

Production of Biodiesel From Microalgae

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Abstract

This study investigates the feasibility of biodiesel production from microalgae isolated from the Gulf of Aqaba employing a number of interdependent steps and focusing on the laboratory scale cultures.

Under certain conditions such as high light intensity or rich nutrient medium, producing a few tens to several hundreds of biomass which used to produce biodiesel which is environmental friendly.

Chlorella was isolated from the Jordan Eastern Ghor Canal and cultivated using a certain media. The cells were harvested and micro algal oil was prepared by extraction with n-hexane and the extracted oil was distilled. The transesterification reaction was carried out for one hour and two hours using the micro algal oil, ethanol, and potassium hydroxide as a catalyst under the condition of closed system, at a temperature of 62 c°, and a mixing speed of 500 rpm. The products through the transesterification reaction were supposed to be biodiesel and glycerin.

Algae has a high capability of absorption and adsorption of sulfur up to 9 g/l, so it can be used to reduce sulfate content in water in the red – dead canal project

Keywords: Biodiesel, Microalgae, Transesterification, Chlorella, Algal oil.

1. Introduction

1-1 Definition

Biodiesel is defined as mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats and now from microalgae which conform to ASTM specifications for use in diesel engines.

A mono alklyester is the product of the reaction of straight chain alcohol, such as methanol or alcohol, with fat or oil (triglyceride) to form glycerol and the ester of long chain fatty acid.

“Bio” represents its renewable and biological source in contrast to traditional petroleum based diesel fuel; “Diesel” refers to its use in diesel engine. Fatty acid alklyester (FAAE) can be used as biodiesel fuel or can be used as an additive or extender to diesel fuel.

Biodiesel is made through a chemical process called transesterification whereby the glycerin is separated from the fat, vegetable oil and algal oil. The process leaves behind two products: methyl esters (the chemical name for biodiesel) and glycerin (a valuable byproduct usually sold to be used in soaps and other products)(Chisti, 2007).

1.2 Description:

Biodiesel is light to dark yellow liquid. It is practically immiscible with water, has a high boiling point and low vapor pressure. Typically ethyl ester biodiesel has a flash point of ~ 144°C, making it rather non-flammable. Biodiesel has a density of ~ 0.872 g/cm³, less than of water. Biodiesel uncontaminated with starting material can be regarded as non-toxic (Hannon, 2010).

It can be used as an additive in formulations of diesel to increase the lubricity of pure Ultra-Low Sulfur Diesel (ULSD) fuel. Much of the world uses a system known as the "B" factor to state the amount of biodiesel in any fuel mix. Biodiesel blends, a mixture of petroleum diesel and biodiesel, can be used in any diesel engine. As biodiesel can be blended with diesel in any concentration, the blend level depends on economics, availability, the desired emissions level, material compatibility and combustion characteristics. Biodiesel in its pure form is known as "neat biodiesel" or B100, but it can also be blended with conventional diesel, most commonly as B5 (5 percent biodiesel and 95 percent diesel) and B20 (20 percent biodiesel and 80 percent diesel). Biodiesel is registered with the U.S. Environmental Protection Agency (EPA) and is legal for use at any blend level in both highway and non road diesel vehicles (Bradley, 2013).

1.3 Reasons for selecting microalgae as a source of biodiesel

Bio energy is currently receiving considerable international attention in politics and the media. In the last 10 years many studies have been conducted on bio fuels, pure or blends, for substituting a meaningful share of fossil fuels, in order to reduce emissions and for achieving efficiency and a certain degree of sustainability. Biodiesel has gained considerable attention as the need to develop alternatives to traditional diesel fuel increases (Pisutpaisal, 2008).

The attention should be centered on the feed stocks (oilseed crops, vegetable exhausted oil, and animal fats) because of their differences in economic, energetic and ecological costs. During the last years there have been few attempts to study and estimate the real feasibility and sustainability of algal biomass utilization in order to produce biodiesel. Some works focus on the use of different species of microalgae because of their high oil yield

with respect to oleaginous plants (Li, 2008).

The idea of using microalgae as a source of fuel is not new but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels. Biodiesel is produced currently from plant and animals oils, but not from microalgae. This is likely to change as several companies are attempting to commercialize micro algal biodiesel (Dung, 2012).

Microalgae have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops. Exploring ways to reduce the high cost of biodiesel is of much interest in recent biodiesel research, especially for methods concentrating on minimizing the raw material cost (Gao, 2012).

