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Original paper

Purification of petroleum wastewater and biodiesel production by Prasinophyte alga Tetraselmis chuii

HEBA S. EL-SAYED[1], LAILA ABD EL-FATTAH MOHAMED[2], MOHAMED ABD-EL-NABY ALY-ELDEEN[2], HANAN M. KHAIRY[3*]

[1]Fish Reproduction and Larval Rearing Lab. (Marine Hatchery), Aquaculture Division, National Institute of Oceanography and Fisheries, NIOF, Egypt
[2]Maine Chemistry Laboratory, Marine Environmental Division, National Institute of Oceanography and Fisheries, NIOF, Egypt
[3]Hydrobiology Laboratory, Marine Environmental Division, National Institute of Oceanography and Fisheries, NIOF, Egypt

Abstract

This study aimed to assess the potential role of Prasinophyte alga, *Tetraselmis chuii* in petroleum wastewater purification (PWE) and biodiesel production. PWE samples with Conway medium were taken on 2 days intervals to determine the growth of *T. chuii*. The results indicated that the using 5% of PWE is recommended for cost-effective microalga culture, as well as for growth promotion and biodiesel content of *T. chuii*. The maximum growth as cell number was 9.1×10^6 cells/ml at the end of exponential phase of growth (day 12). Also, the maximum optical density (OD) of *T. chuii* (1.05 ± 0.16) was sustained (day 12), and the highest protein content amounted 69.4 µg/ml. The content of saturated fatty acids (SFAs) varies in different PWE percentages. The highest percentage of SFA contents was reported at 5% of PWE (96.9%) on day 8 of incubation when compared to other percentages. Cetane number (73.97), iodine value (5 g I_2/100 g oil), viscosity of the fatty acid resembled those recommended by international standards. Thus, *T. chuii* was a promising marine microalga for dual use in biodiesel production.

Keywords Biodiesel, Fatty acids, Petrolium wastewater, *Tetraselmis chuii*.

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*Corresponding author: Dr. HANAN M. KHIRY, Hydrobiology Laboratory, Marine Environmental Division, National Institute of Oceanography and Fisheries, Egypt
E-mail: hanan_khairy@yahoo.com Phone number: 00201006048854

Introduction

In water treatment, algae can use nutrients in the aquatic environment; furthermore, wastewater is rich in nutrients, which are suitable for algae feeding (GOTAAS and OSWALD, 1955). Biological treatment systems gained importance recently and accepted that algae can used as an effective approach for treatment of wastewater. Nitrogen and phosphorus in different forms in wastewater are the main pollutants that threaten the balance of aquatic ecosystem (RASOUL-AMINI et al, 2014). Microalgae can able to reduce nitrogen up to 84% (RASOUL-AMINI et al, 2014).

Heavy metals discharged water via wastes has negative effects on the aquatic system (KHAN et al, 2008). Therefore, they need to develop new, cost-effective, efficient and sustainable methods for the removal of heavy metals from aquatic bodies insurgent (KHAN et al, 2013). Biofuels produced from biomass through several chemical and physical processes, which can be used to replace petroleum fuels (DEMIRBAS, 2009; LI and YU, 2011). Microalgae are photosynthetic microorganisms, which convert carbon dioxide, water by sunlight energy through photosynthesis to produce their own nutrients (OZKURT, 2009).

Recently, algae have a lot of interest as a new source of biomass for renewable energy production in the form of biodiesel (HOSSAIN et al, 2008; ONCEL, 2013). Algal biomass is one of the emerging sources of sustainable energy. The US Department of Energy reports that the yields of biodiesel from algae could be greater than oilseed crops (PADMANABHAN and STANLEY, 2012). Using wastewater to grow algae is probably the most promising approach to produce biodiesel (MAHMOUD et al, 2016). Wastewater mainly contains ammonium, nitrogen, phosphorus and heavy metals, which are needed by microalgae for their growth. Thus, wastewater has the potential of being used as a medium for microalgal culturing as the cells will absorb and use the nutrients from wastewater to grow. This leads to a reduction in the overall cost of microalgal production and serves as an alternative way of wastewater treatment. Microalgae have been reported as one of the best sources of biodiesel (SHAY, 1993).

Helena et al (2018) showed that aquaculture wastewater is considered as a potential candidate for *T. chuii* growth due to their nutrients content and the rapid growth rate of aquaculture sector. Generally, *Tetraselmis* sp. contains 31% protein, 12.1% carbohydrate and 17% lipid (HELENA et al, 2018)). Today algae are used by humans in many ways; such as conditioners, fertilizers and livestock feed. Limitations can, however, be minimized by selecting a suitable algal species and manipulating the initial fatty acid profile by varying the growth conditions and extraction process. They can produce up to 250 times the amount of oil per acre as compared with soybeans amount (Shay, 1993). In fact, biodiesel from microalgae may be the best way for sufficient automotive fuel production to substitute the present petro-diesel (CHISTI, 2007).

According to the US standard specification (American Society for Testing and materials (ASTM) D6751, biodiesel is a fuel consists of mono alkyl esters of long fatty acids chain from natural sources as vegetable oils or animal fats (KEERA et al, 2011). In addition to biomass and lipid productivities, qualitative and quantitative lipid and fatty acid constituents are critical parameters for large-scale production (HUERLIMANN et al, 2010).

Lipid content can be changed for the same algal strain, algal species selection still important steps to reduce time and cost for biodiesel production at large-scale cultivation (CHEN et al, 2012, NASCIMENTO et al, 2013). Microalgal biodiesel contains no sulfur and can replace diesel in today's cars with little or none modifications of vehicle engines (MATA et al, 2010). Oil content of some microalgae exceeds 80% of the dry weight of algae biomass (BANERJEE et al, 2002, CHISTI, 2007). As microalgae grown on PWE, it can absorb heavy metals (CHOJNACKA et al, 2005), and degrade other organic chemicals (MUNOZ and GUIEYSSE, 2006) and subsequently, this process can be considered as treatment step to decrease the impact of discharged wastes on to the environment.

