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A Research Study to Compare the Productivity of Extracted Algal Oil and Production of Biodiesel from Some Local Algae Samples in Libya

Amal Dou Egmea [1*], Nader Kamal Nasar[2] , Khalid Omar Elhaji [3]

amalegmea@gmail.com [1], naderkmal1983@gmail.com [2] elhaji2004@gmail.com[3]

Author Name and address (amalegmea@gmail.com)

[1] Department of Chemical Engineering, Faculty of Engineering, Sabratha University, Libya
[2] Department of Chemical Engineering, Faculty of Engineering, Sabratha University, Libya
[3]Libyan Petroleum Institute (LPI),Tripoly, Libya

ABSTRACT

Renewable energy resources have been an urgent need to overcome fossil fuel recourses issues. Biomass in general and Algae in particular have been the main energy resource to produce biofuel and biogas. This research paper aims to compare the productivity of the algal oil extracted from some local algae samples and produce biodiesel. Algae samples were collected from different spots of Surman and Sabratah shores. They are classified into 2 macro-algae samples (marian types) represented in (Ulva and *Enteromorpha linza*) and one sample of micro-algae represented in *(Spirolina plareassis*). The extracted algal oil is achieved by chemical extraction technique using soxhlet extractor apparatus. Then, it was preheated to make it ready for the final biodiesel conversion step by the acidic esterification using sulfuric acid (H_2SO_4) in the presence of potassium hydroxide solution (KOH) as a catalyst.In terms of algal oil extraction process, the algal oil content extracted from samples of 100g for the macro- samples is poor with 0.058g of *Ulva* and 0.0.02169 g of *Enteromorpha linza*. In contrast, the 100g of *spirolina plareassis* revealed richness in algal oil content with 7.5g extracted oil. Due to the poorness of algal oil content, the tested macro-algae samples might not to be practical candidates for the biodiesel production. In this study, therefore, the biodiesel production process will be only considered for *Spirolina plareassis*. The acidic esterification process has been chosen

to convert it to biodiesel. The productivity of biodiesel using this method has been almost as double as the quantity of extracted algal oil.

Keywords: Renewable energy, algal oil , esterification and biodiesel

1. Introduction

Fossil fuels have been the dominance energy resource for the global energy markets. However, the reliance on renewable energy resources has dramatically increased for critical reasons. These are represented in the continuous rise of the crude oil prices, the depleting fossil fuel reserves day by day and worryingly the adverse impacts of greenhouse gases which mainly contributes to the global warming[1] .The production of biodiesel has recently received much attention worldwide. Because of the world energy crisis [2], many countries have started to take a series of measures to resolve this problem [3]. Finding alternative energy resources is a pressing mission for many countries, especially for those countries lacking conventional fuel resources. In the 1930s and 1940s, vegetable oils have been used as diesel fuels in the emergency situation.

Biodiesel is made from biomass oils, such as vegetable and algae oils. Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and an alcohol that can be used in diesel engines, alone or blended with diesel oil.Biodiesel is the monoalkyl esters of longchainfatty acids, which is derived fromtransesterification of biological matter [4,5]. Table 1 shows the advantages and disadavantages of biodiesel as a fuel [6].

Algae account hugely for the interest of researchers and experts in the production of biofuels [7] Algae exist in numerous forms throughout this ecosystem and have a wide array of properties, such as being capable of photosynthesis. Their ability to produce oil is a topic of interest for chemical engineers, since algae are a renewable energy source as well as CO_2 neutral. Algal organisms can be divided into two distinct groups: macro-algae and micro-algae, growing in aquatic environments. Macro-algae is commonly referred to as “seaweed”, are multicellular plants growing in salt or fresh water. They can be classified into three groups identified by their pigmentation: brown sea-weed (*Phaeophyceae*), red seaweed (*Rhodophyceae*) and green seaweed (*Chlorophyceae*)**.** In contrast, micro-algae are unicellular microorganisms that exist in saline or fresh water environments and live and reproduce by converting sunlight, water and carbon

dioxide to algal biomass through aquatic photosynthesis. Moreover, they are uniquely rich in lipid content and consist of proteins, carbohydrates, lipids and nucleic acids in varying amounts. Their classification and exploration remain a relatively unexplored topic in modern science [8].

