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ARTICLE

Culturing of green photosynthetic microalgae (*Chlorella* sp.) using palm oil mill effluent (POME) for future biodiesel production

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ABSTRACT

Nowadays, the water pollution is leading issue due to palm oil mill effluent (POME), waste from palm oil production is a big problem to Malaysia which is largest palm oil producers. POME containing large amount of chemical oxygen demand (COD) which can cause severe pollution to the environment especially water. This present study designed for culturing green algae (*Chlorella* sp.) isolated from Pantai Balok, Kuantan which is most common microalgae with palm oil mill effluent (POME). *Chlorella* sp. was cultured using BG-11 medium with the addition of POME as experiment and without POME as control. The POME responsible for the growth of green algae is studied by measurement of the growth rate, total cell count and chemical oxygen demand (COD) for both conditions. The composition for control and experiment is also measured and determined by using Gas Chromatography Mass Spectrometry (GCMS). From the results, it is observed that the total cell count and growth rate of *Chlorella* sp. greater in presence of POME since the green microalgae absorbs the essential nutrients from the POME as their nutrients. GCMS revealed that the difference in composition for both the conditions. Results concluded that several COD also vigorously deplete with the help of green algae digestion. Cultivation of *Chlorella* sp. in POME will help in future water pollution treatment.

1. Introduction

The algae are found as the diverse group of photosynthetic organisms. This organism uses photons from the sunlight and chlorophyll (green pigment in algae) to prepare their own food. As a result of photosynthetic activities, algae produce a large portion of oxygen present in this atmosphere and generate huge amount of organic carbon (Bhuyar et al., 2019a). They are microscopic and macroscopic, unicellular or multicellular, mobile and immobile, attached and free-living (Tipnee et al., 2015). The algae can be found in water such as river, sea and estuary at different geographic latitudes. They grow in water with different level of

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salinity, organic matter, hydrogen ions and at variety of temperature. Microalgae, a type of algae are one of the ancient microorganisms on earth. They are single cell organisms, including of both bacteria and eukaryotes. Common microalgae are including in Eukaryotes which have organelles to control the functions of the cell, for the purpose of survive and reproduce (Ramaraj et al., 2014a).

Nomenclature and abbreviation	
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
GCMS	Gas Chromatography Mass Spectrometry
POME	Palm Oil Mill Effluent

There are two ways of microalgae species organization which are unicells and colonies. Unicells occur in a variety of shapes, but coccoid microalgae that take the form of small round balls may have the most common body type found among the algae. Besides, microalgae are able to divide once every 3-4 hours under favorable growing conditions (Saengsawang et al., 2020). This is because microalgae have simple cellular structure and large surface to volume ratio that making them able to absorb high quantity of nutrients from water sources and therefore increase their growth rate (Ramaraj et al., 2016). Microalgae can be divided into four groups depends on the pigmentation, life cycle and basic cellular structure which are diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cynophyceae) and golden algae (Chrysophyceae). Presently, microalgae are used in foods and health foods, as aquaculture feeds and for production of pigments, polyunsaturated fatty acids and other fine chemicals (Gangl et al., 2015).

There are number of industries that contribute to Malaysia's economy and one of the major industries is palm oil industry which is the largest production in the world (Bhuyar et al., 2019b; 2020). Malaysia is the largest producers and suppliers of palm oil in the world. In 2000 and 2008, 8.3 and 16.3 million tonnes of annual production figures respectively were estimated the oil palm fresh fruit bunches processed producing a total of 423 mills up to 89 million tonnes of per year include about 66.8 million tonnes of palm oil mill effluent (Jayakumar et al., 2017). This palm oil production produces various forms of solid and liquid wastes including empty fruit bunches (EFB), palm press fiber (PPF), palm kernel cake (PKC), palm kernel shell (PKS), sludge cake (SC) and palm oil mill effluent (POME) (Lam and Lee, 2011).

