO_{(5)}$)was determined using the Hanna oxygen (HI) model. The Hach method was used to measure the chemical oxygen demand (COD). In which the standard solution and curve, as well as the addition of digestion solutions, catalysts and standard CODs were used [18]. To measure the total amount of organic carbon, water samples were passed through a simple Whatman filter and then digested with 1.6% Hydrochloric acid solution to remove inorganic carbon. A set of equipment (Liqui TOC) model was used to measure the TOC content [19].

2.5. Measuring the biomass, number of algae and chlorophyll A

Microalgae growth was done by single cell count using a hemocytometer lam and the suggested method by Martınez et al. [20] every other day. The dry weight of algae biomass was calculated according to the total Transient Voltage Surge Suppressor (TVSS) Measurement method [21]. 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 ℃, and then centrifuged again. The color 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 Zhang (2008) for chlorophyll a, chlorophyll b and carotenoids [22]:

$$\text{Chlorophyll A} = 13.95A_{665} - 6.88A_{649} \quad (1)$$

2.6. Measuring SGR and DT^2

Specific growth rate and the doubling time of algae are two important indicators for measuring the proliferation of algae in the culture medium. For the specific growth rate (SGR) and the doubling time (DT), the following relationships were used [23].

$$SGR = (LnN2 - LnN1)/ t \quad (2)$$

$$DT = \log 2e / SGR \quad (3)$$

Where N2 is the number of algae counted in the second sample, N1 is the number of algae counted in the first sample and t is the time difference between the two sampling.

2.7. Measuring oil hydrocarbons

On days 1, 7 and 14, the microalgae were removed from the contaminated environment (crude oil diluted in water) Biodegradation of crude oil was investigated by using GCeMS HP 6890 gas carrier helium (1 mL/min). A 15 m _ 0.25 mm I.D. DB-1 HT J&W (J&WScientific, Folsom, CA) fused-silica capillary column with 0.1mm film thickness was used for the experiments. For the light samples the GC oven temperature was programmed from 0 ℃ to 300 ℃ at a rate of 5 ℃/min. For the heavier samples the GC oven temperature was programmed from 50 ℃ to 380 ℃ at a rate of 10 ℃/min. The GC oven remained at the maximum temperature for 10 min. Samples were introduced into the gas chromatograph via a Gerstel injection system (Cooled Injection System-CIS 3,Gerstel, Germany). For the light samples, the injection system temperature was programmed from 0 ℃ to 350 ℃ at a rate of 12 ℃/s. The heavy samples were introduced at 50 ℃. The purification percentage of heavy compounds was calculated as follows:

$$A = [(C_0 - C_1)\, C_0] \times 100 \quad (4)$$

Where A is the percentage of purification of heavy compounds by microalgae, C0 and C1, respectively, indicating the total mass of oil in solution and the mass of light petroleum compounds.

2.8. Statistical analyses of data

Statistical analysis of the experiments was carried out using a completely randomized design in factorial arrangement. The Pearson test was used for the correlation between the parameters using the software Sas 9.4. Drawing diagrams were also done by Excel 2013 software.

3. Results and discussion

3.1. Analysis of statistical analysis

The analysis results of growth parameters variance, water quality and oil hydrocarbons are shown in Tables 1 and 2.

Tables 1 and 2 calculate the degree of freedom DF, and the values of statistics F. By comparing their mean and recognizing their equality or inequality among the communities, one could vote for them to be the same or different. Accordingly, if one of the means is different from the others, we could state that the communities are not similar.

Table 2. Analysis of growth parameters variance and water quality.

Parameter	M1		M2		M3	
	F	DF	F	DF	F	DF
Chl a	115**	19	64.1**	19	18.9**	19
Bio	8.03**	19	1.6^{NS}	19	4.34*	19
Num	1040.51**	19	3724.54**	19	1050.95*	19
PO_4	457.87**	20	772.32**	20	1173.16**	20
NO_3	105.66**	20	97.07**	20	90.47**	20
NO_2	69.04**	20	132.04**	20	84.33**	20
BOD	304.79**	23	85.24**	23	443.98**	23
COD	1546.86**	23	5906.16**	23	751.43**	23
TOC	565.70**	23	378.14**	23	52.68**	23

Notes: ** indicates a significant difference between the values at 0.01; * Indicates a significant difference between the values at 0.05; F stands for ratio of two variances and DF stands for degree of freedom.

