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Screening of Some Algal Oils to Select the Best Algal Biodiesel Resource

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ABSTRACT

Recently, algae seem to be a promising feedstock for the future alternative fuel (green fuel) instead of fossil fuel. Algae (macro- and micro-algae) may represent as a main resource for biofuel and biodiesel due to their high biosynthesis effeminacy to produce lipids and fatty acids comparing to other types of biomass. Algae can be an interesting source of commercial and eco-friendly fuel due to algae local availability and low CO_2 emission, respectively. The current study was conducted to compare the percentage of oil production in three different genera of algae, including *Cosmarium*, *Spirogyra* and *Chara*, and examine the ability of using the algae oil as an alternative fuel for industrial purposes. Soxhlet-based method and n- hexane organic solvent were used for extraction of algae oils followed by biochemical analysis and gas chromatography (GC-Mass) technique for algae oil detection. The algae *Cosmarium* had 2.8 % algal oil yield, and recorded the highest yield of the total fatty acids (65.60 %), whereas *Spirogyra* recorded a low result by producing 32.07 %, noting that *Chara* alga showed the lowest value (21.58). The chemical analysis of algal oils showed that the quality of algae oil fatty acids proved to be the best choice. *Cosmarium* oil recorded the highest ratio (2.05 %) for unsaturated fatty acids (USAFA)/ saturated fatty acids (SAFA) and the degree of unsaturation (DU) was 84.86. While, *Spirogyra* and *Chara* showed that the value of USAFA/SAFA was 1.58 and 0.56 % with DU of 27.74 and 9.68, respectively. In this context, *Cosmarium* alga proved to be the best feedstock for biomass of algal oil production with astounding properties of algal biodiesel.

INTRODUCTION

Green algae constitute the most heterogeneous group of photoautotrophic protoctists inhabiting the biosphere and show an enormously wide variability of shape, size, and habit, and even color may be highly variable. According to the most recent taxonomic tree, the green algae have evolved in two major lineages; the chlorophytes clade (e.g. Cladophora, *Chlamydomonas*) and the Charophytes clade (e.g. *Chara*، *Spirogyra*). The first one includes the majority that is called "green algae", whereas the second contains a smaller number of green algal; taxa Green algae. All are photoautotrophic and their growth depends on nutrients and underwater light availability، which is linked to physical parameters such as transparency, hydrodynamics، temperature, conductivity, and others .Those organisms have dimensions ranging from few micrometers to several centimeters with a substantial difference in their morphological organization from swimming or non-motile unicells

and colonies to filaments and varied levels of tissue organization(Pseudoparenchymatous, Parenchymatous or thalloid), in addition to branching morphologies. Hence, they have many ways of adaptation to the highly variable aquatic environment from freshwater to hypersaline and from very acidic to strongly alkaline **(Naselli-Flores & Baron, 2009)**. Algae are a large and diverse group of organisms growing in aquatic environments able to photosynthetically convert CO_2 and minerals to biomass, although some species grow heterotrophically. Macroalgae and microalgae are two main forms of algae that vary in many characteristics. Macroalgae are fast-growing, multicellular macroscopic nonvascular plants that contain Chlorophyll, with sizes reaching up to 60 m in length. They are classified into groups based on their pigmentation: Chlorophyceae Phaeophyceae and Rhodophyceae. On the other hand, microalgae consist of a wide range of autotrophic organisms that grow through photosynthesis in the same way as plants do, and their classification depends on a revision due to new genetic evidence. Remarkably, macroalgae is the larger algal species, visible to the naked eyes, mostly represented by red, brown and green algae and also known as seaweed. They have a higher protein content as well as carbohydrates, amino acids, minerals, and vitamins **(Singh *et al.*, 2010)**. It was noticed that, the study of microalgae is less developed than that of sea-weeds but their advantage of faster growth, higher photosynthetic efficiency, and indoor production add important features to it **(Villarruel- López *et al.*, 2017)**. It is worth mentioning that, microalgae have a size length of 0.5-30 µm **(Marko & Georgakakis, 2011)**. Generally, microalgae are those algae whose cell size ranges from less than $1/1000^{th}$ of 1 mm to 2 mm. Additionally, they possess a high amount of lipids and high proportion long-chain unsaturated fatty acids like omega 3-polyunsaturated fatty acids (PUFAs). Apparently, algae are regarded important primary producers and early producers of oxygen through oxygenic photosynthesis. Recently, they were determined as efficient and promising biotechnological tool that should be benefited from to reach a better future. Worldwide attention is drawn towards the algal species for their high nutrition profile, therapeutic properties that because of it, they are being used as food, feed fuel fertilizer, colourants, antioxidants, a resource of toxins and bioactive compounds **(Singh *et al.*, 2010).** Terrestrial fuels like coal and petroleum are in the service of humankind since immemorial time. With an asymptotic rise in the use of fuels (with developing thermal power stations and increase in the number of transporting vehicles), the terrestrial coal pits and petroleum vessels are going to be emptied within a short period. Alternative energy sources are under harvest, including solar, wind, geothermal and many others. Thus, in the proteomic and genomic era biotechnology became the source of many inventions through which technology was used to harvest energy from plant materials, without waiting for a long period for the transformation of fresh plant to fossil fuel (Barker,2010). This energy is in the form of liquid fuel and is termed as bio-fuels. Biofuels can be defined as any fuel derived from biomass, including biodiesel, bioethanol, and a product of bioethanol such as ethyl tertiary butyl ether (ETBE), biogas, bio methanol, biomethyether and bio-oil. Biodiesel reduces the emission of smoke, Sulphur, particulate matter, carbon monoxide and total hydrocarbons. While, biodiesel has a higher flash point and offers low storage at ambient temperature, handled like diesel and is nontoxic and biodegradable, so biodiesel is also safe to handle and transport and sustainable partial replacement for diesel. Biodiesel is made through a chemical process called esterification, most being produced using an alkaline catalyzed reaction whereby a fat or oil reacts with alcohol in the presence of catalyze (usually sodium or potassium hydroxide).The biomass oils/ fats can be