POME is a waste that produces from the last stage of palm oil production process that rich in 95-96% water, 0.6-0.7% oil and 4-5% total solids. It is a viscous acidic brownish colloidal suspension (pH between 4 and 5) with unpleasant odor, originating from the mixture of sterilizer condensate, separator sludge and hydrocyclone wastewater in a ratio 9:15:1 respectively (Cheah et al., 2018). POME can affect environment such as may alter aquatic habitats, affect aquatic life and harmfully impact human health if it is discharged untreated into marine ecosystems (Dussud et al., 2018).

This is because it contains the biological oxygen demand (BOD), chemical oxygen demand (COD), oil and grease, total solids and suspended solids that ranges from 25,000 to 35,000mg/L, 53,630mg/L, 8370mg/L, 43,635mg/L and 19,020mg/L respectively (Idris et al., 2018). Since POME is brownish color, it cannot be discharged directly into the natural waterways without proper treatment. There are some treatments that were applied to treat POME such as conventional oxidation ponds (aerobic and anaerobic), open and closed tank digesters with biogas recovery and land application (Yahmed et al., 2016).

Based on the previous studies, anaerobic digestion or treatment of POME with microalgae is more effective (Bhuyar et al., 2019a). It is proved that the microalgae play a significant part in self-purification of natural waters and therefore recommend an alternative means as a tertiary treatment of organic wastewater (Tsai et al., 2015). Although POME is a waste and can cause a big pollution, but in the same time it has its own value added products such as carotenoid which utilize for vitamin A and vitamin E, citric acid, fertilizer, biodiesel and hydrolytic enzymes (Wannapokin et al., 2018). Furthermore, POME was identified as a potential source to generate renewable bioenergy's such as biomethane and biohydrogen through anaerobic digestion. It was found as a good nutrient for the growth of microalgae since it composes of nitrate and ortho-phosphate that are needed for microalgae growth and thus producing the biofuels such as biodiesel and bioethanol (Ramaraj et al., 2014b;2016).

Therefore, the main aim of this study was to identify the POME enhances the growth of green algae (*Chlorella* sp.) and how based on the composition measured for control (*Chlorella* sp. culture without POME) and experiment (*Chlorella* sp. culture with POME). Besides that, the green algae reduce the chemical oxygen demand of POME also is investigated. Thus, the microalgae can expect to remove the pollution of POME.

2. Materials and Methods

2.1. Microalgae sample collection

Algae sample collection was made at Pantai Balok, Kuantan by using plankton net with 0.50 µm of size and was put into a plastic container. Three plastic containers contained the microalgae samples were taken. After the collection, samples were brought to the laboratory for isolation and identification. Then, after the identification, samples were cultured in mass culture. Palm oil mill effluent (POME) was collected at palm mill of Felda Lepar Hilir, Gambang in Pahang.

2.2. Identification and screening microalgae

A single cell of green algae was isolated by spread onto a petri plate containing BG11 medium and incubated at room temperature (250ºC-270ºC) under 24 hours light for two weeks. The sample was analyzed in species level (Bhuyar et al., 2020a,b). For the analysis, slide was prepared. Two drops of sample were added onto a clean slide and attached with a cover glass. Larger form was scanned at low magnifications and the smaller ones needed the use of oil immersion at higher magnifications. Identification was made

using standard manuals and local floras. The species found are *Chlorella* sp. This species has a spherical in shape and no flagella.

2.3. Algae culture media preparation

BG-11 medium was used for mass culture of green algae. BG11 medium which contains $NaNO_3$, K_2HPO, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Citric acid.H_2O, Ammonium Ferric Citrate, $Na_2EDTA.2H_2O$, Na_2CO_3, BG11 Trace Metal Solutions (H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4{\cdot}7H_2$, $Na_2MoO_4.2H_2O$, $CuSO_4{\cdot}5H_2O$ and Co $(NO_3).6H_2O$). *Chlorella* sp. sample was inoculated into 250 ml of BG11 medium in a conical flask to get 500 ml of mass culture under uniform culture conditions at 25-27°C with 24 hours light (Bhuyar et al., 2019a).