Thus, the aim of this study was to compare the growth and evaluate the lipid composition of *T. chuii* when it is cultured with PWE, which can highlight the potential use of wastewater as an alternative to the expensive commercial medium. The results can be manipulated and applied for specific uses and high mass production of *T. chuii*, and integrating biodiesel production with PWE treatment in processes that flexibly can be tailored and linked to local facilities and offer a way to reduce operational costs related to algal cultivation.

Materials and Methods

Quality of petroleum waste effluent

The petroleum waste effluent (PWE) was collected from Gabco Company located in Red Sea, Egypt. PWE was stored at 4°C degree until use. Physio-chemical analyses of the water sample (pH, conductivity, total dissolved solid (TDS), salinity, nitrate, nitrite, silicate and phosphate) were carried out according to the methods described by APHA (2000). Determination of total metals in PWE sample was carried out according to the methods described by EP (2007). The total metals were assessed by using the Agilent 5110 inductively coupled plasma optical emission spectroscopy (ICP-OES) Instrument.

Preparation of different concentrations of PWE

Serial dilutions of the tested solution of PWE were prepared in the following percentages (1, 2, 3, 4, 5, 6 and 7%) with natural sea water enriched with Conway medium (WALNE, 1966), the Prasinophyte alga *T. chuii*, compared with control without any addition of the effluent.

Alga strain and culture media

T. chuii strain that was used in this study obtained from the microalgae culture collection of marine hatchery laboratory, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. The alga was grown on water source from Eastern Harbor of Alexandria (EH) and enriched with Conway medium (WALNE, 1966). Two daylight

fluorescent tubes of 20 W were used to maintain constant illumination and culture temperature was 25 ± 1ºC.

Determination of algal growth

Cell number count of the alga *T. chuii* was determined every 2 days by placing an aliquot of well-mixed culture suspension on a Neubauer haemocytometer (Germany). The cell number in the culture was calculated by dividing the number of cells counted by the volume and the dilution. Optical density of the alga *T. chuii* was measured at 750 nm, by using UV/V Spekal 1300, Analytik Jana AG Spectrophotometer.

Determination of total protein and total lipid

Alga was harvested at the end of exponential growth phase (day 12), triplicate samples of algal material were allocated to each assay (protein and fatty acid contents). Total protein in the algal cells was determined by the Folin-phenol method of Lowry et al (1951), and total lipid content was determined after extraction with chloroform–methanol (2:1) gravimetrically, according to the methods described by Bligh and Dyer (1959).

HPLC analysis for fatty acid analysis

Fatty acid methyl esters (Biodiesel) were analyzed in the algal cells at early exponential (day 8) and late stationary growth stages (day 16) according to Vogel (1975) by using gas liquid chromatography (HP-6890 gas–liquid chromatography).

Biodiesel properties

Physico-chemical properties of FAMEs were calculated. The percentage of unsaturation degree (DU, %), cetane number (CN), iodine value (IV, g I2/100 g oil), saponification value (SV, mg KOH g−1), long chain saturation factor (LCSF, wt%), cold filter plugging point (CFPP, °C) and kinematic viscosity (υi, mm^2 s^{-1}) were calculated according to Ramirez-Verduzco et al (2012) and Mitra et al (2016) as shown in the following equations:

$$DU = \Sigma [MUFA + (2 \times PUFA)] \quad \text{(PAN et al, 2017)}$$

where, MUFA and PUFA represent the percentage of mono-unsaturated and polyunsaturated fatty acids, respectively.

$$SV=\Sigma [(560\times N\%) / M] \quad \text{(ZHU et al, 2017)}$$

$$IV=\Sigma [(254\times N\%\times D) / M] \quad \text{(RAWAT et al, 2011)}$$

$$CN = 46.3 + (5458 / SV) – (0.225 \times IV) \quad \text{(NGUYEN et al, 2016)}$$

where: N% represents the percentage of each fatty acid; M represents the molecular mass of the fatty acid; and D represents the number of carbon-carbon double bonds.

$$LCSF = (0.1 \times C16:0) + (0.5 \times C18:0) + (1 \times C20:0) + (1.5\times C22:0) + (2 \times C24:0) \text{ (ABOMOHRA et al, 2018)}$$

$$CFPP = (3.1417\times LCSF) -16.477 \quad \text{(SHAO et al, 2018)}$$

where: C16:0, C18:0, C20:0, C22:0 and C24:0 represent the weight percentage of the corresponding fatty acids.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. Quantitative data were represented as mean and SD. For normally distributed data, comparison between more than two populations were analyzed by F-test (ANOVA) followed by post hoc test by Duncan's method. Duncan's method was used to find the significant between each two groups. Also, cluster analysis and the correspondence analysis were obtained.

Results

Quality of petroleum waste effluent

The physio-chemical analyses of PWE were showed in Table (1). The levels of different metals which determined in the PWE are found to be within the acceptable levels. Furthermore, the higher concentrations of phosphate, silicate, nitrate and nitrite were 135 µg/l, 6.119 mg/l, 1.823 mg/l and 1.051 mg/l, respectively (Table 1).