One alternative to oil crops is the algae because they contain lipids suitable for esterification \ transesterification. Among many types of algae, microalgae seem to be promising because (Pienskos, 2011):

- Micro-algae grow within all year and has short life cycle. They could be harvested more than once in a year.
- Micro-algae the most fast-growing plant on Earth, grows in 100 times faster, than trees and that means they have high growth rates and microalgae commonly double their biomass within 24h. Biomass doubling times during exponential growth are commonly as short as 3.5h.
- For micro-algae the readily available raw material is required: sunlight, water, carbon dioxide and nutrients (P and N).
- From micro-algae it is possible to receive natural products: pigments, fibers, enzymes, sugar, fats, amino acids, vitamins.
- Depending on kinds of algae (exists more than 30.000 kinds) and conditions of its cultivation, the chosen algae makes about 40-60 % of oil, and microalgae produce 15-300 times more oil for biodiesel production than traditional crops on an area basis.oil content in microalgae can exceed 80% by weight of dry biomass.
- Selection micro-algae grow in all environments, even if the temperature of water makes - 2°C.
- Their lipid content could be adjusted through changing growth medium composition. Salty or waste water could be used.
- Atmospheric carbon dioxide is the carbon source for growth of microalgae and producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide.
- Algae can be fertilized with sewage and waste water.
- Algae Sugars can be fermented to make Ethanol for E85.
- Algae is producing feed for fish and Livestock from waste biomass.
- In addition to the advantages of biodiesel from microalgae.

2. Theory

2.1 Raw materials:

2.1.1 Feedstocks of biodiesel in this work

Microalgae are photosynthetic microorganisms that convert sunlight, water and carbon dioxide to algal biomass. Many microalgae are exceedingly rich in oil which can be converted to biodiesel using existing technology.

Microalgae are unique because they combine the renewable energy-capturing ability of photosynthesis with the high yields of controlled microbial cultivation, making them potentially valuable organisms for economical, industrial-scale production processes in the 21st century so our project studies and estimates the real feasibility and sustainability of micro algal biomass utilization in order to produce biodiesel.

Oil productivity, that is the mass of oil produced per unit volume of the micro algal broth per day, depends on the algal growth rate and the oil content of the biomass. Microalgae with high oil productivities are desired for producing biodiesel. Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils. Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops.

The aim of this project was to obtain high quality biodiesel production from a microalgae *Chlorella* protothecoids through the technology of transesterification (Mata, 2010).

2.1.2 *Chlorella*

Chlorella protothecoides is a microalga that can grow photoautotrophically or heterotrophically under different culture conditions. Heterotrophic growth of *C. protothecoides* supplied with acetate, glucose, or other organic compounds as carbon source, results in high biomass and high content of lipid in cells. With the addition of the organic carbon source (glucose) to the medium and the decrease of the inorganic nitrogen source in the medium,

the heterotrophic *C. protothecoides* was cultivated with the crude lipid content up to 55.2% which was about four times that in photoautotrophic *C. protothecoides*. Therefore, *C. protothecoides* has not only become an important source of many products, such as aquaculture feeds, human food supplements, and pharmaceuticals but also been suggested as a very good candidate for fuel production (Benemann, 2008).

Chlorella is a genus of single-celled green algae, belonging to the phylum chlorophyta. It is spherical in shape, about 2 to 10 μm in diameter, and is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll -a and -b in its chloroplast. Through photosynthesis it multiplies rapidly requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce. Figure (1) shows the *Chlorella* algae taken from the Dead Sea.