3.2. Effect of oil concentration on chlorophyll A, biomass and algae cell density

Chlorophyll A content, performance of biomass and density of algal cell were obtained based on the above methods. The results of Table 3 display the influence of crude oil on the content of chlorophyll A, algae density and biomass in the oil medium. The main chlorophyll amount was detected for M1 treatment during 14 days (P <0.01, 105). Considerably, the content of chlorophyll A, cell density and biomass enhanced gradually from day 7 to day 14. In other words, algae cell has deal enhancing growth with the logarithmic phase inflowing it. This is because of the reality that the growth of algae cell has been limited after 7 days in concentrations more than oil. In reaction to these results, the *S. obliquus* cells metabolic mechanism grown in various oil concentrations by Guo et al. [24] has been studied in prior investigations. The results displayed that both parameters of great oil content and decrease of biological oxygenation in culture medium could prevent the growth of algae cell. It is important that, as well as the main photosynthetic pigments, micro-algae comprising supplementary photosynthesis pigments also enhance bio-degradation in autotrophic situations by light energy [25–27]. For instance, the minimum density of algal cell at the end period was 412013 in M2 treatment, that had an important alteration with M3 treatment (469733, P >0.05). In difference, weight of dry material in M2 treatment was not considerably related with 2 other treatments (P >0.01). A nearer look at the issue has also revealed in related results in the refinement of *S. obliquus* algae in a synthetic wastewater by Xin et al. [28] that the extreme algae biomass performance has enhanced with growing oil concentration and temperature, but when temperature surpassed 20 ℃ and the concentration surpassed 3 mg/L, enhancing in biomass maximum yield was not important.

Table 3. Effect of crude oil on the content of chlorophyll A, biomass and algae density.

Parameter	Time Type of Algae	Day2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Chl a	M1	0.9 ^{Db} ±0.06	1.09 ^{Db} ±0.06	1.34 ^{Cc} ±0.1	2.39 ^{Be} ±0.1	2.92 ^{Bab} ±0.4	3.86 ^{Bde} ±0.1	3.54 ^{Ac} ±0.1
	M2	0.9 ^{Ecd} ±0.03	1.13 ^{Ecd} ±0.03	1.16 ^{Ed} ±0.3	1.37 ^{Df} ±0.05	2.76 ^{Ccd} ±0.4	3.3 ^{Bf} ±0.2	3/62 ^{Aed} ±0.2
	M3	1 ^{Db} ±1.1	1.16 ^{Db} ±1.1	1.16 ^{Dc} ±0.2	1.61 ^{Cc} ±0.06	2.4 ^{CBcd} ±0.8	3.18 ^{Bef} ±0.01	4.15 ^{Aef} ±0.08
Bio	M1	1/7 ^{Db} ±0.06	3.05 ^{Db} ±0.06	4 ^{Cc} ±0.1	5.9 ^{Be} ±0.1	6. 2 ^{Bab} ±0.4	7. 6 ^{Bc} ±0.1	7.6 ^{Ac} ±0.1
	M2	2.05 ^{Ecd} ±0.03	2.62 ^{Ecd} ±0.03	2.36 ^{Ed} ±0.3	3.87 ^{Df} ±0.05	5.2 ^{Ccd} ±0.4	6.1 ^{Bf} ±0.2	6.72 ^{Aed} ±0.2
	M3	2.58 ^{Db} ±1.1	3.13 ^{Db} ±1.1	2.92 ^{Dc} ±0.2	4.8 ^{Cc} ±0.06	6.1 ^{CBcd} ±0.8	6.6 ^{Bef} ±0.01	8.35 ^{Aef} ±0.08
Num	M1	87800 ^{Fe} ±17.8	96700 ^{Fe} ±17.8	210333 ^{Ee} ±15502	324867 ^{De} ±8105	356867 ^{Chb} ±24524	592333 Be ±2516	634733 ^{Ae} ±6300
	M2	876400 ^{Fe} ±3078	112400 ^{Fe} ±3078	206733 ^{Ee} ±7616	310233 ^{De} ±8020	397733 ^{Cg} ±6709	426267 ^{Bg} ±3308	469733 ^{Ag} ±6106
	M3	926200 ^{Fed} ±1342	121533 ^{Fed} ±1342	219333 ^{Ee} ±5507	326067 ^{De} ±3722	357723 ^{Ch} ±6842	384400 ^{Bh} ±18124	412013 ^{Ah} ±3302

Note: The uppercase letters indicate a significant difference between each row during the trial period at the level of 0.05α, and the small letters also indicate a significant difference in each column at the level of 0.05α.