Table 1. Physio-chemical analyses of the collected water sample (petroleum waste effluent; PWE)

Physio-chemical analyses	
pH	5.07 ± 0.14
Conductivity (ms/cm)	82.46 ± 0.92
TDS (g/l)	98.50 ± 0.10
salinity	116.0 ± 1.0
Ca (mg/l)	60.13 ± 0.27
Mg (mg/l)	189.0 ±3.0
Cu (µg/l)	1.20 ± 0.04
Zn (mg/l)	3.01 ± 0.085
Cr (µg/l)	5.00 ± 0.13
Mn (µg/l)	380.0 ±5.0
Fe (mg/l)	1.76 ± 0.02
Cd (µg/l)	2.00 ± 0.08
Pb (µg/l)	15.1 ± 0.21
Ni (µg/l)	2.99 ± 0.09
K (mg/l)	583.3 ± 5.51
Phosphate (mg/l)	0.14 ± 0.001
Silicate (mg/l)	6.11 ± 0.09
Nitrate (mg/l)	1.82 ± 0.01
Nitrite (mg/l)	1.05 ± 0.003

Growth of *T. chuii* under different concentrations of PWE

In the present study, *T. chuii* has been chosen as microalgae species to estimate its biomass production on different effluent petroleum waste (PWE) percentages, due to its fatty acid contents that enhance biodiesel productivity. This microalga was cultivated for 16 days for biomass production. The growth parameters and other analyses were analyzed.

Growth production of microalga *T. chuii* on different PWE percentages were estimated as cell counts and optical density (OD) and as shown in Figure (1) and Figure (2), respectively. The results of cell count showed that the first-day of the culture was in lag phase and there were no differences between control and other microalga that grown on different percentages of PWE, but from the second day onwards, there was a gradual increase in the cell count of culture conditions. After 16 days of the culture, all the cultures begin to start the decreasing phase after day 12.

T. chuii grew well, even when they were cultured using PWE (Figure 1). After 16 days of culture, the microalgae cell numbers which grow on 1% of PWE non-significant changed compared to control, while, 3% and 5% of PWE showed a significant increase in their count. However, *T. chuii* which grown in 7% of PWE showed a significant decreased in cell numbers (Table 2). The highest cell density was found at 5% of PWE ($9.1 \times 10^6 \pm 0.16$ cells/ml) at day 12 of culture (Figure 1).

Optical density (OD) is a common measurement used to simply quantify the ability of light to pass through a sample at a specific wavelength. The culture growth was determined for every 2 days (Figure 1). *T. chuii* grown on each treatment during the cultivation period of 16 days was observed in Figure (2). The results showed that the OD of cultivated alga started with initial OD at 0.089 starting from day 2 of cultivation, the results showed that there were significant differences ($p \leq 0.05$) in the OD directly proportional with the increase of the percentage of the concentration of PWE except with the highest concentration (7%) when compared with their control (Table 3). The maximum growth in terms of OD (1.05 ± 0.16) was observed at day 12 when the concentration of PWE was 5% (Figure 2).

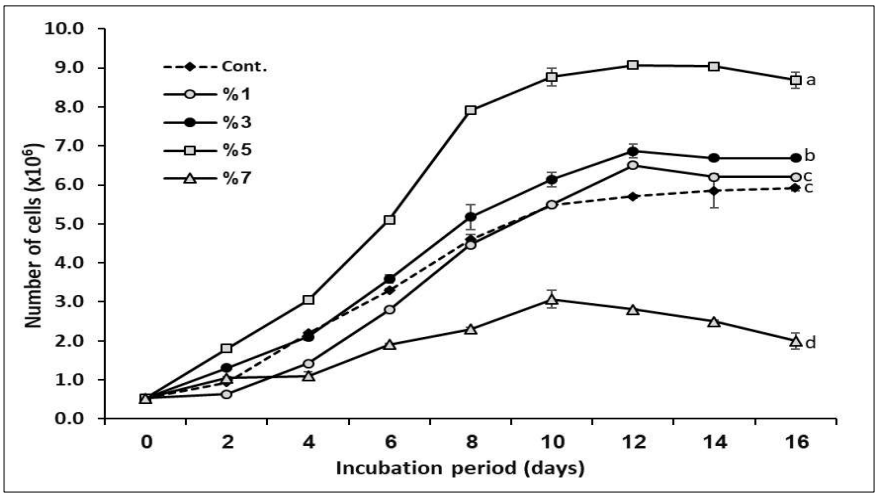

Figure 1. Growth by cell number $\times 10^6$ for microalgae *Tetraselmis chuii* grown on different PWE percentages during 16 days of culturing.
Means that do not share a letter are significantly different.

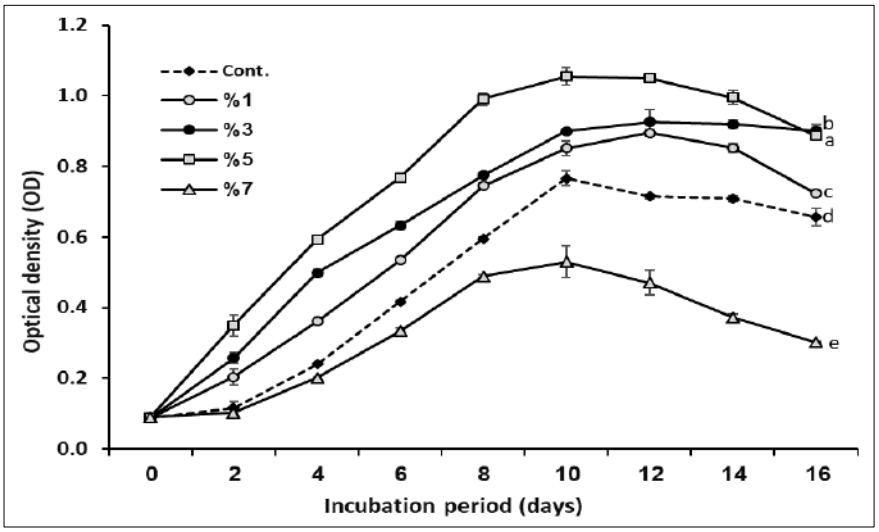

Figure 2. Optical density (OD) measurements for microalga *Tetraselmis chuii* grown on different PWE percentages during 16 days of culturing.
Means that do not share a letter are significantly different.

