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Quality Profile of Spirulina-platensis Oilgae Extraction for Biodiesel Production

KEYWORDS: Algae biodiesel; lipids; biomass; extraction; algal oil; Spirulina-platensis; Soxhlet; safety assessment.

H. I. El Shimi	N. K. Attia	A. A. Abdel Allah
Chemical Engineering Department, Faculty of Engineering, Cairo University, Egypt	Chemical Engineering and Pilot Plant Department, National Research Center, Egypt	Microbiology Department, Soil, Water and Environment Institute, Agricultural Research Center, Egypt

S. T. El Sheltawy	G. I. El Diwani
Chemical Engineering Department, Faculty of Engineering, Cairo University, Egypt	Chemical Engineering and Pilot Plant Department, National Research Center, Egypt

ABSTRACT *Algal biodiesel has received much attention in recent years; due to the need for energy and declining of fossil fuels. Biodiesel production from microalgae appears to be suitable biofuel; as microalgae are the fastest-growing plants on the earth, important lipids source (10–70 % by wt) and can be cultivated with the primary treated municipal wastewater in desert areas. Extraction of algal oil is costly process which determines the sustainability of microalgae-based biodiesel. This paper investigates the chemical extraction of the algal oil from Spirulina-platensis using different solvents under various conditions on bench scale. Results reported that Soxhlet extraction using methanol gave maximum algal oil recovery (98.5% wt.) at 1h using biomass-to-solvent ratio of 1:110 by wt. Extracted oil specifications, showed that density is 0.89 kg/L, viscosity is 58 mPa.s at 40oC while acid number is 37.4 mg KOH/g oil. The dominant saturated fatty acid in Spirulina-platensis is palmitic acid (48.35%) which makes it a promising feedstock for biodiesel production. Extracted biomass characterization and uses are involved.*

1. INTRODUCTION

Petroleum based fuels account more than 90% of transportation energy, since transportation is the lynch pin of our society, powering the economies of the world everyday (Hawash, S., *et al.,* 2008). Fossil fuels are the most used form of energy; however the world reserves are declining, and will be exhausted in the next 40 to 50 years (El Shimi, H., *et al.,* 2013). In addition, the global warming effects of fossil fuels, and pollutes the environment, so a viable energy source that eliminates petroleum dependence and eliminates green house gas emissions must be found (Huang, G., *et al.,* 2010). Today, the world interest is the research about renewable energy sources such as biofuels (Dragone, G., *et al.,* 2010; Koh, P. & Ghazoul, J., 2008). Biofuels (like biodiesel and bioethanol), hydrogen, solar and wind are alternative fuels, renewable, and environmentally friendly (El Shimi, H., *et al.,* 2013; Demirbas, A., 2003).

Biodiesel is an attractive fuel for diesel engines that it can be made from any vegetable oil (edible or non edible oils), used cooking oils, animal fats as well as microalgae oils (Aullón, A., 2010). It can be used in conventional diesel engines, without excessive modifications (Refaat, A., *et al.,* 2008). Pure biodiesel (100% biodiesel) reduces CO_2 emissions by more than 75% over petroleum diesel while using a blend of 20% biodiesel is more common, and reduces CO_2 emissions by 15% (Shah, G., *et al.,* 2012). EGYPT dependence on imported oil and the world dependence on petroleum products can be reduced by biodiesel. Biodiesel is the most desirable biofuel technically and economically (Kumar, M. & Sharma, M., 2014; Demirbas. A., 2002). In EGYPT we prefer to make biodiesel from non – edible vegetable oil such as microalgae; to eliminate any competition for oil uses in food industry (Chisti, Y., 2007; Datta, S. & Pandey, R., 1993).

Microalgae are photosynthetic microorganisms like plants that convert sunlight, water and carbon dioxide to sugars, from which biological macromolecules, such lipids, can be obtained, these lipids are the source of biodiesel. The residual biomass can be used as animal and fisheries feed, and after anaerobic digestion can be used as fertilizers and composts (Rodolfi, L., *et al.,* 2009). Microalgae are a potential feed stock of biodiesel (El Shimi, H., *et al.,* 2013). The utilization of microalgae for biofuels production offers a lot of advantages over higher plants: microalgae synthesize and accumulate large quantities of neutral lipids (10–70 % dry weight of biomass) and grow at high rates (58700 Liter Oil/ha.Year). In addition, microalgae cultivation does not require other than waste water, sunlight, and large areas (Aullón, A., 2010). The government in EGYPT has been interested in greening and investment of the desert, and finding solutions for municipal wastewater, so cultivation of microalgae in EGYPT desert seems successful and responsible source for biodiesel production (El Shimi, H., *et al.,* 2013).