3.3. Influence on DT and SGR

By enhancing the special growth rates, the rate of growth will be lesser and the tolerance power will growth. In difference, the more the time it doubles, it shows the high reproductive power in the medium [23]. According to the results attained in Figure 1a, b, the maximum rate of particular rates and the maximum propagation was detected in M3 ($P > 0.05$).

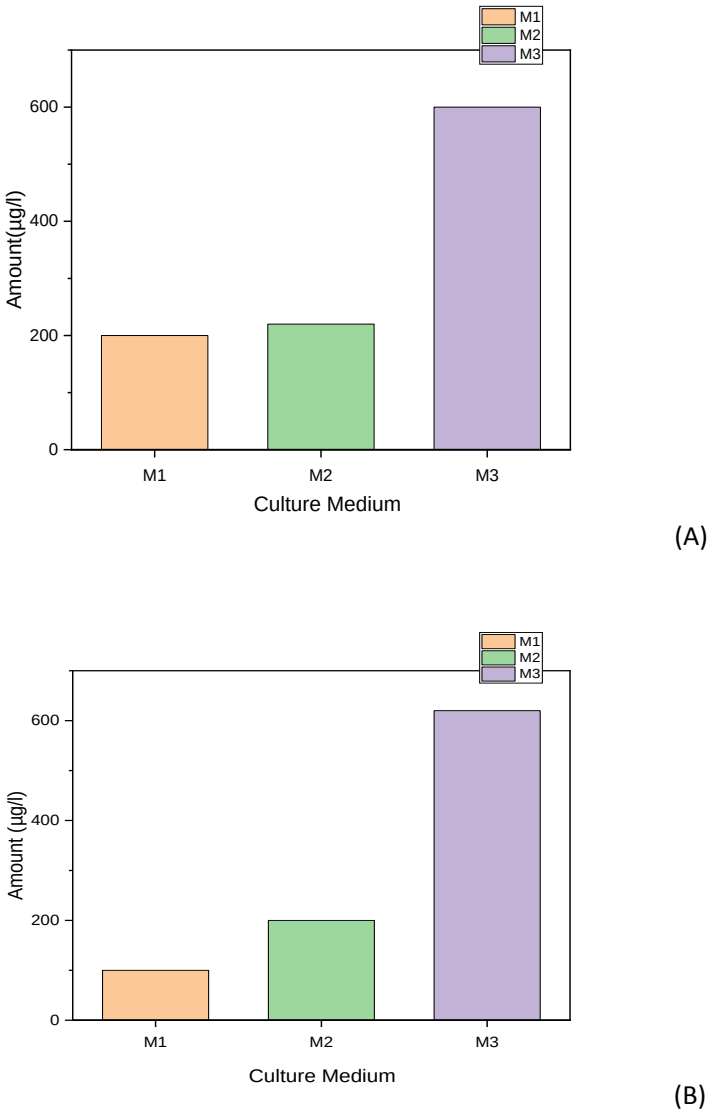

Figure 1. A. Change in the rate of the growth rate of algae (SGR) under three different culture media M1, M2 and M3. B. Change in Doubling Time (DT^2) under three different culture media M1, M2 and M3.

3.4. Nutrient changes procedure in the wastewater

In Table 4, the procedure of amount of nutrients changes in the synthetic wastewater could be found. Amongst treatments, at the end of the period of culture, the minimum phosphorus volume in the S1 treatment with 0.19 rate (mg/mL) has been respectively found followed by nitrate and nitrite with 0.3 and 0.6 rate (mg/mL), (P >0.01). Temporarily, amongst the nutrients between Po4-2, No-3, No-2 Po was the maximum elimination rate for composition of nitrite in S3 treatment (P >0.01). Nitrogen in the nitrate form is immersed in the microalgae cells and is implemented as a source of nutrient for algae growth. The upper biomass growth in situations of nitric oxide existence might be due to its participation in the DNA formation together with increased growth. Good growth of algae biomass with CP composition might be owing to the both nutrients single function in simplifying cell division of algal cells [29]. Alternatively, CNP combinations are vital for growth in aquatic

conditions. Also, these constituents participate to the tissues and fibers growth, therefore participating to the enhancement of microalgae growth [30]. The results of tests indicate that from the 10th to the end of the period of culture at concentrations of 1.5 and 2.5 oil, the biodegradation volume of nitrate and phosphate in the treatments was decreased similarly, there was a positive relationship among these 2 ratios P <0.05). Fried et al. [31] observed that nitrates, potassium and phosphates have positive impacts on algae growth. These variables affect algal growth individually of each other without any interactions among them. Though, more studies are required to explain the influences of nutrients on each other in the absorption procedure.