Total protein contents

Total protein content of microalga *T. chuii* grown on different PWE percentages were estimated as shown in Table 2. Under the experimental conditions, total protein contents (µg/ml culture) of microalga *T. chuii* ranged from 34.85 ± 1.95 µg/ml to 69.7 ± 3.9 µg/ml culture. The highest protein content (69.7 µg/ml) was recorded at 5% of PWE at the end of exponential phase of growth (day 8). The results also showed that, the increase of the total protein content in the microalgae increased by the increase

of the percentage of PWE until 5%, however the total protein content of the alga which grown in 7% was decreased even below the control condition (Table 2).

Table 2. Total protein contents (μg/ml culture) for microalga *Tetraselmis chuii* grown on different percentages of PWE during exponential growth phase (day 8).

PWE percentage	Total protein
Control	43.73 ± 2.44^{d}
1%	$47.51 \pm 2.66^{c,d}$
3%	$54.67 \pm 3.06^{b,c}$
5%	69.71 ± 3.90^{a}
7%	34.85 ± 1.95^{e}
F-Value	41.98
P-Value	0.000

*The same small letters indicate that there was no significant difference between the two groups, while the difference letters indicate that there was a significant difference between these two groups at $P < 0.05$ level of probability.

Fatty acids and biodiesel contents

In this study, the possibility of producing algal biofuel using different PWE percentages was investigated. As represented in Table (3; 4) and Figure (3; 4), the results showed that the contents of saturated fatty acid were higher than unsaturated fatty acids. Their comparison with the sum of individual fatty acids from day 8 (early exponential) and day 16 (late stationary phase) were represented. *T. chuii* showed high percentage of saturated fatty acid (SFA) contents at 5% of PWE (96.9%) at day 8 of incubation as compared to all other conditions. Gas chromatography-mass spectrometry (GC-MS) indicated that the presence of high content of palmitic (C16:0) which ranged from 22.3-52.6% of the total fatty acids at the end of exponential growth phase (day 8) as shown in Table 3.

Table 3. Fatty acid composition (% and mg/100 g microalga) of *Tetraselmis chuii* grown on different percentages of PWE at day 8.

	PWE percentages				
Fatty Acid	**Control**	**1%**	**3%**	**5%**	**7%**
Saturated fatty acid					
C4:0	0	0	0.03	0.24	0
C6:0	0.08	0.02	0.14	0.29	0
C8:0	0.03	0.11	0.6	1.19	0.14
C10:0	0.05	0.06	0.07	0.09	0.02
C11:0	0.04	0.06	0.13	0.17	0.1
C12:0	0.46	0.1	0.3	0.11	0.1
C13:0	1.11	0.11	0.09	0.11	0.05
C14:0	0.49	0.14	0.14	0.15	0.11
C15:0	0.63	0.08	0.07	0.06	0.03
C16:0	1.68 **(22.3%)**	3.97**(52.6%)**	3.07**(40.7%)**	2.73**(36.2%)**	3.48**(46.1%)**
C17:0	0.21	0.05	0.05	0.12	0
C18:0	0.80 **(18.5%)**	2.51**(33.3%)**	2.27**(30.1%)**	2.02**(26.8%)**	2.91**(38.6%)**
C20:0	0.03	0.03	0	0	0.11
C21:0	0	0	0	0	0
C22:0	0	0	0	0	0
C23:0	0	0	0	0	0
C24:0	0	0	0	0	0
Monounsaturated FA					
C14:1	0.15	0.06	0.04	0.1	0.02
C15:1	0.45	0.04	0.03	0.03	0.02
C16:1	0.19	0.02	0.03	0	0
C17:1	0.02	0.04	0.06	0.04	0.05
C18:1n9c	0	0	0	0	0
C20:1	0	0	0	0	0
C22:1	0	0	0	0	0
Polyunsaturated FA					
C18:2n6c	0.56	0	0.02	0	0.24
C18:3n3	0.11	0.08	0.1	0.07	0.04
C20:2	0	0	0	0	0
C20:3n3	0	0	0	0	0
C20:3n6	0	0	0	0	0
C20:4n6	0.06	0	0.04	0	0
C20:5n3	0.2	0	0.1	0	0
C22:2	0.1	0	0	0	0.08
C22:6n3	0.1	0.06	0.13	0	0.04
Total SFA	5.61**(74.6)**	7.24**(96.0)**	6.96**(92.6)**	7.28**(96.9)**	7.05**(93.5)**
Total UFA	1.94	0.3	0.55	0.24	0.49
Total MUFA	0.81	0.16	0.16	0.17	0.09
Total PUFA	1.13	0.14	0.39	0.07	0.4
Total PUFAs-n3	0.41	0.14	0.33	0.07	0.08
Total PUFAs-n6	0.62	0	0.06	0	0.24

Table 4. Fatty acid composition (% and mg/100 g microalga) of *Tetraselmis chuii* grown on different percentages of PWE at day16.