Table 1: *Advantages and disadvantages of Biodiese l* [6]

Advantages	**Disadvantages**
Renewable fuel obtained from vegetable oils or animal fats.	Slightly higher fuel consumption due to the lower calorific value of biodiesel.
Low toxicity in comparison with petroleum diesel.	Slightly higher nitrous oxides(NO_x) emissions than petroleum diesel.
Lower emissions of contaminants, CO, CO_2	Higher freezing point than conventional diesel. This may be inconvenient in cold
No sulfur dioxide SO_2 emissions.	It is less stable than diesel fuel,
Higher flash point (100^oC minimum).	May degrade plastic and natural rubber gaskets.
May be blended with diesel fuel at any proportions.	May degrade plastic and natural rubber gaskets.
Excellent properties as a lubricant.	It dissolves the deposits of sediments and other contaminants in storage tanks and fuel lines which can cause problem in the valves and injection systems.
It can be used in any conventional diesel engines without any modifications.	

The importance of algae could be summarized for several reasons. First, Algae use enormous amount of CO2 removing from power plant emissions. Allied to this is the enormous capacity of the algae to convert CO2 into biomass, liberating via photosynthesis more oxygen for the atmosphere than forests. Second, algae depollute the waters by absorbing the urea expelled by these animals and at the same time increase the CO2 conversion into biomass. The algae can then be converted into various kinds of biofuel using liquefaction, pyrolysis, gasification, extraction and transesterification, fermentation, and aneorobic digestion [9–11]. Transesterification to produce biodiesel is more energy-efficient than fermentation to produce ethanol [12]. One hectare algae farm on wasteland can produce over 10–100 times of oil as compared to any other known source of oil-crops. Moreover, algae can start producing oil within 3–5 days and thereafter oil can be harvested on daily basis. Algae can be grown using sea water and non-potable water on wastelands where nothing else grows. It is firmly reinforced that algae' farming for biofuels is expected

to provide a conclusive solution to food vs. fuel debate [13]. The carbon dioxide fixation and the main steps of algal biomass technologies are illustrated in Figure. 1.