Extraction of algal oil from *Spirulina-platensis* is one of the most costly processes which can determine the sustainability of microalgae-based biodiesel. It is common to apply dehydration of algal biomass to increase its shelf-life and for the final product. For biodiesel production, lipids and fatty acids have to be extracted from the microalgae biomass. Extraction and processing of microalgae biomass is similar to that of other oil seeds such as Jatropha oil or rapeseed oil (Aullón, A., 2010). Oilgae can be extracted mechanically using simple expeller press, while chemical extraction or solvent extraction by Soxhlet apparatus gives higher oil yield, and overcomes the drawbacks of the small scale basis (Sharma, M., *et al.,* 2005). Commercial manufactures use a combination of mechanical press and chemical solvents in extracting oil. Most of solvents used

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can provide 90% to 99% of the total available lipids. Although extracting oil using organic solvents is the most efficient method, but it is more costly and advanced technology which cannot be carried out economically on a small scale (Hawash, S., *et al.,* 2008). Pressing of oil from dried biomass may not be appropriate, particularly for small or decentralized operations.

More trials have shown that *Spirulina* can serve as a supplementary cure for many diseases. *Spirulina* capsules have proved effective in lowering blood lipid level, and in decreasing white blood corpuscles after radiotherapy and chemotherapy (Mata, T., *et al.,* 2009), as well as improving immunological function. *Spirulina* also is used for health food, feed and for the biochemical products since 1980s (Ringer, M., *et al.,* 2006). Cake produced after oil extraction and solvent recovery may be used as animal food or fertilizer, since it has a considerable amount of protein so suitable for animal food as well as promising plant nutrition element content to be used as P – K fertilizer (Mata, T., *et al.,* 2009). The use of *spirulina-based fertilizers* is impeded by the low cost, ready availability and preferred use of inorganic fertilizers.

Thus, the aim of this study is to reach the best conditions for oil extraction from Egyptian *Spirulina-platensis* microalgae on bench scale. Investigate the oil physicochemical properties; hence determine its suitability as a feed stock for biodiesel production. Biomass losses in grinding and extraction as well as solvent recovery will be discussed through a process mass balance.

2. MATERIALS AND METHODS

2.1. Materials

Spirulina-platensis microalgae were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst., Agricultural Research Center (ARC), Giza, Egypt. The culture media used was the same of Zarrouk's medium that published (Ringer, M., *et al.,* 2006). *Spirulina-platensis* was cultivated in around 25 mini-tanks, the dimensions of which are similar to that mentioned by (Pelizer, L., *et al.,* 2001) supplied by agitation of 180 rpm provided by revolving blades. The biomass cultivation was carried out at 30 °C, 3.5 klx of illuminance provided by fluorescent lamps and pH = 8.5 ± 0.5. Biomass was collected at 21 days old and subjected to the following steps:

Algal suspensions were homogenized (Homogenizer WisetisÒ HG-15D) for 10 minutes at 180 rpm; to disrupt the cells and ease the oil extraction.

Suspension was separated through Centrifuge (Beckman CS-6 Centrifuge 3500 rpm, Germany).

Collected microalgae were dried to a constant weight using solar beds (~32°C).

The resulting biomass was analyzed by a Spectrophotometric method and chemical composition was determined. Algal biomass was prepared for extraction by grinding using a mortar and produced powder was stored under 20°C until use.

Solvents used in this investigation are illustrated below (Table 1). All these solvents are purchased from El-Nasr Pharmaceutical Chemicals Company (ADWIC), EGYPT.

2.2. Methods

Oilgae was extracted using static-hexane and soxhlet methodologies.

2.2.1. Lipids extraction using Static-hexane methodology

For straight hexane extraction, 300 ml of n- hexane was added to 4g of microalgal powder into a suitable conical flask. For evaluating the addition of alcohol to hexane on extraction process, a 3/2 v/v single phase mixture of n-hexane and isopropanol (also 300 ml) was added to 4g microalgal powder into the same flask. The conical flasks were sealed with aluminum foil to reduce solvent evaporation. Extraction mixtures were agitated at 500 rpm at ambient conditions for 8.5 hours.