Table 4. Effect of crude oil on Nitrate, Nitrite and Phosphate.

Parameter	Time Type of Algae	Day2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
	M1	20_{Ac} ±3.6	18.67_{Bc} ±4.1	16.73_{Ced} ±0.2	6.43_{Dc} ±1.2	5.2_{Ddbc} ±1.2	1_{De} ±0.07	0.19_{Dd} ±0.02
Po_4	M2	3526_{A} ±2.6	33.95_{Aa} ±2.9	28.95_{Ba} ±1.8	13.20_{Cab} ±3	12.67_{Ca} ±1.5	4.65_{Dbc} ±1.43	1.33_{Dbc} ±0.5
	M3	40.39_{Aa} ±2	20.29_{Bc} ±2.7	19.58_{Bcd} ±3.7	14.27_{Ca} ±2.8	7.99_{Db} ±3.6	4.98_{DEb} ±1.8	1.76_{Eb} ±1.7
	M1	32.73_{Af} ±0.5	25.16_{Bd} ±2	18.5_{Cc} ±1.8	5.9_{De} ±0.6	2.9_{Ec} ±0.8	1.16_{EFe} ±0.5	0.33_{Fcb} ±0.5
NO_3	M2	39.1_{Ab} ±1	40.3_{Ba} ±2	33.3_{Cb} ±1.57	22.56_{Dc} ±1.5	20.96_{Ea} ±1.5	12.96Fcb ±0.7	8.2_{Gab} ±3
	M3	42.13_{Aa} ±1	24.56_{Bc} ±1.1	21.53_{Ca} ±1.6	11.8_{Dc} ±0.6	10.43_{Da} ±1.7	7.6_{Ea} ±0.3	3.26_{Fa} ±1.06
	M1	31.1_{Ad} ±2.8	28.24_{Ac} ±4.02	31.04_{Aa} ±1.8	15.7_{Bcb} ±1.5	11.59_{Cb} ±1.9	2.6_{Dde} ±0.5	0.46_{Dc} ±0.3
NO_2	M2	56.31_{Ab} ±4.6	61.52_{Aa} ±3.8	27.24_{Bb} ±5.7	26.68_{Ba} ±6.5	23.03_{Ba} ±4.9	9.1_{Cb} ±1.8	1.7_{Cb} ±0.5
	M3	75.41_{Aa} ±6.6	33.1_{Bb} ±5.7	24.19_{BCb} ±5.5	10.93_{Cdb} ±6.5	24.74_{DEb} ±4.9	1.87_{FEc} ±1.9	2.01_{Fb} ±0.5

Note: The uppercase letters indicate a significant difference between each row during the trial period at the level of 0.05α, and the small letters also indicate a significant difference in each column at the level of 0.05α.

3.5. Water quality improvement (COD, BOD, TOC) using microalgae

It is presumed that microalgae have a role in the Aquatic Bodies treatment [32]. The microalgae implementation has been revealed to improve quality of water in wastewater [33,34]. The reduction rate of water quality factors in Table 5, along with their changes trend in Figures 2, 3 and 4, are presented respectively. Based on the results, the maximum decrease in BOD values was 49.4 percent for M3 treatment, with 84 percent and 94 percent for COD and TOC for M1 treatment (P > 0.01). There was a substantial variance among COD and BOD compared to days 1 and 14 (P > 0.01). So, high oxygen concentrations because of the photo oxidation intensification could cause damage to microalgae cells. For instance, Johnson et al. [35] stated a 98 percent reduction in photosynthetic activity at concentrations of 0 to 30 mg/L. On the other hand, great oxygen concentrations could be a good reason to entirely remove pollutants [36]. Carbon is one of the nutrients needed in microalgae. Owing to the organic matter biological oxidation, it could be used to more reduce TOC and COD. The results of the changes in carbon dioxide volume in the entire cultivation period display that the carbon concentration reduced steadily and considerably (P <0.01).

Table 5. Reduction of water quality parameter.