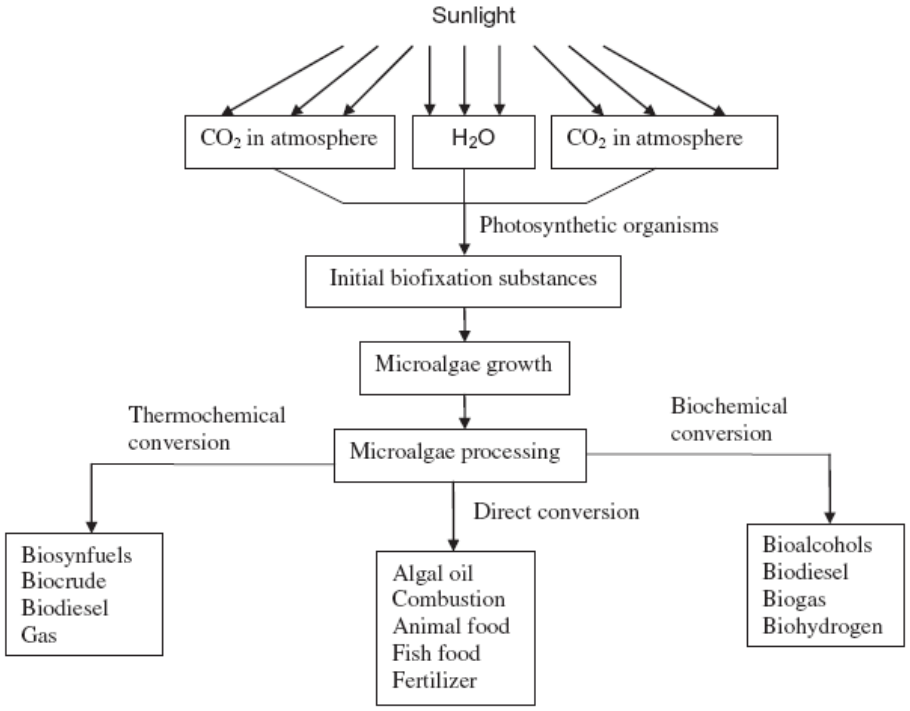

Figure (1) carbon dioxide fixation and main steps of algal biomass technologies [14]

This research paper aims to compare the productivity of algal oil extracted from some selected samples of macro-algae and micro-algae. Another target is to convert their algal oil into biodiesel.

2. Theory of Experimental Research:

The experimental methodology of this research will focus on collecting two local marian macro-algae species and compare their extracted algal oil content with a micro-algae specie. The physical treatment processes of the strain algae will be displayed in this paper. Then, procedures to practically produce biodiesel from extracted algal oils will be another target of this research.

3. Materials and Methods

3.1 Collection of Algal Samples:

In February 2019, two samples of marine algae were collected from the shores of Sabratha and Sorman in the state of Libya. They were collected from the mentioned shores on the sea rocks at a depth of 1.5 m with a quantity of 18 kg of each sample

before drying and then placed in plastic bags. Then, they were kept in a freezer before transferring it to the workplace. Then, they were analyzed to be identified in the Faculty of Science of Sabrata Univiersity . This has lead to the knowledge of their scientific names which are *Ulva* and *Enteromorpha linza.* Figure 2 and 3show algae samples of *Ulva* and *Enteromorpha linza* respectively. Lastly, in March 2019 a sample of micro-algae was brought from Tunis; Institute of Biological Algae[15], specialized in Algae treatment to produce nutritional supplements and cosmetics. This sample is shown in figure 4 and has a biological name of *SpirulinaPlantensisLonar.*

Figure 2.A sample of*Enteromorphalinza*

Figure 3.A sample of*Ulva*

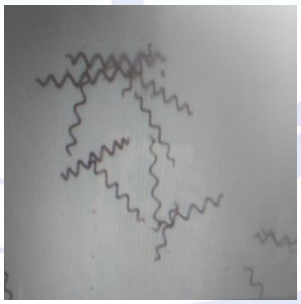

Figure 4.A microscopic analysis of *Spirulina Plantensis Lonar*.

3.2 Physical Treatment Methods of Algae strain Samples

3.2.1 Drying Process of algae strain samples:

The drying process of these samples was then carried out in an oven for 24 hours at a temperature of 60 ° C. This process was done at the Libyan Oil Institute in Tripoli. The purpose of drying process is to remove humidity and evaporate the water content in algae samples.

3.2.2 Grinding Process of the dried algae strain samples:

The treated drying samples were first grinded using an electrical grinder tool. Then, it was sifted to be converted into a powder using first, a sieve drop with a diameter of 500µc for the purpose of removing the suspended granules and impurities.

3.2.4 Extraction of Algal Oil from its Algal Powder Samples:

The chemical extraction process of algal oil will be applied for all prepared algal powder from all tested algae species whether from the macro-algae species or from the micro-specie. A quantity of 100g of algal power from all tested algal species will be chemically extracted as the following. First, it will be placed in a soxhlet apparatus and heated and treated with n-hexane as a solvent to dissolve algal oil inside its powder content. This process can be shown in figure 5. Then, the resulted algal oil dissolved in n-hexane was heated in a rotary-evaporator as showed in figure 6 in order to evaporate n-hexane and to get pure algal oil (see figure7).

Figure 5.Soxhlet apparatus to extract algal oil with chemical solvent (hexane)

Figure 6. Drying algal extracted oil using rotary evaporator.