2.4. Culturing green algae (Chlorella sp.) using palm oil mill effluent (POME)

For this stage, 300 mL of *Chlorella* sp. mass culture was used. The mass culture in the conical flask was divided into two new conical flasks equally, 150 mL for each flask. The first flask was marked as a control (contained 150 mL mass culture of *Chlorella* sp. plus 100 mL BG11 medium) and the second flask as an experiment (contained 150 mL mass culture of *Chlorella* sp., 100 mL BG11 medium and plus 4 mL of palm oil mill effluent (POME)). Both conical flasks were incubated at proper environment as stated. The parameter involved were chemical oxygen demand (COD) by COD meter, total cell count by hemocytometer and growth rate by spectrophotometer.

2.5 Measurement of chemical oxygen demand (COD)

Wastewaters chemical oxygen demand (COD) was measured by using COD meter with low range solution (Wang et al., 2010). The COD meter that was used is Hach spectrophotometer (DR 2800). Both samples, control and experiment were pipetting in two ml (2000 µl) of amount and were inserted into prepared low range (LR) standard solution of COD meter respectively. The standard solutions were then heated in a heater (DRB 200) for two hours at 150°C. After the heating, let them to cool before measured the COD by Hach spectrophotometer. The COD was measured two times at initial (first day) and final (ten days) of cultivation. COD of control and experiment samples were recorded and compared. COD meter was used to measure the chemical oxygen demand content in water like pond water, lake, river, sea and wastewaters. It consists of two separate parts which were a heater and spectrophotometer. The samples must be heated first by mixed it with low range or high range standard solution before measured their concentration. The spectrophotometer showed result in unit of mg/L.

2.6. Total cell count

The total cell count of *Chlorella* sp. culture with POME was calculated by using hemocytometer under a microscope (Nikon-E100). Hemocytometer was designed to calculate the small cells like algae to ensure that a reasonably large count (example: 50-100 organisms). Firstly, the hemocytometer was cleaned by 70% ethanol to remove the dusk and another unneeded substance. The sample was pipetting in 10 µl of amount and put on hemocytometer with a cover slip. The algae cells were counted using a microscope (Nikon-E100) at 40x magnification (Andersen and Lin, 2005). Total cell count of control and experiment samples were recorded and compared. Microscope was helped to observe the small particle especially cells that were too small and cannot be seen by the naked eyes. A specimen was observed by put it on a glass slide and seen through the objective lens started from low magnification (4x) to high magnification (100x) with immersion oil applied.

2.7. Growth rate

The culture solutions were observed from day to day and measured the growth rate by using a spectrophotometer (Thermo-GENESYS 20) at 680 nm wavelength to get the concentrations (Ramaraj et al., 2014b). The growth rate of control and experiment samples were recorded and compared. Spectrophotometer was used to measure the amount of light (concentration) that absorbed by a sample solution. A beam of light was passed through a sample and once it reached a detector, its light density was measured. The light intensity depends on wavelength used.

2.8. GC-MS analysis

The *Chlorella* sp. samples for both of control and experiment were injected into HP-5 column (30 m x 250 µm x 0.25µm), Agilent Technologies, 7890A QC System model. Chromatographic conditions were as follows: helium as carrier gas, flow rate of 34.5 ml/min; injector and column oven temperature 350 °C and 80 °C; injection mode, Split and Split ratio 1:20. Oven temperature was isothermal at 80 °C for 1 min, then increased to 300 °C at 5 min with a rate of 4 °C/min and held isothermal at 61 min. MS conditions were as follows: ionization voltage of 70Ev; ion source temperature of 230 °C; interface temperature of 250 °C; mass range of 39-144 mass units (Bhuyar et al., 2019).

2.9. Statistical analysis

The statistical analysis was performed by an application of SPSS software 16.0 (Statistical Program for Social Sciences). The means of total cell count, growth rate and chemical oxygen demand (COD) were compared between the control and experiment samples by One-way Anova analysis of variance in conjunction with Tukey's test.