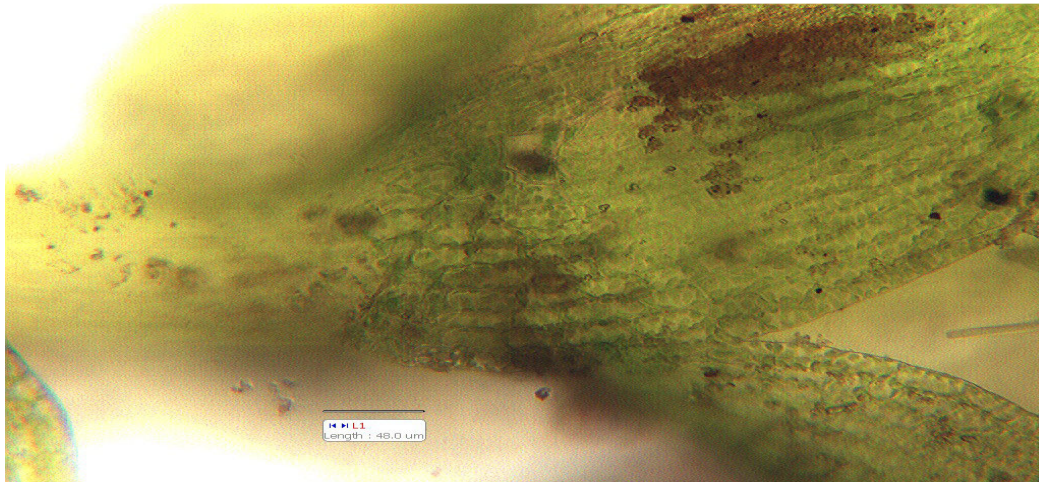


Fig (1) *Chlorella* algae

2.1.3 Filamentous (*Spirogyra*)

Spirogyra is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas, and there are more than 400 species of *Spirogyra* in the world. *Spirogyra* measures approximately 10 to 100 μm in width and may stretch centimeters long.

Spirogyra is unbranched with cylindrical cells connected end to end in long green filaments. The cell wall has two layers: the outer wall is composed of cellulose while the inner wall is of pectin. The cytoplasm forms a thin lining between the cell wall and the large vacuole it surrounds. Chloroplasts are embedded in the peripheral cytoplasm; their numbers are variable (as few as one). The chloroplasts are ribbon shaped, serrated or scalloped, and spirally arranged, resulting in the prominent and characteristic green spiral on each filament. Each chloroplast contains several pyrenoids, centers for the production of starches, appearing as small round bodies.

Spirogyra is very common in relatively clean eutrophic water, developing slimy filamentous green masses. In spring *Spirogyra* grows under water, but when there is enough sunlight and warmth they produce large amounts of oxygen, adhering as bubbles between the tangled filaments. The filamentous masses come to the surface and become visible as slimy green mats. *Mougeotia* and *Zygnema* are often found tangled together. *Spirogyra* can reproduce both asexually and sexually. In asexual reproduction, fragmentation takes place, and *Spirogyra* simply undergoes intercalary mitosis to form new filaments.

Spirogyra uses photosynthesis for the production of food and figure (2) shows the filamentous algae under microscope.

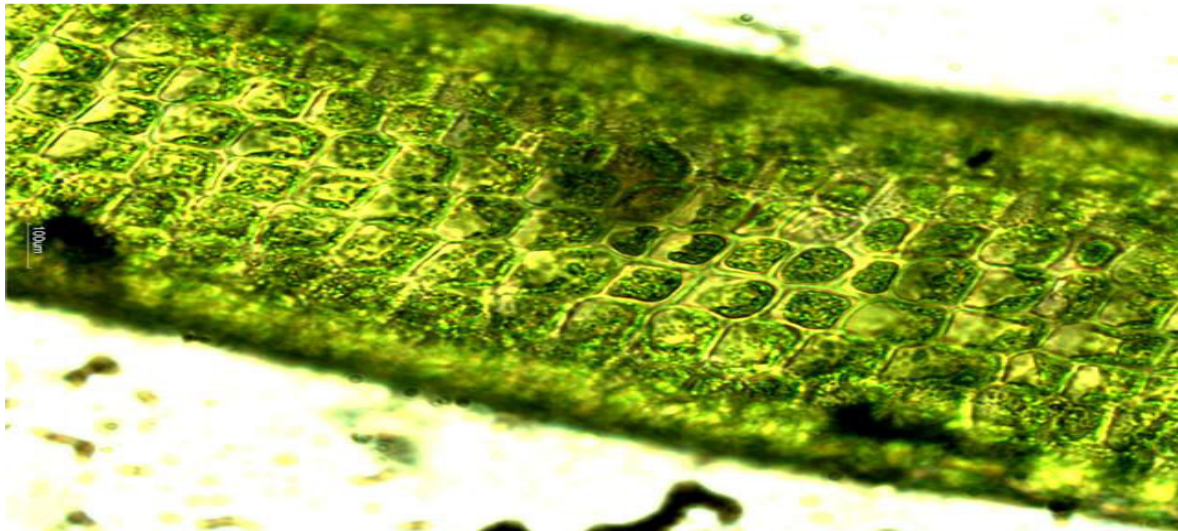


Fig (2) Filamentous algae (Spirogyra)

And this figure below shows the algae given from the Aqaba Gulf.