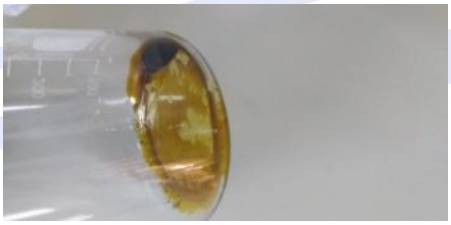

Figure 7.A sample of the extracted algal oil after drying it.

3.2.5 Esterification Method (Algal oil conversion to biodiesel):

The conversion process of algal oil to biodiesel depends on the content of free fatty acid, molar ration of methanol: oil, reaction temperature, reaction time and agitation [16]. According to Gerard et al., 2003, direct etherification of algal oil with alkyl catalyst is applied, when its free fatty acid content is less than 5ppm. In this process,

algal oil reacts with methanol in the presence of sodium hydroxide as a catalyst. Meanwhile, if it has a FFA content higher of 5ppm, the acidic catalyst esterification is recommended to avoid soap and water formation. According to Gregory W. et al., 2016. The acidic catalyzed esterification is more complicated than the alkyl catalyst tranesterificantion. it is used when the algal oil is rich with free fatty acid content. In particular, if it has a free fatty acid content more than 5%wt of algal oil. It can be mentioned that the next practical procedures has been followed as practiced to produce biodiesel form reference [17].

3.2.5.1 Saponification of algal oil

The main target of this process is to isolate the free fatty acid content of algal oil through the saponification reaction between dissolved algal oil and aqueous solution of Potassium hydroxide KOH.

The practical procedures are outlined as the following:

1. Add 360ml of methanol CH_3OH and 180ml dichloromethane to 18g of algal oil. This step aims to redissolve the algal oil in this solvent mixture.
2. The resulting dissolving mixture of algal oil in the previous step is mixed with 48.06 g of water and 9 g KOH and stirred and heated to 60 ^oC for 3 hours.
3. Once the reacted mixture has been cooled, the removal of organic solvents (methanol + dichloromethane) will be started immediately by heater at temperature of 60^oC.
4. The remaining mixture from the previous step was put into a 1-L separatory funnel, and then25ml of hexane was added and shakes it well together as showed in figure 8.
5. The shaked mixture inside the funnel was left for a while in order to settle down and forming two layers. Then, the bottom layer was dragged into a measured flask and the above organic layer was put into a separate flask.
6. Repeat the steps 4 to 5 until the organic layer is colorless.

Figure 8.The separation process of algal oil after saponification process.

7. Combine the collected top organic extracts and heat it on a rotary evaporator at a temperature ranged of (60-70^{o}C) this action will lead to the isolation of the neutral lipids which have a green solid shape.
8. 5ml of HCl solution (6M) was added as drops to the collected bottom layers mixture until the PH meter has indicated that the acidic mixture has reached an acidity of PH=2.
9. Add 30ml of hexane to the treated acidic mixture and put them in a 1L seperatory funnel and repeat the steps 4 to 5. This process will results in the extraction of the free fatty acids (FFAs).
10. The free fatty acids (FFAs) will be purified as a result of the removal of hexane from the resulting extracts in step 9 by heating them using a rotary evaporator. The obtained FFAs have a dark green oil residual form.

3.2.5.2 The Acidic catalyzed esterification of free fatty acids:

This treatment considers being the final stage to get a green biodiesel due the reaction between methanol and the extracted purified FFAs in the presence of an acidic catalyst.