Table 1. Solvents and solvents mixtures used in this study for lipid extraction from dried Spirulina-platensis biomass

Solvent	Reference
Chloroform/methanol 2:1, solvent:sample> 20:1 (v/v) (Folch method)	(Folch, J., *et al.,* 1951)
Chloroform/methanol/water 1:2:0.8 (Bligh & Dyer method)	(Sheng, J., *et al.,* 2011)
Chloroform/methanol 1:1 (Modified Bligh & Dyer method)	(Lee, A., *et al.,* 2009)
Methanol	(Smedes, F. & Thmasen, T., 1996)
n-Hexane	(Xu, H., *et al.,* 2006)
Ethanol	(Fjerbaek, L., *et al.,* 2009)
Iso-propanol	Non-published before
Hexane/Isopropanol 2:3	(Molina, E., *et al.,* 1994)
Hexane/Isopropanol 3:2	(Molina, E., *et al.,* 1994)
Butanol	(Lee, S., *et al.,* 1998)

Cells' residue was removed by filtration through Whatman GF/C paper. For hexane/isopropanol extraction from dried microalgae, the filtrate was transferred into a separating funnel and sufficient hexane and water (approximately 40 ml each) were added to induce biphasic layering. After sufficient time, the solvent mixture is partitioned into two distinct phases: a top dark-green hexane layer containing most of the extracted lipids and a bottom light-green aqueous-isopropanolic layer containing most of co-extracted non-lipid contaminants. The hexane phase from which extracted oil was collected in a pre-weighed flask before it was heated to dryness in the rota-vapor (<60°C) to enable gravimetric quantification of the lipid extract. The crude lipid was re-dissolved in hexane (approximately 20 ml) and transferred into a sealed glass vial for storage. The vials containing the lipid extract solutions were stored in the incubator at 20 °C for use in the transestrification process.

2.2.2. Oilgae extraction using Soxhlet system

A Soxhlet apparatus made by Quick fit, England was used for extraction of oil. It con sists mainly of a round flask, 0.25 L capacity, mounted by a special tube in which the dried biomass (microalgae) were placed with a capillary side tube which permits solvent drainage and circulation in closed loop from the round flask to the algae. Above this tube, a condenser is quick – fitted to prevent any losses of solvent and permits its condensation and circulation through the biomass, as shown in (Fig. 1).

About 1.0 g of grinded biomass was placed in the Soxhlet. A known volume of the solvent was placed in the round flask and was boiled using a water bath Julabo model

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TWB 20 provided with a temperature controller for keeping used solvent temperature at its boiling point. Evaporation and further condensation of solvent on the algal biomass which is accumulated till certain height then it drains carrying the extracted oil into the round flask by siphoning effect. Visual observation was used for extraction terminal, when the drained solvent became very clear and colorless; this indicated that all oil in microalgae was completely extracted in the solvent. Different types of solvent were tested on bench scale and reported in (Table 1).

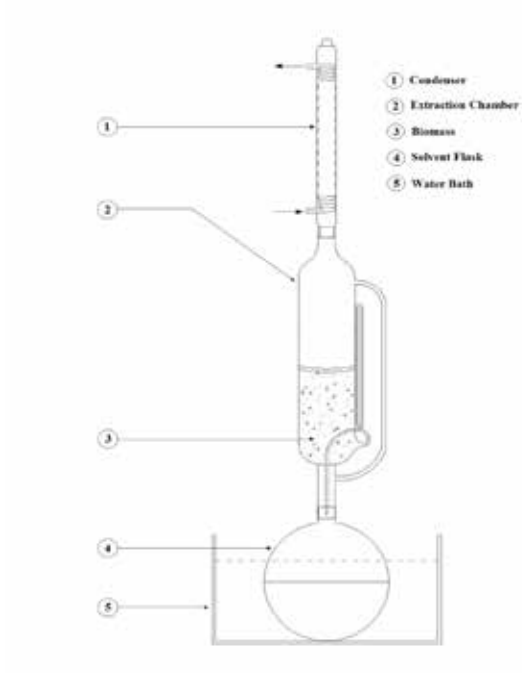

Fig. 1: Soxhlet Extraction System

After complete oil extraction from biomass, liquid – liquid extraction step was conducted for solvent recovery and oil purification. Oilgae obtained was then characterized through fatty acids composition analysis, viscosity measurement and acid number analysis.