Fig (3) Microalgae from the Aqaba Gulf

2.1.4 Diatoms

A major group of eukaryotic algae, and are one of the most common types of phytoplankton. Most diatoms are unicellular, although they can exist as colonies in the shape of filaments or ribbons (e.g. *Fragillaria*), fans (e.g. *Meridion*), zigzags (e.g. *Tabellaria*), or stellate colonies (e.g. *Asterionella*). Diatoms are producers within the food chain.

A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica (hydrated silicon dioxide) called a frustule. These frustules show a wide diversity in form, but usually consist of two asymmetrical sides with a split between them, hence the group name. Fossil evidence suggests that they originated during, or before, the early Jurassic Period. Diatom communities are a popular tool for monitoring environmental conditions, past and present, and are commonly used in studies of water quality.

There are more than 200 genera of living diatoms, and it is estimated that there are approximately 100,000 extant species. Diatoms are a widespread group and can be found in the oceans, in freshwater, in soils and on damp surfaces. Most live pelagically in open water, although some live as surface films at the water-sediment interface (benthic), or even under damp atmospheric conditions. They are especially important in oceans, where they are estimated to contribute up to 45% of the total oceanic primary production. Spatial distribution of marine phytoplankton species, are restricted both horizontally and vertically. Diatoms occur in all oceans from the poles to the tropics; polar and sub polar regions contain relatively few species compared with

temperate biota. Although tropical regions exhibit the greatest number of species, more abundant populations are found in polar to temperate regions. Usually microscopic, some species of diatoms can reach up to 2 millimetres in length and figure (4) shows these diatoms algae.

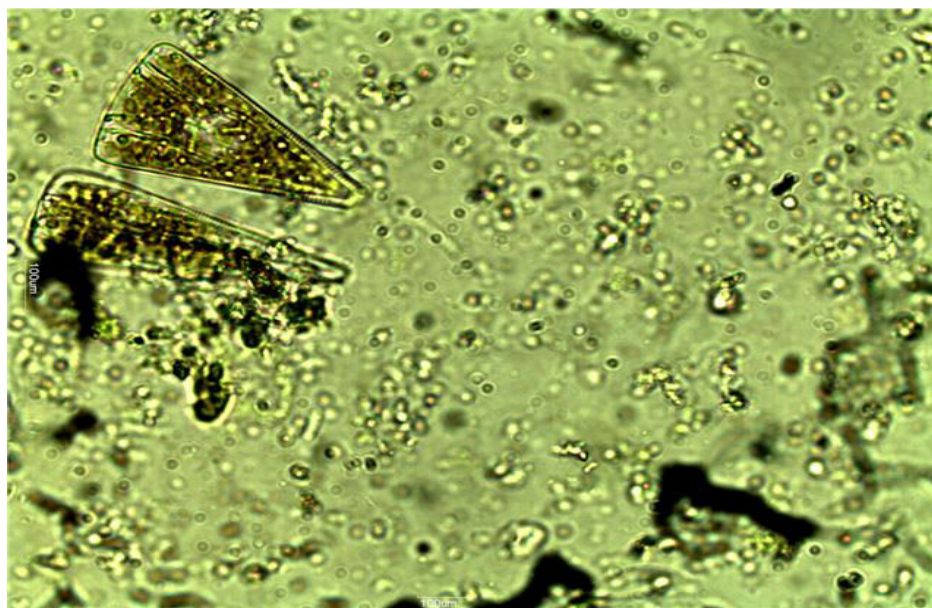


Fig (4) Diatoms

2.2 Transesterification

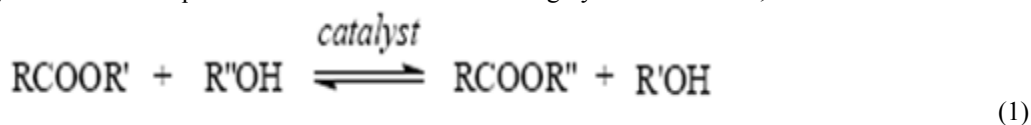
2.2.1 General:

There are four primary ways to make biodiesel, direct use and blending, micro emulsions, thermal cracking (pyrolysis) and transesterification. The most common way is transesterification as the biodiesel from transesterification can be used directly or as blends with diesel fuel in diesel engine so any future production of biodiesel from microalgae is expected to use the same process.

2.2.2 General Aspects of Transesterification

Transesterification is the general term used to describe the important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety. When the original ester is reacted with an alcohol, the transesterification process is called alcoholysis (Scheme I). In this review, the term transesterification will be used as synonymous for alcoholysis of carboxylic esters, in agreement with most publications in this field.