Fatty Acid	PWE percentages				
	Control	1%	3%	5%	7%
Saturated fatty acid					
C4:0	0	0	0	0	0
C6:0	0.07	0	0	0	0.01
C8:0	0.33	0.71	0.01	0.01	0.03
C10:0	0.21	0.5	0.02	0.01	0.01
C11:0	0.03	0.09	0.02	0.04	0
C12:0	0.32	0.41	0.15	0.11	0.15
C13:0	0.53	0.17	1.42	0.77	1.31
C14:0	0.26	0	0.71	1.14(15.0)	1.31(17.3)
C15:0	0.3	0.45	0.96	0.47	0.9
C16:0	2.70**(35.4)**	0.44**(5.8)**	1.57**(20.8)**	2.33**(31.0)**	1.16**(15.3)**
C17:0	0.15	0	0.34	0.15	0.14
C18:0	2.09**(27.7)**	0.38**(5.1)**	0.78**(10.4)**	1.74	0.6
C20:0	0.04	0.61	0	0.03	0.02
C21:0	0	0	0.17	0	0.04
C22:0	0	0	0	0	0
C23:0	0	0	0	0	0
C24:0	0	0.78	0	0	0
Monounsaturated FA					
C14:1	0.11	0.31	0.13	0.1	0.64
C15:1	0.21	0.28	0.69	0.34	0.65
C16:1	0.09	0	0.3	0.13	0.31
C17:1	0.06	0.52	0.02	0.04	0.01
C18:1n9c	0	0	0	0	0
C20:1	0	0	0	0	0
C22:1	0	0	0	0	0
Polyunsaturated FA					
C18:2n6c	0	0.44	0.08	0.04	0.13
C18:3n3	0.03	0.31	0.04	0.04	0.03
C20:2	0	0	0	0	0
C20:3n3	0	0	0	0	0
C20:3n6	0	0	0	0	0
C20:4n6	0.01	0	0.01	0.01	0.04
C20:5n3	0	0	0	0	0
C22:2	0.02	0.45	0.05	0	0
C22:6n3	0	0.67	0.04	0.04	0.06
SFA	7.03**(93%)**	4.54**(60.1%)**	6.15**(81.8%)**	6.8**(90.1%)**	5.68**(75.2%)**
UFA	0.53	2.98	1.36	0.74	1.87
MUFA	0.47	1.11	1.14	0.61	1.61
PUFA	0.06	1.87	0.22	0.13	0.26
PUFAs-n3	0.03	0.98	0.08	0.08	0.09
PUFAs-n6	0.01	0.44	0.09	0.05	0.17

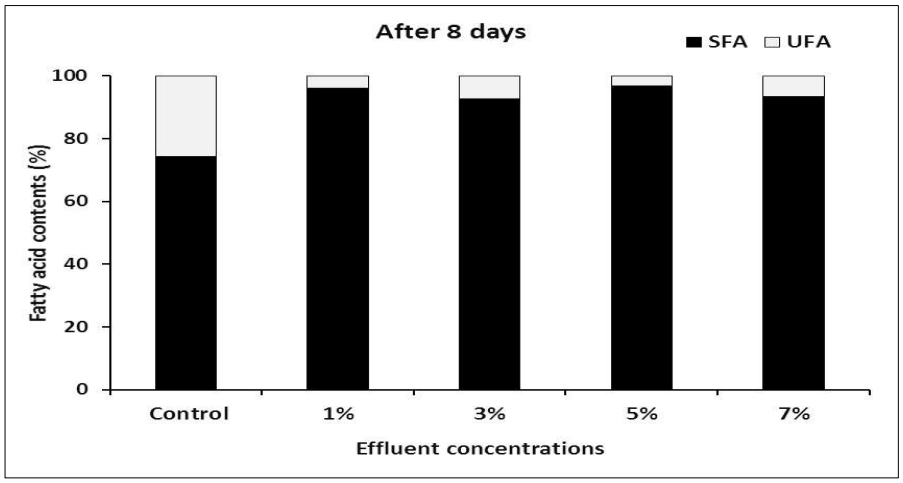

Figure 3. Percentage of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) of microalga *Tetraselmis chuii* grown on different percentages of PWE in early exponential (day 8).

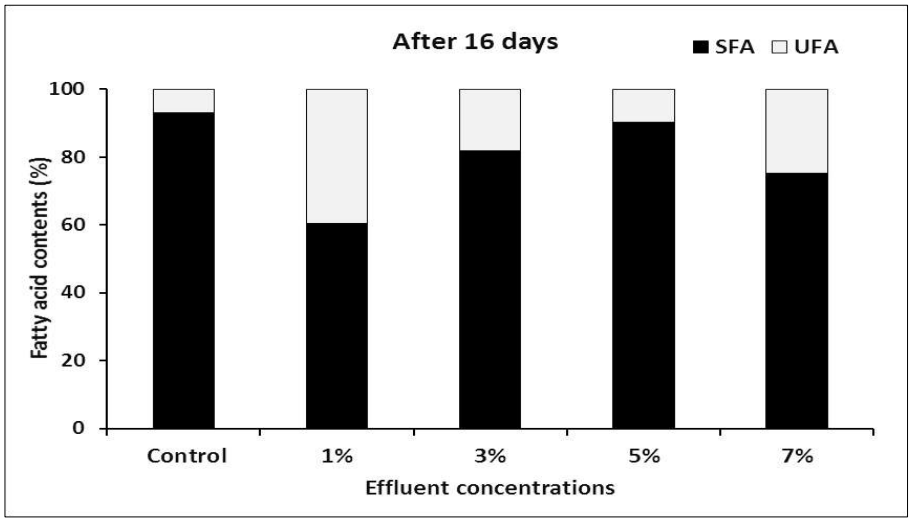

Figure 4. Percentage of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) of microalga *Tetraselmis chuii* grown on different percentages of PWE in late stationary phase (day 16).

Biodiesel obtained from *T. chuii* grown on different percentages of PWE at day 8 and day 16 was listed in Table 5. The higher biodiesel content was observed at 5% of PWE (0.741 ± 0.010 g/l culture) compared to all other conditions.

Table 5. Contents of biodiesel (g/l culture) of microalgae *Tetraselmis chuii* grown on different percentages of PWE in early exponential (day 8) and late stationary phase (day 16).