2.2.3. Analytical methods

Fatty acids composition of the extracted *Spirulina-platensis* microalgae oil was determined using gas liquid chromatographic analysis of the oil ethyl esters. Modification of the oil to its ethyl esters was made using 2 % H_2SO_4 as catalyst in the presence of dry ethyl alcohol in excess. The chromatographic analysis was made using Hewlett Packard Model 6890 Chromatograph. A capillary column 30 m length and 530 µm inner diameter, packed with Apiezon® was used. Detector temperature, injection temperature and the column temperature were 280 °C, 300 °C and 100 to 240 °C at 15 °C/min, respectively.

3. RESULTS AND DISCUSSION

3.1. Characterization of Spirulina-platensis microalgae

As mentioned before, the microalgae biomass was analyzed by a Spectrophotometric method; to detecte the percentage of lipids content. Chemical composition was determined on dry matter basis and reported in (Table 2). According to the culture conditions used in this study, *Spirulina-platensis* samples were determined to have a total lipid content of 10.95% wt. of biomass. The oil content of the used microalgae strain is highly dependent on the specific growth conditions not only influenced by the microalgae specie (Canela, A., *et al.,* 2002). The microalgae culture conditions, nutrients and light intensity can be optimized to increase the oil content of the biomass, and hence increases in the biodiesel production (Canakci, M. & Van Gerpen, J., 2001).

Extracted oil yield from biomass (%) and oil recovery (%) at different solvents and solvent mixtures are calculated according to the following equations respectively:

$$\text{Oil yield from biomass (\%)} = (M_e / M) \times 100\ \% \quad (1)$$

$$\text{Oil recovery (\%)} = (M_e / M_a) \times 100\% \quad (2)$$

where: M_e is the mass of extracted oil

M is the mass of dried algae biomass

M_a is the mass of available (theoretical amount of) oil in the algae biomass

Table 2. Characterization of Spirulina-platensis used in this study

Compound	% wt.
Proteins	62.3
Lipids	10.95
Carbohydrates	12.8
Minerals	6.95
Fibers	4
Moisture	0.5
Nucleic Acid	2.5

3.2. Effect of solvent selection on algae oil extraction

The effect of solvents selection on algae oil extraction was studied using ten solvents namely isopropanol, methanol, butanol, mixtures of chloroform and methanol, hexane, and also two mixtures of hexane and isoproponol with two different volume ratios as illustrated in (Table 3). A biomass-to-solvent ratio of 1:95 by weight, and extraction temperature (around 68°C) using bench-scale Soxhlet apparatus for 60 minutes assumed to be sufficient for equilibrium.

Results are plotted in (Fig. 2), in which the right axis represents the percentage of crude extracted oil from biomass: wt. of oil/wt. of biomass, and the left axis represents the percentage of oil yielded of the available oil in the biomass: wt. of extracted oil/wt. of maximum amount of oil in biomass. Each solvent used in the oilcake extraction was tested two times with an analytical error of ±1%. From the results, it may be observed that using methanol; oil extraction may reached 98.5% which is a satisfactory result, while (chloroform-methanol 2:1) mixture was enhanced extraction to 99.4% of microalgae extraction with an increase of less than 1% of oil yield, but this increase cannot compensate this advantage due to the well-known toxic effect of associated chloroform.

Table 3. Extracted Oilgae yield from Spirulina-platensis biomass at different solvents and solvent mixtures

Solvent (s)	% Crude Extract/ biomass	% Oil Yield from Available
n-Hexane	7.953333333	72.63318113
Isopropanol	8.77826087	80.16676593
Hexane:Isoprop. = 2:3	9.008130081	82.26602814
Hexane:Isoprop. = 3:2	9.751219512	89.05223299
1-Butanol	9.887391304	90.295811

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Ethanol	9.954545455	90.90909091
Methanol	10.782	98.46575342
Chloroform/methanol 2:1	10.88226415	99.38140777
Chloroform/methanol/ water 2:1:0.8	10.67213208	97.46239338
Chloroform/methanol 1:1	10.62727273	97.05271897

Oil extraction results from *Spirulina-platensis* microalgae using the different solvent systems are reported before in (Fig. 2). The Folch extraction method yields 99.39%, wt.%, and the Bligh & Dyer methods perform 97.5%, wt.%, both using chloroform + methanol, had the highest oil yields based on the oil available in the dry biomass. The water content in the Bligh & Dyer method played a quite important role in oil extraction.