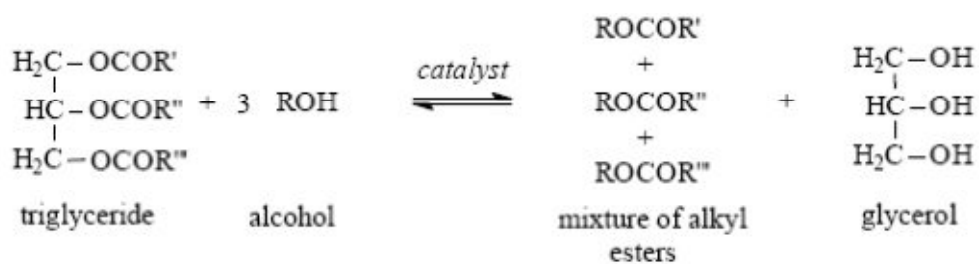
The transesterification is an equilibrium reaction and the transformation occurs essentially by mixing the reactants. However, the presence of a catalyst (typically a strong acid or base) accelerates considerably the adjustment of the equilibrium. In order to achieve a high yield of the ester; the alcohol has to be used in excess.



SchemeI: General equation for transesterification reaction.

The applicability of transesterification is not restricted to laboratory scale. Several relevant industrial processes use this reaction to produce different types of compounds.

In the transesterification of algal oils, a triglyceride reacts with an alcohol in the presence of a strong acid or base, producing a mixture of fatty acids alkyl esters and glycerol (Scheme II). The overall process is a sequence of three consecutive and reversible reactions, in which di and monoglycerides are formed as intermediates. The stoichiometric reaction requires 1 mol of a triglyceride and 3 mol of the alcohol. However, an excess of the alcohol is used to increase the yields of the alkyl esters and to allow its phase separation from the glycerol formed. Several aspects, including the type of catalyst (alkaline or acid), alcohol/ oil molar ratio, temperature, purity of the reactants (mainly water content) and free fatty acid content have an influence on the course of the transesterification .



Scheme II : Transesterification of algal oils. (2)

Direct acid catalyzed esterification of the oil with methanol.

Conversion of the oil to fatty acids, and then to Alkyl esters with acid catalysis.

3. Methodology

3.1 cultivation

Chlorella protothecoides in exponentially period was inoculated (10%, v/v) in a liquid medium. the cultivation was initially carried out in 500-ml Erlenmeyer flask containing 300 ml medium at $28 \pm 1 \text{ }^\circ\text{C}$ with a certain nutrients for along time extend for a few month in a large container and a source of light should be focused on the container .

Figure (5) below shows the beginning of the cultivation in the first week, and figure (6) shows the cultivated algae after several weeks.



Fig (5) The first week of Chlorella cultivation



Fig (6) The last week of Chlorella cultivation.

3.2 Extraction

The wet paste of algae was collected to evaluate the lipid extraction yield and then was prepared to be extracted by weighing and filtration by piece of cloth then micro algal lipid was extracted with n-hexane in a soxhlet extractor operated at 80°C for one hour for each batch, after that the mixture of the extracted oil and n-hexane was separated from the biomass by filtration, and then was distilled by simple distillation at 75°C for one hour for each batch to separate the n-hexane (which was reused in other batches to minimize the cost) from the oil needed to the next stage which is the transesterification. Figure (7) shows the soxhlet extractor and it was filled with algae and n-hexane.



Fig (7) Soxhlet Apparatus.

Figure (8) shows the extracted oil level.



Fig (8) Algal oil extraction (oil level)

After extraction which lasted for one hour the mixture was taken and the oil was separated from the algae. Figure (9) shows the oil with n-hexane before separation.



Fig (9) Algal oil with n-hexane

A distillation was carried out after extraction to separate the extracted oil from the n-hexane and to recover the n-hexane and reuse it as shown in figure (10).



Fig (10) Solvent recovery by distillation.

The extracted oil is shown in figure (11) below



Fig (11) Extracted algal oil.

3.3 Transesterification

Alkali-catalyzed transesterification was carried out at approximately 62°C under atmospheric pressure under 500rpm of stirring speed, under these conditions the reaction takes about 90 min to complete. Ethanol and oil do not mix, hence the reaction mixture contains two liquid phases, other alcohols can be used but ethanol is the least expensive. To prevent yield loss due to saponification reaction (i.e. soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids.

Biodiesel was separated from ethanol – glycerin mixture and figure (12) shows the transesterification reaction lasted for one hour and two hours.