3. Results and discussion

3.1. Isolation and identification of microalgae

The microalgae that have been collected from Teluk Cempedak that were isolated were observed under fluorescent microscope to identify according the microalgae manual book. The identification was based on their morphology, color, shape and the physical of the microalgae. According to the algae manual and morphological identification the microalgae was identified as *Chlorella* sp. as

observed in Fig. 1. The microalgae that have been isolated were identified by referring to the study done by Selvarajan et al. (2015). The microalgae observed under the fluorescent microscope (Fig. 1) have the same characteristics as *Chlorella* sp. From the observation, the microalgae were green in color thus it indicated that the microalgae are in the division of chlorophyta. Chlorella has round or oval shape with the diameter between 2-15 µm (Corliss et al., 1977). *Chlorella* belongs to the class Trebouxiophyceae, order Chlorellales, family Oocystaceae and genus *Chlorella* sp. This species usually found cluster or single form.

They can be found in salt or fresh water as well as in soil (Beuckels et al., 2015). *Chlorella* can be used as food source, biofuel and for the wastewater treatment as they can efficiently remove nitrogen, phosphorus, COD and at the same time BOD. This microalga can produce oxygen during their photosynthesis and the oxygen can be used by the bacteria present in the wastewater to converts the nutrients in wastewater into biomass. They can also utilize nutrients available in the wastewater for their growth and metabolism (Ahmad et al., 2013) as *Chlorella* can with stand highly saline environment (Nurul et al., 2013).

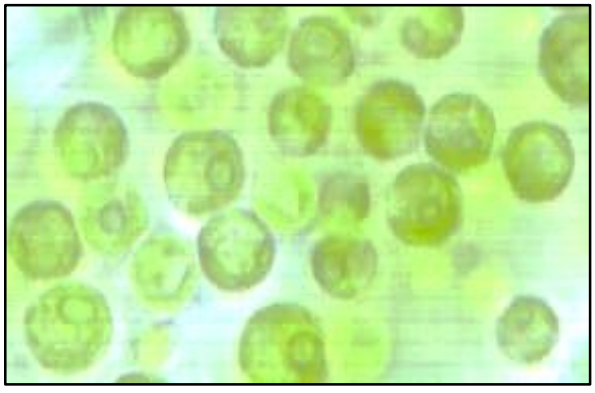

Fig. 1. Morphological image of *Chlorella* sp. green microalgae under fluorescent microscope at 100 X magnification.

The *Chlorella* was exposed to the artificial light for 24 hours as the light play an important role in the growth of microalgae. The light energy will be converted into chemical energy. The phase of converting the light energy into chemical energy is called photochemical phase. *Chlorella* produced adenosine triphosphate (ATP), Nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and oxygen (O_2) (Al-Qasmi et al., 2012). The *Chlorella* can use the oxygen released from the photosynthesis for their respiration and produce carbon dioxide (CO_2).

3.2 Growth rate analysis

The previous studies measured the concentration of algae biomass that was cultured with POME by using spectrophotometer at 680 nm wavelength (Hwang et al., 2016). Based on Table 1, it showed for the reading of spectrophotometer at 680 nm wavelength for the growth rate of *Chlorella* sp. culture (control and experiment). The growth rate of both samples was always increased for each day. The green algae were facing a proliferation reaction. Roughly, the growth rate of experiment sample was always greater than control sample started from day one until day ten. That means, the algae in experiment sample was growth vigorously than the algae in a control sample. A bar chart (Fig. 2) was represents the data in Table 2. Growth rate (A) was represented by y-axis while the x-axis represents the day (first until tenth). The bar showed an increasing growth rate for both control and experiment samples for each day with a maximum value at day ten. Although that, experiment sample always had a higher value than control sample.

Palm oil mill effluent was proved that it was enables to enhance the growth of microalgae since the contents of some nutrients such as nitrate and ortho phosphate in it that really needed by the microalgae.