Parameter	M1	M2	M3
BOD_5 Reduction	45.8%	47.2%	49.4%
COD Reduction	87%	85%	87%
TOC Reduction	93%	96%	90%

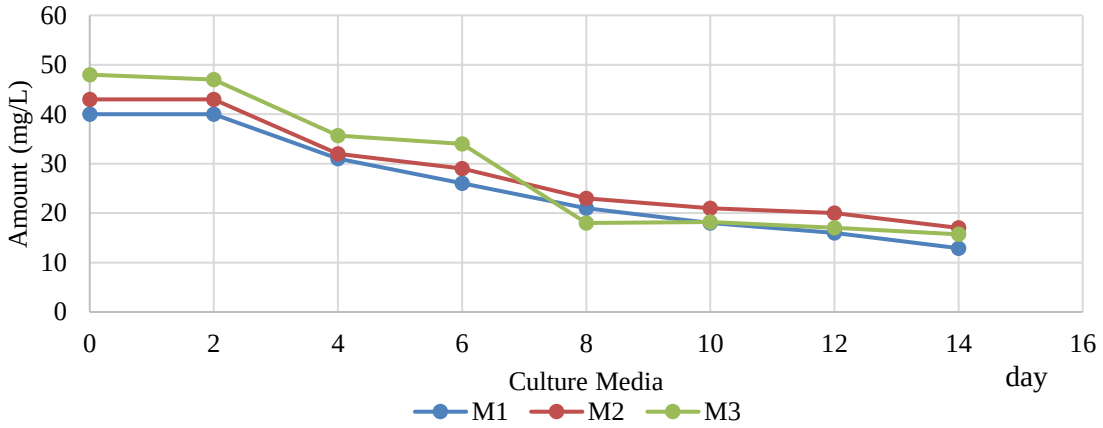

Figure 2. Concentration changes of BOD in culture media M1, M2 and M3 during 14 days of cultivation.

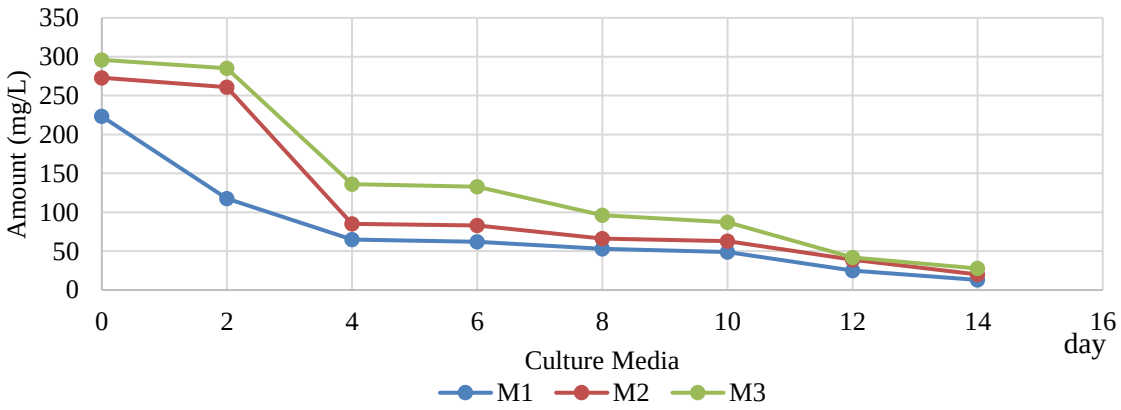

Figure 3. Concentration changes of COD in culture media M1, M2 and M3 during 14 days of cultivation.

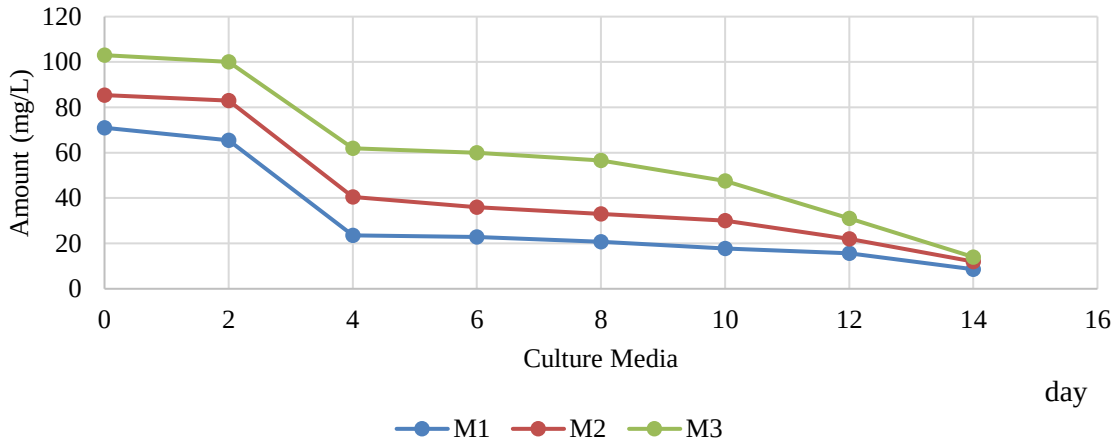

Figure 4. Concentration changes of TOC in culture media M1, M2 and M3 during 14 days of cultivation.