The practical steps are outlined here as the following:

1. The purified FFAs will be dissolved with 108ml solvent mixture (methanol +chloroform). Then, the resulting dissolved mixture of FFAs will be put in a thick-walled high pressure reaction flask equipped with a stir bar.
2. 3.6 ml of concentrated solution of sulfuric acid (H_2SO_4) will be added to the dissolved mixture in step1 and the reactor flask will be sealed and then heated to a temperature of 90^{o}C with stirring for an hour.
3. Once the chemical reaction has finished, the resulting mixture will be left to cooled to the room temperature. Then, it will be poured into a separatory funnel.
4. 36ml of (H_2O) will be added to the cooled mixture in the funnel. This mixture will be shacked properly and then, it will be left to be settled down to allow phases to be separated as it is seen in figure 9.
5. The settled bottom layer will be dragged into a pre-weighed round flask. Then, it will be heated on a rotary evaporator. Then, the remaining product is the main target of this research which is biodiesel as showed in figure 10. The mass of produced biodiesel mass was 32.5g

Figure 9.The resulting mixture of acidic esterification process to be separated.

Figure 10: the final produced biodiesel product

4. Results and Discussion:

It can be seen from table 2 that all the tested macro-algae species has not reached even 1% wt of extracted oil out of 100g raw algal powder. In fact, this revelation of extraction results of tested macro-algae samples is not encouraging to be used for the biodiesel production due to the very limited content of algal oil. This could be affected with seasons timing of growing and climate where the collection of samples were in the winter season (in the period between November and Febraury). This outcome might be a prove is that micro-algae species have higher algal oil content form macro-algal species which would be make it a strong completive to be a sustainable resource to produce biodiesel.

On the other hand, it can be noticed that *Spirulina Lonar* is the richest sample among them with algal oil content. However, all these current 6 macro- algale investigated samples could not be a major source to produce algal oil to be converted to biodiesel due to the very limited content of algal oil. Ihsanullah et al., 2015 extracted algal oil from *spirogyra* algae with 4%wt. In contrast, the tested *spirulina Lonar* algae productivity of algal oil has almost similar algal oil productivity with 4.003 %wt.

Table 2:*algal oil productivity from 100g of algal powder of all tested algae species.*

Tested Algae species	Extracted algal oil quantity, g
Ulva	0.0581
Enteromorphalinza	0.0216
SpirulinaLonar	4.003

In terms of acidic esterification, the main reasons why it has been decided to carry on this method to convert the extracted algal oil to biodiesel from SpriulinaLonar algae

are it has a high content of lipids and FFAs (more than 2%) which was proved with the formation of soap. Another reason is to ensure to use a less amount of catalyst (H_2SO_4) instead of using larger amounts of NaOH for the base esterification process.
There have been some obstacles during performing the acidic esterification which can be outlined as the following: performing this reaction requires a high operation temperature of 90^oC and a sealed batch reactor therefore, this reaction could be dangerous during operation which has indeed the reactor exploded at the first trial due to weakness of its glass wall material. Another difficulty is that this reaction requires a reaction time of 3hours to be completed which is considered a long time because these reactions are slow in terms of reaction rate which is a disadvantage of this method. Therefore, it must be warned that this method is quite dangerous unless personal and lab safety tools are provided.
However, according to Refaat A. 2010, the most abounded composition of micro-algal oil transesterified with methanol is $C_{19}H_{36}O_2$, which is suggested to accord with the standard of biodiesel. This is a good advantage for the esterification process that the productivity of biodiesel from extracted oil can be twice or triple the amount of algal oil [19]. This has been observed in table 3.

Table 3 *Experimental productivity of green biodiesel*

Mass of treated algal oil, gm	Mass of produced Biodiesel , gm
18	32.5

It can be seen from table 4 that there have been a relatively similar properties between produced biodiesel in terms of its density and viscosity. These results could be due to the intensive search which had been made to find potential references that have described how to produce biodiesel.
On the other hand, Figure 11 represents the result of the Gas chromatograph (GC-FID) analysis test for a sample of 5g produced biodiesel.
This test has indicated that this produced biodiesel has a carbon number ranged from C9 to C22 in the organic chain.
Table 4:*physical properties of produced biodiesel compared with literature Data of petroleum diesel and ASTM standard biodiesel* .