Fig (12) Production of biodiesel from algal oil

After the transesterification reaction two layers were formed, the upper layer was a mixture of glycerin and ethanol, the lower layer is supposed to be the biodiesel formed after 10 hours settling for complete separation as shown in figure (13)



Fig (13) Formation of two phases

4. Results

Sources of algae:-

- 1- Chlorella (by cultivation)
- 2- Algae from cooling tower of Jordan petroleum refinery
- 3- Algae from Aqaba Gulf

Table (1) Extraction of algae:

| Source of algae | Weight of algae (gm) | Volume of extracted mixture (ml) | RI |
|----------------------------|----------------------|----------------------------------|-------|
| Chlorella | 70 | 8 | 1.386 |
| Aqaba gulf algae (trial 1) | 150 | 75 | 1.347 |
| Aqaba gulf algae (trial 2) | 300 | 140 | 1.347 |
| Aqaba gulf algae (trial 3) | 300 | 145 | 1.347 |
| Aqaba gulf algae (trial 4) | 300 | 67 | 1.35 |
| Aqaba gulf algae (trial 5) | 400 | 150 | 1.35 |
| Aqaba gulf algae (trial 6) | 450 | 190 | 1.35 |
| Cooling tower algae | 800 | 3 | 1.38 |

Table (2) Transesterification of the extracted mixture

| Vol (ml) | Sample (1) Reaction time 1 hour | Sample (2) Reaction time 2 hours | RI |
|-------------------------|------------------------------------|-------------------------------------|------|
| Vol. of the upper layer | 1560 | 975 | 1.36 |
| Vol. of the lower layer | 840 | 525 | 1.36 |

Table (3) Tests of the lower layer (biodiesel formed) properties

| TEST | SAMPLE (1) | SAMPLE (2) |
|--------------------------------------|------------|------------|
| Density @15 c° [gm/cm ³] | 0.8671 | 0.8918 |
| Total sulfur [%wt] | 0.296 | 0.901 |
| Flash point [c°] | 25 | 25 |

5. Discussion

Three kinds of microalgae were used in the experiments, the third one was a mixture of three different kinds of micro algae which was:-

- 1- Chlorella
- 2-Filamentous (Spirogyra)
- 3-Diatoms

As can be seen from the table that the amount of the extracted oil from each sample were slightly small, because the mixture contains water and biomass beside the oil. According to the lipids and oil content in each kind, Chlorella algae has the largest content of lipids (14-20 %), and the spirogyra (11-21 %). According to these percentages the amount of oil was small in the mixture.

As can be seen from table (2), transesterification reactions were run for one hour and two hours, after the reaction two layers were formed, the upper layer contains ethanol and side product which was the glycerin, and the lower layer supposed to be the wanted biodiesel after 10 hour settling.

After running the tests of the properties of the lower layer, the density and the sulfur contents differ in each sample because the second sample was exposed to heat source so a certain amount of water was evaporated from the sample, this explains the increase in the density and sulfur values in the second sample, and the flash point was the same in both samples.

As a result for these tests, the sulfur content in the third sample which contains a composition of three kinds of microalgae is relatively high; this is attributed to the contamination of Aqaba Gulf by phosphogypsum which contains 44% sulfur. There are 31 million tons of phosphogypsum stored under the open atmosphere, and in windy days some of this phosphogypsum is carried away to contaminate water in the Gulf of Aqaba which indicates that these compositions of three kinds of algae has the capability to absorb a large amount of sulfur which was according to the tests (9000 ppm). So as an advantage, treatment of water from the sulfur content can be carried out using these microalgae in the Red-Dead canal project.

6. Conclusions

1. The alga Chlorella is suitable for the production of biodiesel, since it contains the largest oil content among the experimented three types of microalgae.
2. A further usage of these algae can play a major role to solve the problem of the red – dead canal project, in absorbing the sulfur content from the Red Sea before transporting it to the Dead Sea which will prevent the formation and settling of gypsum in Dead Sea.
3. The produced biodiesel has a density which is very close to the density of petroleum diesel.
4. Algal biodiesel has a very strong economic potentiality.
5. Algal biodiesel does not affect the feed stock of the people.

7. Recommendations

As a recommendation for further projects and researches to produce biodiesel from microalgae, a specific type of microalgae should be cultivated in a suitable media with particular nutrients.

The transesterification reaction can be run on a molecular scale and that by using different method like high frequency magnetic impulse cavitation and the nanotechnology technique which provide a higher yield with reducing time period of the reaction.

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