In approval of this discussion, Boyer [37] have exposed the existence of carbon at different concentrations as an important factor in the algae growth pattern in both photoautotrophic and photoheterotrophic systems.

3.6. Algal purification influence on petroleum hydrocarbons

Table 6 displays the removal trend of some light hydrocarbons (with a boiling point more than 350 ℃) of crude oil by *C. reinhartii* and *S. obliquus* algae. Algae were refined in various oil concentrations and various periods. Therefore, the removal percentage of all light petroleum hydrocarbons in M1 treatment was 100 percent on day 14 ($P > 0.01$). Microorganisms could prevent some of oil combinations, to decrease their metabolism. Though, aromatic combinations (like fluorine and naphthalene) and light hydrocarbons like Eicosane and Nonadecane with 100 percent efficiency in the initial concentration of 0.5 g/100 mL of crude oil was refined more efficiently by microorganisms ($P > 0.01$). Consistent with prior investigations by Xaaldi Kalhor et al. [38] with the goal of studying the chlorella algae potential in oil elimination at different concentrations, the efficacy of biological elimination of light hydrocarbons reached 100 percent at the end period of the algal culture. The results of this paper show that combining of *Clamydomonas* and *Scenedesmus* algae have a suitable performance in light petroleum hydrocarbons in small oil concentrations. After this, though algae have used a substantial volume of these hydrocarbons as a nutritional supplement at more concentrations till the seventh day, but after this time its damage has been decreased. Algae have polluted some toxic contaminants as in the investigation on *Chlorella algae*, these microalgae were incapable to endure 10 mg/L of phenanthrene, whereas the Pseudomonas bacteria and *Scenedesmus* algae had a suitable resistance to more concentrations against organic contaminants [39].

Table 6. Removal of light hydrocarbons during 1,7 and 14 days.

Culture Medium	M1	M2	M3	M1	M2	M3	M1	M2	M3
Type of carbon	Time Day1	Day7	Day14	ay1	Day7	Day14	ay1	Day7	Day14
Decan	0Aa ±0	83.7Ab ±0.1	3.9Aab ±0.1	2.2C**c** ±0	2Aa ±0.1	21.1Bb ±0	0Bb ±0	0B**b** ±0	0Bb ±0
Ondecan	14.8Aa ±0.1	6.4Ba ±0	6.4Ba ±0	10.7Ab ±0.1	6.1 Bb ±0	32.3Cc ±0	0Aa ±0.1	0 ±0Ba	14.0Cc ±0
Dodecan	21.6Aa ±0.1	20.6Ba ±0	30.6Ba ±0	13.3Aa ±0.1	18.3Ba ±0	22.6Cb ±0	0Ac ±0.1	13.3Ba ±0	12.1Cc ±0
Tridecan	27.1Aa ±0.1	10.7Ba ±0	10.7Ba ±0	9.9Ab ±0.1	9.2Ba ±0	45.8Ca ±0	0.3Ac ±0.1	6.3 Bc ±0	6.2Cc ±0
Nanodecan	21.2Aa ±0.1	57.9Ba ±0	37.9Ba ±0	15.0Aa ±0.1	41.6Bb ±0	28.7Cb ±0	0Aa ±0.1	13.6 Ba ±0	12.1Cc ±0
Eicosane	27.7Aa ±0.1	6.3Ba ±0a	6.3Ba ±0	19.0Aa ±0.1	58.1Bb ±0	5.0Cb ±0	0.4Aa ±0.1	6 Ba ±0	0.1Cc ±0
Tricosane	21.5Aa ±0.1	15.8Ba ±0	5.8Ba ±0	12.5Aa ±0.1	13.6B ±0	13.8Cb ±0	0.7Aa ±0.1	12.5Ba ±0	0.2Cc ±0
Tetracosane	11.2Aa ±0.1	0Ba ±0	0B**b** ±0	4.3Aa ±0.1	0.1Ba ±0	0Cb ±0	0.9.3Aa ±0.1	0 Ba ±0	0Cc ±0

Note: The uppercase letters indicate a significant difference between each row during the trial period at the level of 0.05α, and the small letters also indicate a significant difference in each column at the level of 0.05α