PWE percentages	Biodiesel (g/l culture)	
	8 day	16 day
Control	0.090 ± 0.004^{f}	0.132 ± 0.005^{c}
1%	$0.171 \pm 0.007^{d,e,f}$	0.031 ± 0.003^{e}
3%	$0.255 \pm 0.013^{c,d}$	0.071 ± 0.003^{d}
5%	0.741 ± 0.010^{a}	0.214 ± 0.030^{a}
7%	$0.140 \pm 0.005^{e,f}$	$0.040 \pm 0.002^{d,e}$
F-Value	102.31	110.44
P-Value	0.000	0.000

*The same small letters indicate that there was no significant difference between the two groups, while the difference letters indicate that there was a significant difference between these two groups at $P < 0.05$ level of probability.

The physico-chemical properties of biodiesel of *T. chuii* grown on different petroleum waste treatments and control medium in comparison to the recommended international standards were shown in Table (6). *T. chuii* that grown in 1% of PWE showed significant increase in the unsaturation degree reached (62.1) when compared with control (7.8), while, *T. chuii* that grown in 5% of PWE showed increase in the cetane number with the value equal 73.97 when compared with control (63.9). In contrast, *T. chuii* that grown in 5% of PWE showed significant decrease in the saponification and iodine values (189.5 mg $KOHg^{-1}$ and 5 g I2/100 g oil), as compared to their control (209.6 mg $KOHg^{-1}$ and 37.5 g I2/100 g oil), respectively. The long chain saturation factor and CFPP cold filter plugging point were significantly increased when *T. chuii* that grown in 7% of PWE.

Table 6. Estimated physicochemical properties of biodiesel of *Tetraselmis chuii* grown on different petroleum waste treatments and control medium in comparison to the recommended international standards.

Treatments	Du		SV(mg $KOHg^{-1}$)		IV (g I_2/100 g oil)		CN		LCSF (wt %)		CFPP °C	
	Exp	Stand.	Exp	Stand.	Exp	Stand.	Exp	Stand.	Exp	Stand.	Exp	Stand.
Cont.	40.66*	7.8	209.6	233.5	37.5	7.88	63.9	67.9	11.59	17.9	19.9	39.7
1%	6.05	62.1*	222.9	226.9	6.9	84.8	69.24	89.4	22.01	32.23	52.67	84.7
3%	12.45	21.02	197.8	226	12	21.4	71.19	65.6	19.12	7.28	43.6	6.39
5%	4.12	11.44	189.5	218.5	5	12.8	73.97	68.4	17.02	14.6	36.99	29.4
7%	32.51	28.2	224.4	241.0	16.6	51.13	66.8	57.5	25.41	2.03	63.35	11.0
EN 14214	-		-		≤ 120		≥ 51		-		≤ 5/≤ −20	
ASTM	-		-		-		≥ 47		-		-	

DU unsaturation degree, SV saponification value as mg $KOHg^{-1}$, IV iodine value as g I_2/100 g oil, CN cetane number, LCSF long chain saturation factor as wt %, CFPP cold filter plugging point as °C and percent of fatty acids with double bonds equal and/or higher than 4 as % of total fatty acids.

Cluster and correspondence analysis

Cluster analysis of different PWE percentages according to their degree of similarities based on the values of total protein contents, fatty acid and biodiesel contents (Figure 5). The results showed two main groups, the first one include control, 1% 3% and 5% PWE) the other group includes 7% PWE. Within the first group 3% and 5% PWE, have the highest degree of similarity among other PWE percentages.

The correspondence analysis plot (Figure 6) is from a table consisting of 5 rows, each representing different concentrations, and 11 columns represent the values of total protein contents, fatty acid and biodiesel contents. The correspondence analysis plot shows protein and biodiesel contents after 8 and 16 days are associated with 3% and 5% PWE percentages.

The percentages of saturated, mono and poly unsaturated fatty acids after 8 day showed a high degree of association with control, on other hand after 16 days saturated and mono unsaturated fatty acids are associated with 7% PWE. However, poly unsaturated fatty acids are associated with 1% PWE.

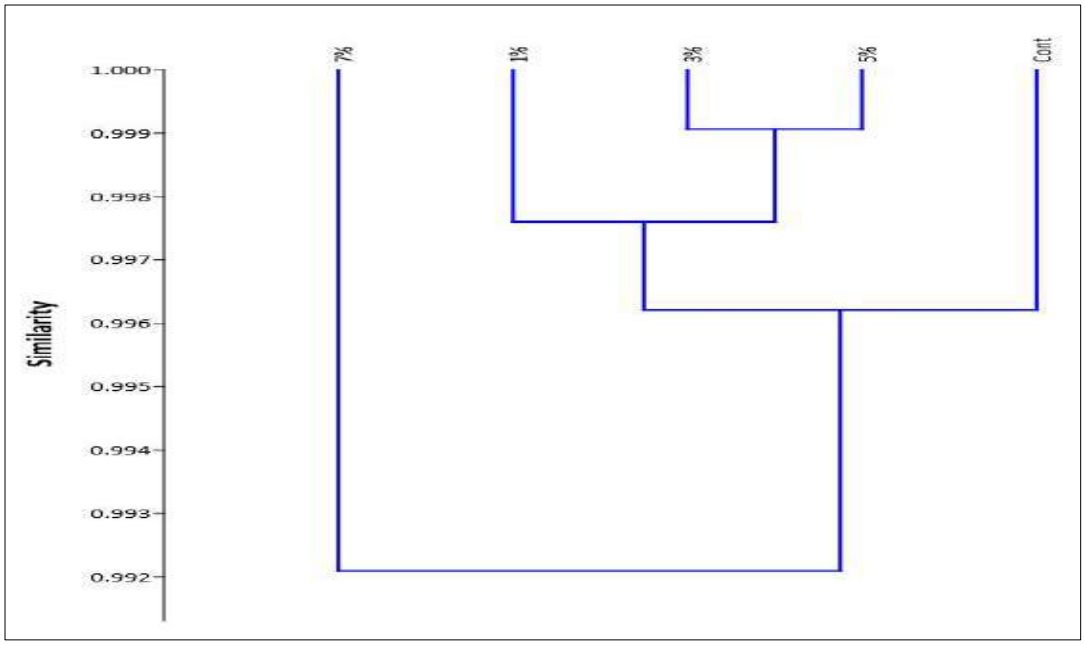

Figure 5. Cluster analysis of different PWE percentages according to their degree of similarities based on the values of total protein contents, fatty acid and biodiesel contents.