converted into biodiesel by a process called transesterification, that is formed by hydrolyzing triglycerides present in oils or fats to produce fatty acids with glycerin, followed by neutralization of the free fatty acids, removal of glycerin, and esterification of acids by using alcohol. Algae have emerged as one of the most promising sources of biodiesel production. Algae are eco-friendly organisms due to their role and their ability to convert CO_2-enriched air to oily substances. In a study which was set out to characterize the proper transesterification, the amount of biodiesel production (ester) and physical properties of biodiesel using common species as *Oedogonium* and *Spinagyra* were determined, and hence, concluded that *Oedogonium* had higher content than *Spirogyra* sp., however, biomass after extraction was higher in *Spirogyra* than *Oedogonium* sp., as well as glycerin, water and pigments. The aforementioned study reported that biodiesel could be produced from both species *Spinagyra* and *Oedogonium*, noting that the latter is better than *Spirogyra* sp. Among biomass, algae (macro and micro) usually have higher photosynthesis efficiency than other biomass, and they are the highest yielding feedstock for biodiesel; thus, it is one of the best sources of biodiesel . Remarkably, algae can produce up to 250 times the amount of oil per acre as soybean. Algae produce 7 to 31-time greater oil than palm oil and it's very simple to extract oil from algae, especially microalgae due to their small diameter (less than 2mm). In comparison, macroalgae is not as widely used for the production of biodiesel because microalgae have much more oil faster and easier to grow. Additionally, microalgae can provide several different types of renewable biofuels derived from microalgal oil (Barker,2010).

The idea of using microalgae as a source of fuels is not new, but is now being taken seriously because of the escalating price of petroleum, and the emerging concern about global warming that is associated with burning fossil fuels **(Barker, 2010).** The composition of microalgal lipids is similar to those of vegetative oils. The total lipids include three major classes: neutral lipids, glycolipids and phospholipids. Microalgal lipids are generally esters of glycerol and fatty acids with a chain length of C14 to C22 which may be either saturated (SF) or unsaturated. The green algae contain primarily C16 and C18 fatty acids with a high degree of unsaturation. Some of algal lipids are also rich in C18 linoleic acid (18:2 Ω6) and linolenic acid (18:3 Ω3) and their derivatives, eicosapentaenoic acid (20:5 Ω3) and arachidonic acid (20:4 Ω6). The microalgal fatty acids composition depends on not only the species and strain used but also on factors related to culture conditions that include the composition of the medium, light intensity, photoperiod, salinity and temperature **(Kaur *et al.*, 2010).** Recovery of microalgal biomass from the broth is necessary for extracting the oil. This step was done by filtration, centrifugation, and other means. The typical biomass concentration produced in photo-bioreactors is nearly 30 times that obtained in raceways. For user acceptance, microalgal biodiesel will need to comply with existing standards. In the United States the relevant standard is the ASTM biodiesel standard D 6751. In European Union, separate standard exit for biodiesel intended for vehicle use (Standard EN 14214) and use as heating oil (Standard EN 14213). Microalgal oils differ from most vegetable oils in being quite rich in polyunsaturated fatty acids with four or more double bonds **(Barker, 2010).** Consequently, this study was organized to evaluate lipids productivity and detect its fatty acids quality and quantity within Chlorophyta: *Cosmarium* as microalgae, unicellular form and filamentous form *Spirogyra* with *Chara* as macroalgae. The quantity and quality of fatty acids are the most important points in the quality of Algae oil. Globally and locally, limited researches were conducted on that species of algae in the field of biodiesel

production. To fill this gap in literature, the current study aimed to determine the most suitable algal oil by comparing the content of lipids of microalgae and macroalgae with its fatty acid chemical analysis.