Tested property	Produced diesel in this research	Diesel Petroleum[20]	ASTM standard biodiesel[20]
Density g/cm^3	0.8950	0.838	0.84-0.90
API Gravity	26.6		
Kinematic Viscosity (mm^2/s)/s	5.7	1.9-4.1	3.5-5.0

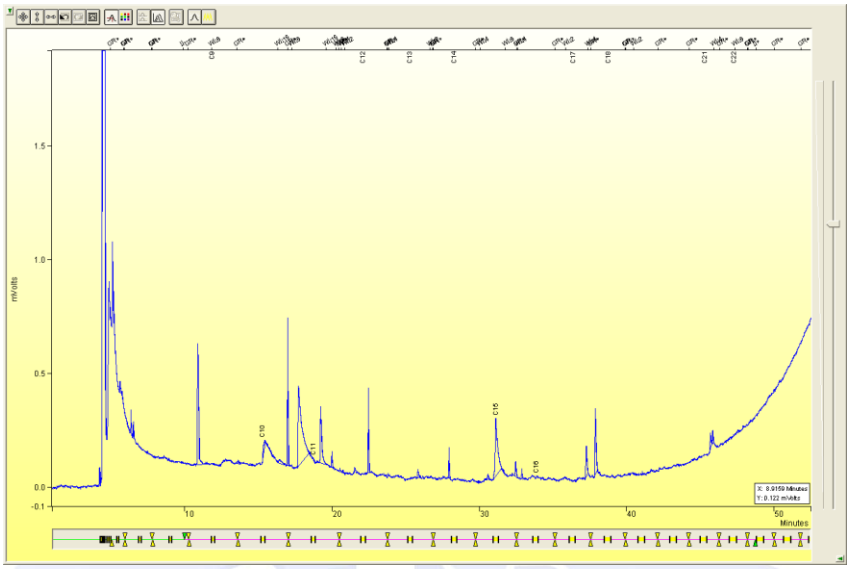

Figure 11.The Gas chromatograph (GC-FID) analysis test for produced biodiesel

5. Conclusion:

Algae play an important role in our daily life in many aspects. It is considered to be a sustainable energy resource to provide us with biodiesel, biogas and bioalcohols and bioglyrcol. It can be farmed and grown in even difficult conditions where it can live in sea, rivers and waste waters.

This research has focused on how to extract algal oil from some selected macro and micro species. In addition, it has also aimed to convert its extracted oil into green biodiesel.

In terms of algal oil extraction, it has been revealed that *the* both tested macro-algae species showed very limited algal oil content. This unfortunately was not encouraging to consider the tested macro-algae species to be a major source for biodiesel production. On the other hand, the tested micro-algae specie represented in *Spriulina Lonar* showed promising algal oil content at which it can give 7%wt algal oil to be extracted. This an encouraging finding to considered Spriulina Lonar as a biological source to produce biodiesel.

In terms of converting *Spriulina`s* tested algal oil into biodiesel, the acidic catalyzed esterification had been implemented. It was found this conversion process was directly successful and effective to produce biodiesel. However, it should be warned to implement personal and lab safety tools while dealing with it.

In terms of the produced biodiesel tests analysis,it was found that almost all the analysis tests have almost similar to the literature ASTM stand biodiesel.

6. Recommendations:

This study research has many recommendations which could be stated as the following:

- The algal oil content for the tested macro-algal species should be re-measured again in summer season for future research studies. This has been based on that their algal oil depends on their grow which could be affected with seasons.
- This research recommends making a future research to implement new untested technologies to extract algal oil for the same tested macro-algal species. This was based on to find an effective extraction method which could be better than this research method.
- It should be recommended to cooperate with local and international institutions and companies specialized in this provision which could stimulate and encourage using biodiesel in particular and renewable energy in general in Libya.This will certainly help to greatly reduce Libya`s reliance on petroleum to provide energy.
- Implementing renewable energy in general and biofuels in particular will hugely post Libya`s economy and clean its environment.

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