As revealed in Figures 5, 6 and 7, heavy combinations with 33.99% at a concentration of 5 g/L and 66.87% at an early concentration of 15 g/L and 66.77% at a concentration the early 25 g/L crude oil was eliminated by *S. obliquus* ($P > 0.05$). Based on these data, these algae efficiently damaged heavy compounds. However, the harmful influence of the early concentration of oil is obvious in the purification of heavy crude oil constituents. Analysis of variance approved that decomposition is significantly a continuous procedure over a period of time (Table 4). Inside 14 days, the degradation percentage was further than 40% greater than purgation for 7 days. In investigation by Kessler [40], the analysis of naphthalene was done by 2 algae and revealed the capability of these algae to decompose this compound and custom it as a sulfur source. Not just does algae eliminate contaminants with a simple structure of hydrocarbon in the freshwater environment, it is also capable of decomposing circular hydrocarbons in salty environments. In a study done by Arias et al. [41], with the goal of analyzing the capability of the 3 compounds, Pyrene, Phenanthrene and Fluoranthene, implementing *S. obliquus* under salinity stress situations in various oil concentrations, the capability of removing these compounds was well revealed to be 70 percent. The results display that microalgae customs carbon to grow well. In difference, there is a restriction in the elimination of some ring compounds like naphthalene in high concentrations. In addition, the volume of carbon intake enhanced gradually over the course of 7 days. In the meantime, at this step, algae obtained dietary supplements (e.g. phosphorus and nitrogen) from an oil source, thus it might be owing to reduce these 3 nutrients (CNP) Microalgae has been capable of maintaining its growth steadily until the end of the period.

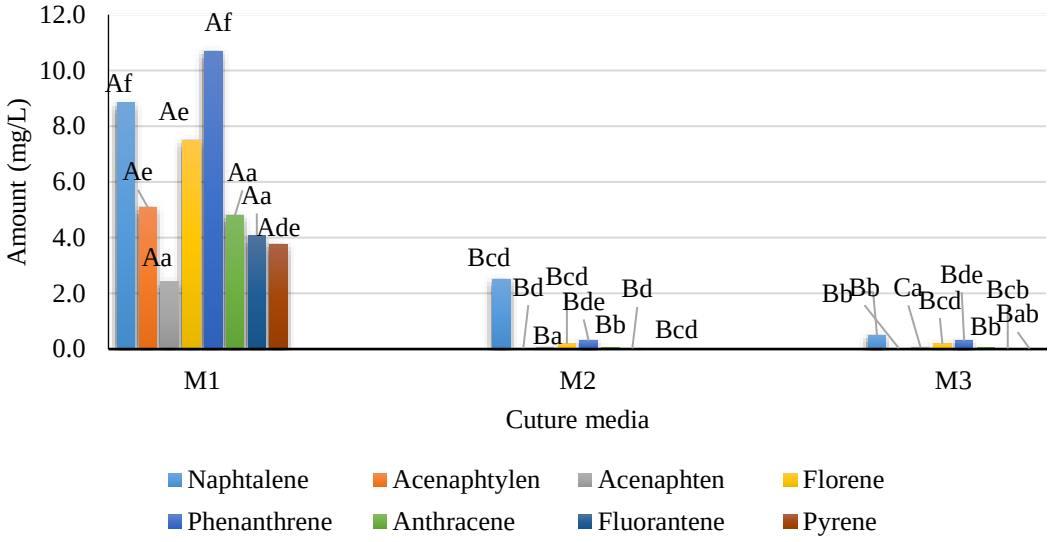

Figure 5. Effect of cultivation of algae at different concentrations of Synthesis oil effluent (1.5 gr/100mL) in three time periods (1, 7 and 14 days). (The uppercase letters indicate a significant difference between each row during the trial period at the level of 0.05α, and the small letters also indicate a significant difference in each column at the level of 0.05α)