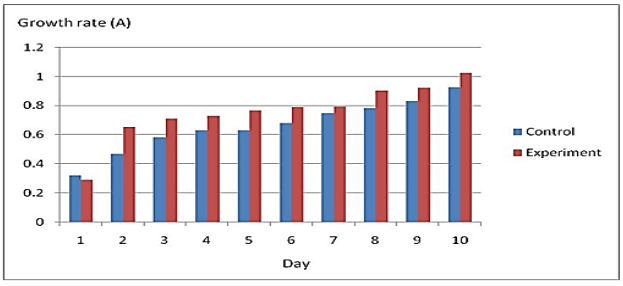

Fig. 2. The growth rate of microalgae cultivated in POME for 10 days' incubation absorbance at 680 nm.

The peak of spectrums showed that the different composition contained in both control and experiment samples. The growth rate was measured three times for each day (triplicate data). Based on an above Table, One-way Anova analysis by Tukey test showed that the data of growth rate for control and experiment samples of *Chlorella* sp. mostly were significant (p value < 0.05).

Table 1

Growth rate for control and experiment of *Chlorella* sp. samples.

Day	Growth rate (A) for control	Growth rate (A) for experiment
1	0.323	0.291
2	0.469	0.654
3	0.583	0.712
4	0.633	0.730
5	0.634	0.768
6	0.683	0.793
7	0.749	0.795
8	0.785	0.906
9	0.831	0.924
10	0.927	1.026

Based on previous research, the growth rate and biomass productivity of microalgae (*Chlorella* sp.) was affected by POME (Cheah et al., 2018). Diluted palm oil mill effluent was allowed the growth rate, biomass, lipid content and chlorophyll in different species of microalgae to increase. Besides that, POME was a good and better carbon source for microalgae culture (*Chlorella* sp.) to growth vigorously compared than another municipal wastewater (Cheah et al., 2018; Nayak and Vyas, 2019).

Table 2

Tukey test (One-way Anova) of growth rate.

		No	Std. Deviation	Significance
1	Control	3	0.004163	0
2	Control	3	0.004041	0
3	Control	3	0.007024	0
4	Control	3	0.004933	1
5	Control	3	0.004619	0
6	Control	3	0.003512	0
7	Control	3	0.004509	0
8	Control	3	0.002646	0.294
9	Control	3	0.001528	0
10	Control	3	0.004163	1
11	Experiment	3	0.002082	0
12	Experiment	3	0.002646	0
13	Experiment	3	0.003055	0
14	Experiment	3	0.002309	0
15	Experiment	3	0.003786	0
16	Experiment	3	0.003786	0.817
17	Experiment	3	0.001528	0.623
18	Experiment	3	0.005508	0
19	Experiment	3	0.003786	1
20	Experiment	3	0	0

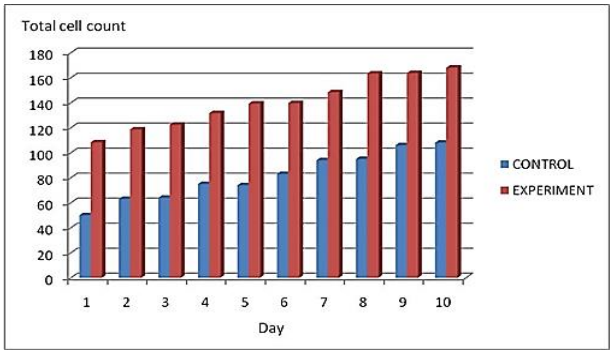

Fig. 3. The braph representing the bar chart of total cell count of microalgae.

Based on previous research, the growth rate and biomass productivity of microalgae (*Chlorella* sp.) was affected by POME (Cheah et al., 2018). Diluted palm oil mill effluent was allowed the growth rate, biomass, lipid content and chlorophyll in different species of microalgae to increase. Besides that, POME was a good and better carbon source for microalgae culture (*Chlorella* sp.) to growth vigorously compared than another municipal wastewater (Cheah et al., 2018; Nayak and Vyas, 2019).

3.3 Total cell count

The Based-on Table 3, the total cell count for both of experiment (*Chlorella* sp. culture with POME) and control

Table 3

Total cell count for control and experiment of *Chlorella* sp. Samples.