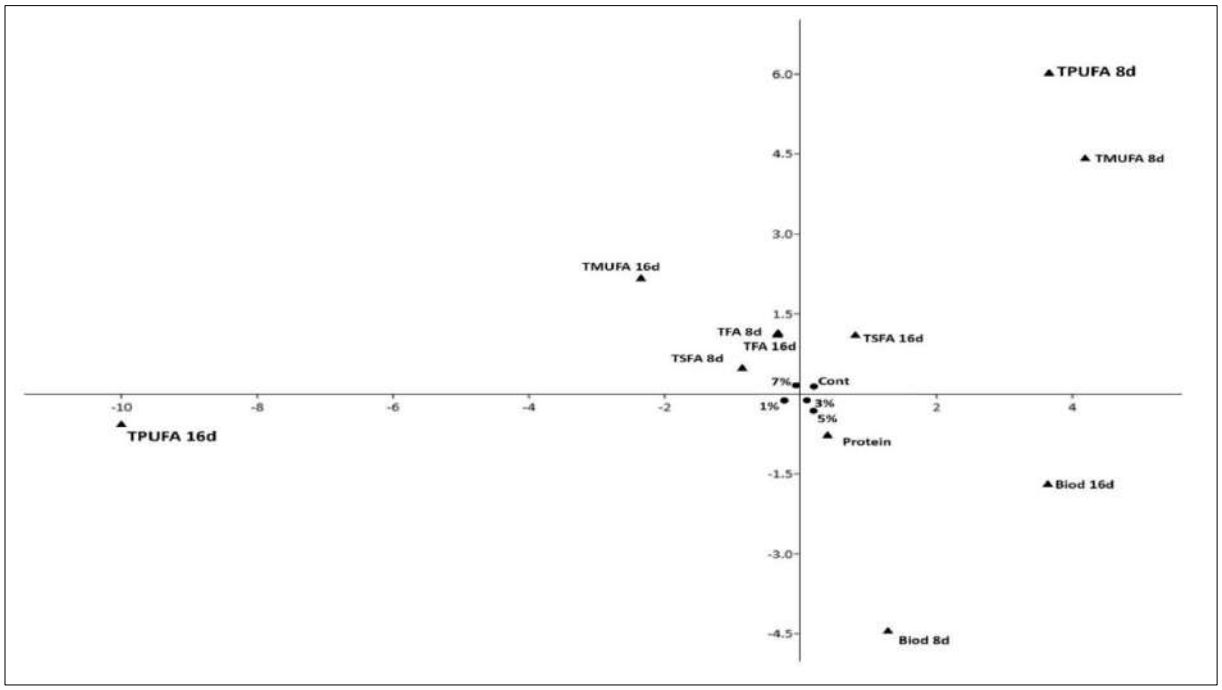

Figure 6. The correspondence analysis

Protein = **Total protein contents**
Bio 8d = **biodiesel contents after 8 days**
Bio16d = **biodiesel contents after 16 days**
TSFA_8 day = Total saturated Fatty acids **after 8 days**
TSFA_16 day = Total saturated Fatty acids **after 16 days**
TMSFA 8d = Total **Monounsaturated FA after 8 days**
TMSFA 16d =Total **Monounsaturated FA after 16 days**
TPUFA 8d= Total **Polyunsaturated FA after 8 days**
TPUFA 16d= Total **Polyunsaturated FA after 16 days**
TFA 8d= Total Fatty acids **after 8 days**
TFA 16d= Total Fatty acids **after 16 days**

Discussion

Several strategies have been investigated for renewable energy sources, to develop renewable energy industries that operate sustainably and competitive with other energy options is still challenged. Water pollution results from unwanted materials enter into water, changes the quality of water and causes environment and human health problems (ALRUMMAN et al, 2016). World health organization (WHO) reported that, 80% of diseases are water borne. Microalgae have wide availability, low cost and reliable quality which is able to absorb high metal pollutants such as zinc, uranium and lead ions from wastewater (MOGHADDAM et al, 2013, KUMAR et al, 2015). The effect of different PWE percentages on the growth rate of microalga *T. chuii* is considered an important factor for assessing the viability of using this waste as a growth media. This study aimed to investigate whether fatty acid profiles of microalgal are suitable for algal species selection and biodiesel characterization.

Fatty acid methyl ester (FAME) profiles were used to calculate the fatty acid composition, it is considered as a major factor affecting the quality of the produced biofuel (KWAN, 2013). The present levels of metals which determined in the PWE are found to be within the acceptable levels according to EEAA (2009). Although, the pH value was lower than the international acceptable limits for aquatic life (6-9), the current species tolerate the decreasing in pH value (LEAVITT, 1999). Who reported that, lowering the pH from 6.6 to 5.0 increasing the algal abundance. In some case, the high stress salinity value may be useful because of the natural response to salinity exploited to manipulate the bioactive compounds in microalgae such as the increased in fatty acid content with increasing salinity in the marine and freshwater microalgae (RENAUD and PARRY, 1994; SALAMA et al, 2014). Previous studies indicated that many microalgae able to tolerate salinity fluctuations (KIRST, 1989, VON ALVENSLEBEN et al, 2013). As reported by Behnaz et al (2013), various physiochemical properties such as light intensity, pH, salinity, temperature, nitrogen, salinity influence the lipids content of microalgae.