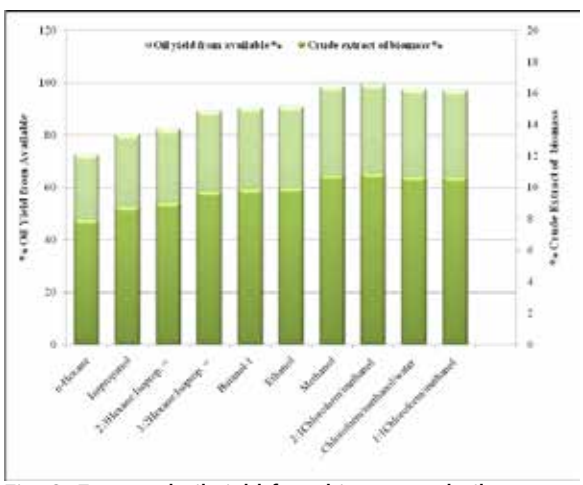

Fig. 2: Extracted oil yield from biomass and oil recovery at different solvents and solvent mixtures

Although these chloroform + methanol-based methods were developed for extracting neutral lipids like TAG, we show that they also are suitable for extracting the polar lipids that exist in *Spirulina-platensis*. Because they had the highest extraction efficiencies, the Folch or Bligh & Dyer methods can be considered the "gold standards" for bench-scale oil extraction from *Spirulina-platensis* strain. However, the carcinogenic effects of chloroform + methanol make them unsuitable for large-scale extraction, which also makes it essential to compare the efficiencies of other solvent systems.

Several other solvents by Soxhlet apparatus achieved lipid extraction efficiencies of at least 90% of that achieved by the Folch and Bligh & Dyer methods. Although it was reported to be ineffective for TAG extraction, methanol alone had an extraction yield of around 98.5% of the *Spirulina-platensis* lipids extractable by Folch and Bligh & Dyer methods.

3.3. Effect of different solvent-to-biomass ratios on extraction of Algae Oil

The effect of solvent-to-biomass ratio on the extraction of microalgae oil using methanol as solvent was conducted at five different ratios (59:1, 79:1, 95:1, 119:1, and 150:1) at temperature above the methanol boiling point (~ 68°C). Results are plotted in (Fig. 3) with a standard error of ±0.507%. The reported results observed that increasing in solvent ratio; increase the amount of extracted oil. Equilibrium was attained for a solvent-to-biomass ratio of 110:1 by weight of biomass; this ratio is equivalent to 120ml methanol-to-1g microalgae, with oil recovery of 98.5%.

3.4. Effect of extraction time on Algae oil recovery

The effect of extraction time on the percentage of oil recovery from *Spirulina-platensis* using methanol as a solvent was conducted at five different times (20, 30, 60, 90, and 120 minutes) at temperature above the boiling point (around 68°C) with solvent-to-biomass weight ratio of 110:1. Results are plotted in (Fig. 4) with a standard error of ±0.56%. From which, it may be observed that increasing in extraction time increase the percentage of extracted oil and equilibrium was fulfilled for an extraction time of 60 minutes with %oil recovery of 98.4; since no recommended increase for the yield of extracted oil at time above 1 h as shown in (Fig. 4).

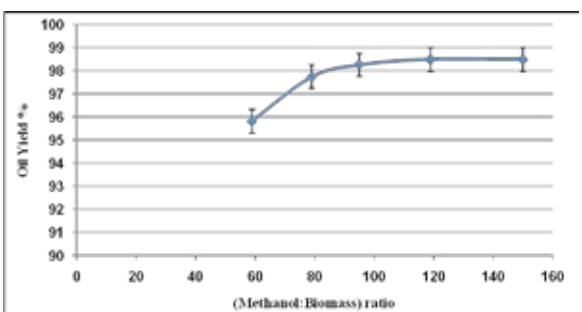

Fig. 3: Effect of methanol: biomass ratio on % Oil yield

(Temp. = 68°C, time = 60 minutes)