Day	Total cell count for control (number of cells)	Total cell count for experiment (number of cells)
1	50	108
2	63	119
3	64	122
4	75	132
5	74	139
6	83	140
7	94	148
8	95	163
9	106	164
10	108	168

Table 4

Tukey test (One-way Anova) of total cell count.

		No	Std Deviation	Significance
1	Control	3	2	0
2	Control	3	4.3589	1
3	Control	3	0.57735	1
4	Control	3	2.08167	0.013
5	Control	3	4.58258	0.0015
6	Control	3	2.08167	0.0075
7	Control	3	2.3094	0
8	Control	3	0	0
9	Control	3	4.50925	0
10	Control	3	1.73205	0
11	Experiment	3	1.52753	0
12	Experiment	3	2.51681	0.895
13	Experiment	3	1.1547	0.002
14	Experiment	3	0.57735	0.012
15	Experiment	3	1.1547	0.012
16	Experiment	3	1.1547	0.009
17	Experiment	3	1.52753	0.004
18	Experiment	3	0.57735	0.583
19	Experiment	3	2.3094	0.704
20	Experiment	3	1	0.644

(*Chlorella* sp. culture without POME) were always increased started from day one until day ten. When compared between control and experiment, total cell count in experiment sample was larger than control sample from day one until day ten. That means there was lot of algae cells in BG11 medium with POME compared to the algae cells in BG11 medium only. A bar above (Fig. 3) was represents the data from the Table 3. Day (one to ten) was represented by x-axis and total cell count was represented by y-axis. The bar showed an increasing total cell count for both control and experiment samples for each day with a maximum number at day ten. Although that, experiment sample always had a higher value than control sample. The existence of palm oil mill effluent in BG11 (Hariz and Takriff, 2017).

The number of cells were counted three times for each day (triplicate data). Based on an above Table 4, One-way Anova analysis by Tukey test showed that the data of total cell count for control and experiment samples of *Chlorella* sp. mostly were significant (p value < 0.05).

3.4. Chemical oxygen demand (COD)

Table 5 showed the reading for chemical oxygen demand (COD) at initial and end of *Chlorella* sp. cultivation. At initial, COD for experiment sample was high than control sample while at the end of cultivation, COD for experiment sample was less than control sample. There was a reduction at both of period but the COD in experiment sample was vigorously decreased. A bar above (Fig. 4) was represents the data from the Table 5. X-axis was representing a period (initial and end of cultivation) and y-axis was representing COD measurements. The bar showed that the reducing amount of COD for both control and an experiment sample. Even though, the reduction for experiment sample was more rapid than a control sample. Digestion of palm oil mill effluent by green algae can reduced a lot of number of chemical oxygen demand (COD) where the high COD of water, the high pollution occurred in that water which contained a greater amount of organic and inorganic matter.

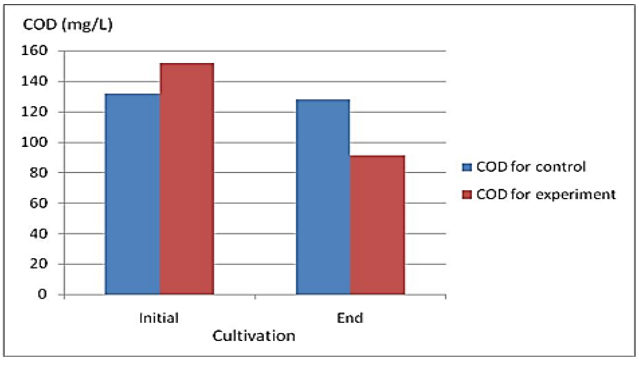

Fig. 4. The COD (mg/mL) level of microalgae and wastewater proportions.

Based on the previous study, palm oil mill effluent (POME) in an anaerobic bioreactor (Lam and Lee, 2011). They found that the anaerobic digestion played a major role in removing most of COD concentrations in POME. The growth of microalgae in POME led to a reduction in chemical oxygen demand (COD) and can return the acidic pH of POME to alkaline (Wang et al., 2010). After 20 days treatment of palm oil mill effluent by anaerobic microorganisms, there were a reduction in the COD by 75%, BOD by 90% and the total solids by 70% (Lam and Lee, 2011). Bhuyar et al. (2020) was cultured green algae, *Chlorella* vulgaris with wastewater namely palm oil mill effluent for the intention to remove COD. In their results, the rate of COD was depleted from day one until day twelve.