Physical and chemical properties of biodiesel are influenced by features of the fatty acids structures, such as unsaturation degree, chain length and carbon chain branching (ISLAM et al, 2013). According to Hii et al (2011), microalgae have been widely used for nutrient removal from wastewater, which is considered to be one of the most efficient, environment-friendly and simple treatments with a low cost compared to other physical and chemical treatments.

Furthermore, total phosphorus and toxic metals from wastewater can be removed efficiently when it is used for microalgae culture compared to chemical treatment which is usually at a high cost (PATEL et al, 2012). Culturing *Tetraselmis* sp. by using wastewater as a medium is better in removing total phosphorus, nitrates and total nitrogen (SIRAKOV and VELICHKOVA, 2014). Thus, it may serve as an effective way of treating and reusing wastewater before releasing to environment. Nitrogen is one of the important components for microalgae growth and is related to protein biosynthesis. In this study the growth medium was rich with nitrate 1.819 ± 0.011 mg/l (VASILEVA et al, 2015). Some organisms cultured in nutrient-rich condition can absorb the extra nitrogen from the culture medium and store it as proteins (ANDERSON, 2013). This explains the higher protein content in Conway + 5% PWE compared to other percentages. Interestingly, microalgae have proportions of proteins (6–52%), lipids (7–23%) and carbohydrates (5–23%) that are dependent on the species and environmental conditions (ELSER et al, 2000). Carbon, nitrogen, sulphur and phosphorus are major constituents needed for microalgal growth, some other nutrients such as iron, cobalt, zinc, are essential for good feedstock (GROBBELAAR, 2007).

Growing *T. chuii* on different percentages of PWE during the log phase, the total protein contents were increased by increasing the percentage of PWE. The previous study showed the chemical composition and protein contents of marine and freshwater microalgal biomass grown in photobioreactors was in agreement with our data (TIBBETTS et al, 2015). The major saturated and unsaturated fatty acids in microalgae are palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids (KNOTHE, 2009). The present study showed that the saturated fatty acids composition particularly, palmitic and oleic acid of *T. chuii* that grown on different percentages of PWE at different periods (8 and 16 days) were higher than the unsaturated ones. The higher degree of saturation of a biodiesel led to the lower the tendency of the biodiesel to oxidize. There are, however, other parameters which also define the oxidation stability of the fuel, for example natural anti-oxidant and free fatty acid content (KNOTHE, 2005; LAPUERTA et al, 2009). Our data was consistent with the previous study which reported that the good quality biodiesel should have a 5:4:1 ratio of C16:1, C18:1 and C14:0 fatty acids as recommended (SCHENK et al, 2008).

Cetane number indicates the ignition delay time and combustion quality of the diesel fuel. It has been reported that the higher cetane number the better ignition quality (MEHER et al, 2006). European standard for biodiesel fuel EN 14214 require the minimum cetane number for the biodiesel is 51 and as for ASTM D-6751 requires minimum cetane number 47. Cetane number for biodiesel produced form *T. Chuii* is 63.7 which comply with EN 14214 and ASTMD 6751. Cetane number for the microalgae species

varied between species due to the variation in the fatty acid composition for each microalga species. Amount of iodine in grams, which taken up by 100 grams of oil or fat is the iodine value (IV). Iodine value was not included in the ASTMD 6751 but it is included in EN 14214 standards which specify the maximum IV is 120 g I_2/100 g^{-1} oil, since heating high amount of unsaturated fatty acid may contribute to formation of deposits of the oil (ERIKA et al, 2009). Previous study by Chen et al (2012) on the fuel properties of *Chlorella protothecoides*, which reported that the IV obtained for the microalgae species, is 112.2 g I_2/100 g^{-1} oil which may due to high content of unsaturated fatty acids, 90.7%. However, in this research, IV obtained for *T. chuii* biodiesel is 59.3 g I_2/100 g^{-1} oil which is lower than IV obtained for *C. protothecoides*. Thus it is suggested that higher the IV indicates higher amount of unsaturated fatty content in the lipid. Our results showed that, the percentages of saturated, mono and poly unsaturated fatty acids after 8 day showed a high degree of association with control, on other hand after 16 days saturated and mono unsaturated fatty acids are associated with 7% PWE. However, polyunsaturated fatty acids are associated with 1% PWE. The percentage of concentration of SFA acid was high as compared with the unsaturated one and subsequently, this may improve the quality of obtained biofuel.

Saturated fatty acids and chain length of algae lipids cause noticeable changes in the biodiesel properties. They have significantly higher melting points than unsaturated fatty acids. Therefore, biodiesel fuels derived from fats or oils with significant amounts of saturated fatty compounds display poor cold flow properties (DUNN, 2005). In accordance to the present results, Knothe (2009) reported that the most common fatty acids methyl esters (FAMEs) present in biodiesel burning qualities, are palmitic (C16:0) and oleic acid (C18:1), which were also the major fatty acids in both studied microalgae in the present study. High percentage of those saturated fatty acids increases the biodiesel quality due to increasing of cetane number which decreased by increasing the degree of unsaturation or the number of double bonds (KINOSHITA et al, 2006).

Kwan (2013) stated that, saturated fatty acids are ideal for biofuel production. As no double bonds and functional group along the carbon chain and therefore the obtained biofuel will have high oxidative stability. Helena et al (2018) evaluated the growth, proximate composition and carotenoid production of *T. chuii* cultured in different aquaculture wastewater media. Their results reported that significant ($p < 0.05$) higher growth (4.3×10^5 cells/ml), carbohydrate (20% of dry weight), lipid (44% dry weight) and protein (56.4% dry weight) contents were detected in *T. chuii* when they were cultured on both of wastewater and Conway media.

Conclusion

This study concluded that the microalgae *T. chuii* can grow in the PWE till 5% wastewater + Conway, however, their growth starts to decline at 7% and this finding support using PWE as a media for growing and producing biodiesel from tested microalga.

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