3.5. Soxtherm extraction system compared to static hexane method

Static hexane method (extraction without Soxtherm system) yield 90% of fatty acids from available oil in the dry biomass, but it consumed about 8.5 h which is much long time in compared with 1 h for the solvent extraction by soxhlet apparatus. Soxhlet extraction by hexane solvent was obtain 72.63% of the available oil in the biomass as shown in (Fig. 2), which is lower than 90% obtained by static hexane method; this can be understand by that, the heat is not helpful for all solvents, as nearly all the lipids can be extracted out at room temperature. Heating may promote solvent recovery and shorten extraction time, but it's a large energy burden for the extraction process. In this study, Soxtherm extraction system using methanol as a chemical solvent seems to be the best one with 98.5% lipids recovery, short operation time of 1 h and relatively less toxic effects. But it consumed higher energy compared to the static hexane methodology, so extraction processes are still need to be optimized; since it represents the key process for the success of biodiesel business.

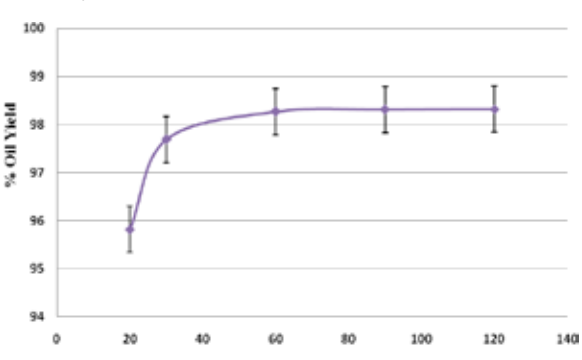

Fig. 4: Effect of time on % Oil recovery

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(Temp. = 68°C, biomass: solvent 1:110 wt./wt.)

3.6. Quality Assessment of Extraction Products
Laboratory tests were conducted to establish the properties of extraction products (Oilgae and biomass cake) from Egyptian *Spirulina-platensis* microalgae.

3.6.1. Characterization of Produced Oilgae
Viscosity, Density and Acid Number of Oilgae

After the Oilgae extraction process using the experimental conditions that illustrated below in (Table 4), algal oil was characterized and compared to ASTM standards as shown in (Table 5); since the extracted oil may be used as a fuel without further processes in case, if it has low viscosity.

The viscosity difference forms the basis of an analytical method, viscometry, applied to determining the possibility to use the microalgae oil as a fuel directly without further transesterification process. The viscosity of the microalgae oil was calculated using Brookfield viscometer Model DV-II. The results obtained denote satisfactory need to the oil transesterification process; to reduce its viscosity.

Table 4. Best conditions for oil extraction obtained from this study

Oilgae Extraction Parameter	Value
Extraction methodology	Soxtherm system
Solvent type	Methanol (CH_3OH)
Biomass-to-solvent	1:110
Temperature (°C)	Doesn't matching 68
Time (min.)	60

The density of the produced oilgae was 0.892 (kg/l) at 27°C. This is in compliance with ASTM standards (0.86-0.90). Density measurement is lying within the range confirm the quality (Table 5).

Table 5. Microalgae oil properties compared to ASTM biodiesel's Standards

Properties	Microalgae Oil	ASTM biodiesel's Standard
Density (kg/L)	0.892	0.86 – 0.90
Viscosity (mPa.s , 40°C)	58	3.5 – 5.0
Acid Number (mg KOH/g oil)	37.4	Max. 0.5

Acid number of *Spirulina-platensis* oil was determined by titration; however, it uses a dilute ethanolic KOH solution with phenolphthalein as an indicator for the detection of the titration endpoint. The oilgae acid number was 37.4 mg KOH/g oil, which is higher compared to the biodiesel standards, therefore the microalgae oil cannot be used directly as a suitable fuel, but it must be transesterificated to biodiesel using the appreciated methodology.