The COD reading were taken three times at the initial and end of cultivation (triplicate data). Based on an above table, One-way Anova analysis by Tukey test showed that the data of chemical oxygen demand (COD) for control and experiment samples of *Chlorella* sp. mostly were significant (p value < 0.05).

Table 5

Tukey test (One-way Anova) of COD.

	Sample	No	Std. Deviation	Significance
1	Control (initial)	3	0.2887	0.001
10	Control (end)	3	0.3606	0.536
11	Experiment (initial)	3	0.1528	0.001
100	Experiment (end)	3	0.495	0

Table 6

GC-MS analysis of sample (POME treated microalgae).

Peak	Retention time (min)	Name of compounds
3	17.142	Propanamide
5	24.806	Phthalic acid, di (3,4-dimethyl) ester
7	27.476	7-chloro-3-[4-cylcohexylphenyl]-3 4-dihydro-10-hydroxy-1,9(2H, 10H)-a cridinedione
8	30.706	5-(2-Aminopropyl)2-methylphenol
9	34.297	3-Methoxyamphetamine
10	37.842	Phenethylamine,p,.alpha.-dimethyl
11	45.583	Silane,diethyldodecyloxy(3-phenyl propoxy)
12	45.902	2,2,8,8,12,13,17,18-Octamethyl-2,3,7,8,22,24-hexahydro-porphine-5-carbonitrile
1	3.875	Tetratriacontane
6	24.936	Hexadecanoic acid

3.5. GC-MS analysis

Table 6 shows the compounds that were detected by Gas Chromatography Mass Spectrometry (GCMS) for the experiment

samples of *Chlorella* sp. respectively. The present study revealed that many compounds found in experiment sample. The compositions in palm oil mill were consumed by the *Chlorella* sp. since that POME act as additional nutrients for the green algae to grow. In a cultivation process, organic compounds in palm oil mill effluent was faced a degradation that lead to an increase of inorganic nutrients that contributed in greater microalgae cell density. Raw POME had a higher concentration of nitrate and orthophosphate that can be used as nutrients for green algae growth. That nutrients will be depleted as resulted from green algae utilization, hence at the end of culture, the pollutants have been removed (Bhuyar et al., 2020c,d; Govinadan et al., 2020).

The peak number of 2, 3, 4, 5, 6, 7, 8 and 9 for a control sample having the same compounds with the peak number of 3, 5, 7,8,9,10,11 and 12 for an experiment sample (Table 6). Even though, there were four peaks with number 1, 2, 4 and 6 (3.875, 16.835, 21.903 and 24.936) that was detected by GCMS in an experiment sample only. A peak number of 6 was recorded for a compound namely hexadecanoic acid. This component is an acid that can be found in oil from palm trees and behave as a major component (McCurdy et al., 2014). In Table 6, the peak number of 2 and 4 (16.835 and 21.903) were cannot be detected what the compounds presence because the retention time of 16.835 and 21.903 did not covered in the listed chemical compounds for GC-MS analysis.

4. Conclusions

In the present study wastewater from palm oil industries namely palm oil mill effluent (POME) must be discharged well and with a good treatment because it can lead to a water pollution that will affect an aquatic organism. Treatment by microalgae is a good solution since POME has some nutrients that can enhance the microalgae growth. The results of total cell count and spectrophotometer shows that the cell number and growth rate of green algae culture with palm oil mill effluent were higher than the normal culture of green algae. At the same time, anaerobic digestion of microalgae by consuming the POME as a carbon source for them, lead to POME depletion. Due to the results obtained, it shows that the green algae were absorbs the components in that effluent. Palm oil mill effluent has a high chemical oxygen demand that can pollute the water. From this study, the green algae that was collected from Sungai Balok, Kuantan has an ability to reduce the COD content in POME, hence decrease or slightly remove the pollution.

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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