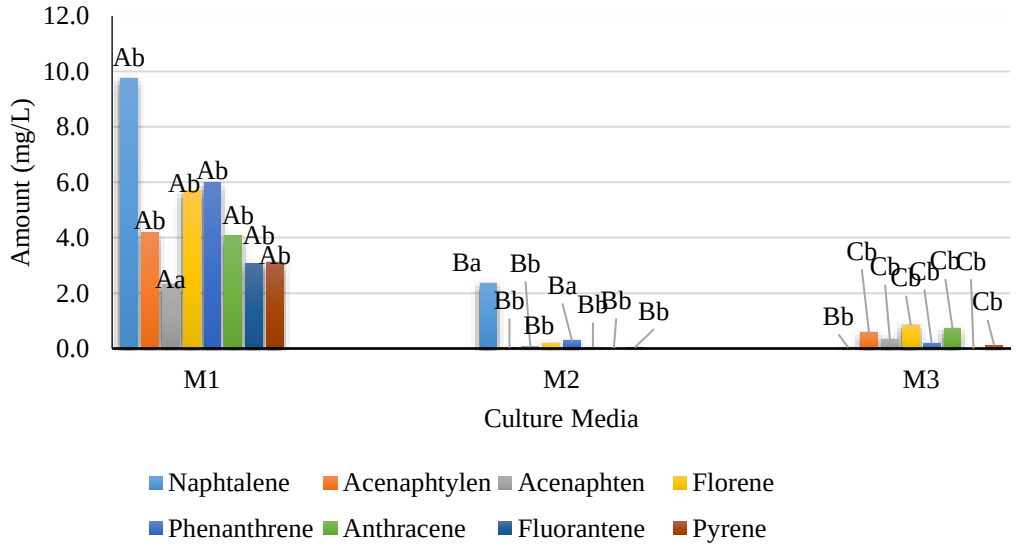

Figure 6. Effect of cultivation of algae at different concentrations of Synthesis oil effluent (1.5 gr/100mL) in three time periods (1, 7 and 14 days). (The uppercase letters indicate a significant difference between each row during the trial period at the level of 0.05α, and the small letters also indicate a significant difference in each column at the level of 0.05α)

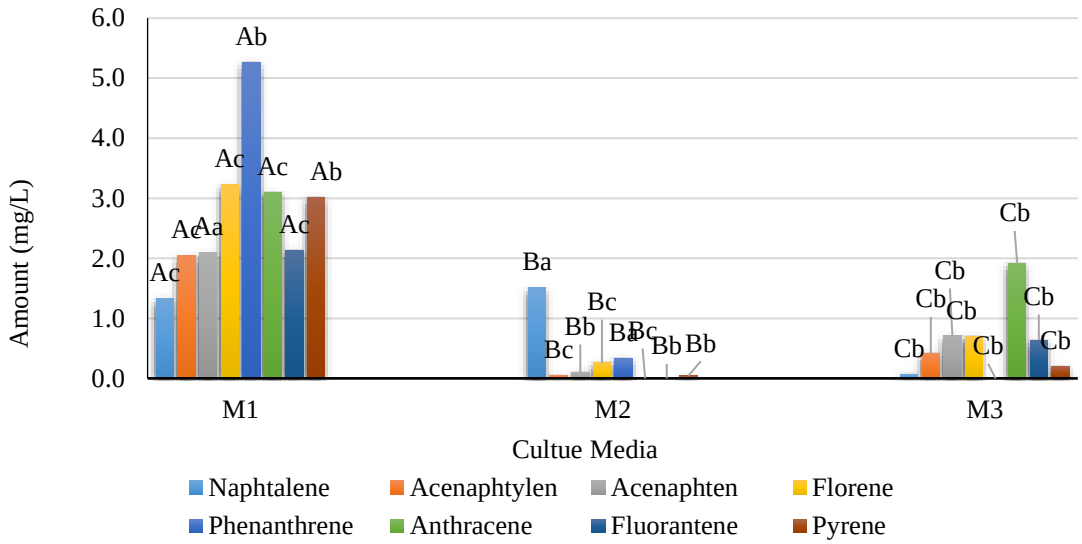

Figure 7. Effect of cultivation of algae at different concentrations of Synthesis oil effluent (1.5 gr/100mL) in three time periods (1, 7 and 14 days). (The uppercase letters indicate a significant difference between each row during the trial period at the level of 0.05α, and the small letters also indicate a significant difference in each column at the level of 0.05α)

4. Conclusion

The results showed that the highest biodegradation rate of light petroleum hydrocarbons with 100% efficiency was observed in M1 treatment. The highest reduction of BOD values was obtained by 49.4% for M3 treatment with 87% and 93% for COD and TOC for treatment M1 belonged. Growth parameters showed that the highest amount of chlorophyll was related to M3 treatment. Evidence from this study suggests that increased cell growth rates and qualitative improvement of water parameters mean that algae species can use distinct organic compounds as nutrients. However, the toxicity of chemical compounds can be considered as a deterrent to growth rates. However, further research is needed to find optimal laboratory conditions for converting the crude oil hydrocarbons needed for algae as a culture medium.

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