Fatty Acid Composition of Microalgae Oil
The fatty- acids composition of microalgae extract is presented in (Table 6). C16:0 (palmitoyl fatty acid group) was the predominant fatty acid group in *Spirulina-platensis*, constituting about 48.35% of the total fatty acids (by wt.). Other abundant fatty acids groups included C14:0 (~20.9%), C16:1 (~2.66%), C18:0 (~2.02%), C18:1 (~2.41%), C18:2 (~5.37%), C18:3 (~7.84%) and C20:1 (~4.36%). Some other natural fatty acid groups- such as C16:2, C17:0, C18:4 and C20:2 are not shown because they were detected in trace amounts. The percentages of the major fatty acids in the Spirulina used in this study were in accordance with previous works by other authors (Collaa, L., et al., 2004; Olguin, E., et al., 2001; Quoc, K., et al., 1994), as the principal fatty acids present were palmitic, gamma-linolenic and linoleic acid as reported in (Table 6). The fatty acid profiles extracted by Bligh& Dyer, Folch, methanol, ethanol or by static hexane are expected to be similar (Canakci, M. & Van Gerpen, J., 2001), even when the total amounts of lipid extracted differed. For biodiesel-fuel production, shorter chain and saturated fatty acids are preferred, as they have lower melting points and higher cetane numbers and are less prone to oxidation. However, the dominance of C16:0 in Spirulina-platensis makes it a promising feedstock for biodiesel fuel production.

3.6.2. Characterization of Produced Cake
As previously mentioned in the literatures, cake produced after oil recovery may be used as animal food or fertilizer (Mata, T., et al., 2009). Produced biomass waste (meal) of Spirulina-platensis was dried and characterized as shown in (Table 7). It may be observed that the microalgae cake has a considerable amount of protein, so suitable for animal food as well as promising plant nutrition element content to be used as P – K fertilizer (Andrade, M. & Costa, J., 2006).

Table 6. Fatty acids composition of Spirulina-platensis Oilgae measured by GC

Fatty acid	Compositions (wt.%)
Mystic (C14:0)	20.9
Palmitic (C16:0)	48.35
Palmitoleic (C16:1)	2.66
Stearic (C18:0)	2.02
Oleic (C18:1)	2.41
Linoleic (C18:2)	5.37
Linoleuic(C18:3)	7.84
Ecosanoic (C20:0)	1.26
Eicosic (C20:1)	4.36

3.7. Proposed Microalgae Management System
After a rough testing technique using chemical treatment and trials to obtain the optimum engineering and operating conditions for algae oil extraction using methanol as a solvent, an algae management system has been proposed with zero waste taking into account all observed parameters that listed in (Table 8). Algal oil management system has been shown in (Fig. 5).

Table 7. Produced Cake Characteristics

Case	Value
Moisture (%)	1.5
Protein (%)	51.5
Ash (%)	7.5
Ca (mg/100 g)	400
P (mg/100 g)	900
Fe (mg/100 g)	70
K (mg/100 g)	1475

Table 8. Optimum Operating Parameters

Selected parameter	Average value
Losses in grinding Spirulina-platensis biomass	0.01% by weight
Methanol losses in apparatus	1% by volume
Methanol in cake (extracted biomass)	2% by volume
Moisture content in Microalgae (after 24 hours drying at 105 °C)	0.5% by weight
Methanol recovered	97%

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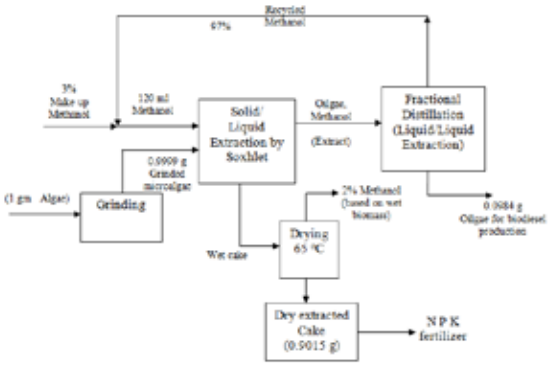

Fig. 5: Proposal of 21 days old Spirulina-platensis Microalgae Management System

4. CONCLUSIONS

From the above study, it may be concluded that:

Currently, algal biodiesel seems to be the golden alternative fuel to reduce the petroleum dependence.

Pure methanol is a suitable solvent for Oilgae extraction with 98.5% (by wt.) oil recovery using biomass-to-solvent ratio of 1:110 for 1h.

The dominance of C16:0 in *Spirulina-platensis* oil makes it a promising feedstock for biodiesel production.

Microalgae oil has an acid number of 37.4 mg KOH/g oil, and viscosity of 58 mPa.s. These values are very high, so algae oil cannot be used directly as a suitable fuel, but it must be transesterificated to biodiesel using the appreciated methodology.

The cake (extracted biomass) is a good feed for domestic animal, and as complex fertilizer containing P and K nutrition elements.

A proposed biomass management system may be followed to obtain a zero waste project.

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