MATERIALS AND METHODS

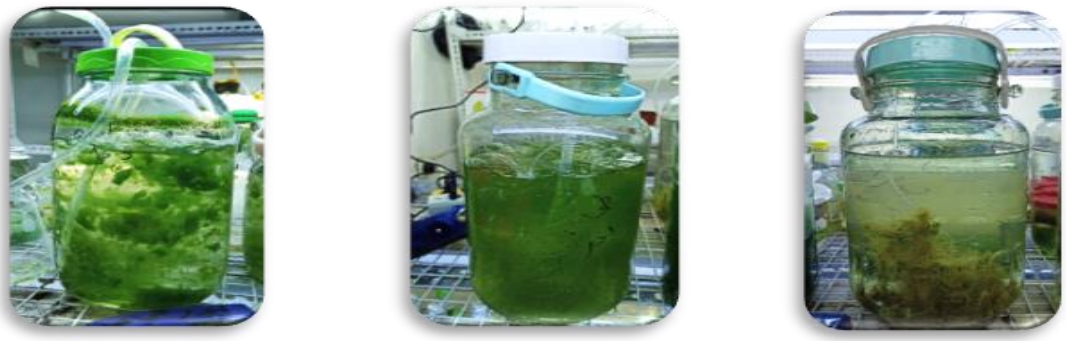
1-Algae Samples

The feedstock of biodiesel extracted from algae oil was originated from Chlorophyta species (micro and macroalgae): *Cosmarium*, *Spirogyra*, and *Chara*. *Cosmarium* algae were collected from the home water tank which previously contains tap water (Fig. 1A) , while algae (*Spirogyra* and *Chara*) were obtained from different environments in the Nineveh governorate in Bartella sub-district / Shaquli village (East of Mosul city) stream (Fig. 1B) .The narrow stream estimated at (1.0 m) coming from the connection of a small spring of water (freshwater) to a spring of water smaller than Ein al-Awwal (sulfuric water) that pass through the village of Shaquli toward the village of Karmelos in the disrtrict of Hamdaniya, near the mountain of Ain al-Safra. To the southwest, it is bounded by the Tigris River, and the Bartella district that represents a raining area of 425 mm/year.

A: *Cosmarium* in home water tank B: *Spirogyra* and *Chara* Bartella stream

Fig. 1: Habitats of algal samples in this study

The algae samples were collected, washed and dried in an oven at 40C° to obtain algae dry weight (DW) for oil extraction. Furthermore, algae samples were cultured in simple photobioreactors that were made manually. For *Cosmarium* culture, modified Chu10 medium was used in growing the algae following the method of **AL-Katib *et al.* (2015)**. For *Spirogyra* and *Chara* cultures, tap water was used. The simple photobioreactors consist of a glass container (5L) to cultivate algae with the addition of an aeration system (Fig. 2). For the culture media that was used in 3L volume, 300 ml inoculum was added in the case of *Cosmarium* photobioreactors and many filaments of *Spirogyra* and *Chara*. Algal photobioreactors were incubated at constant condition 25±2 C° temperature, 16:08 light: dark photoperiod.



A: *Cosmarium* B: *Spirogyra* C: *Chara*

Fig. 2: Simple photobioreactors of algae samples growing in laboratory.

2- Algal growth and Biomass

Algae growth in photobioreactor was recorded for *Cosmarium* algae by using a spectrophotometer at different absorption lengths (436,650,750nm). Nevertheless, in the first week of experiment, it was observed that the biomass of the algal macroalgae samples (*Spirogyra* and *Chara*) was difficult to record with the spectrophotometer method due to its big size filament form compared to *Cosmarium* microalgae. Furthermore, a healthy and young sample was chosen to obtain good biomass for extraction-microalgae species biomass obtained from the original habit that was used later in oil extraction besides culturing it under laboratory condition to safeguard a continuous offering through all days. Water features (T. Alk., T.Hard, Ca.H., Mg.H., HCO_3^-,Cl^-,SO_4^-ions) in which macroalgae grew, were recorded after measuring according to the method of **Shugar (2000)** and **APHA (2017)**, similar procedures were taken with regards to the water in which miccroalgae grew (Table 1).

Table 1. Fresh water stream parameters showing *Spirogyra* and *Chara* habits.

No.	Parameter	Measurements (mg/L)
1	T. Hard	720
2	Ca. Hard	400
3	Mg. Hard	320
4	(Cl^{-1})	200
5	$(SO_4^-)^2$	244L
6	T.Alkal	10.1L
7	$(HCO3)^-$	289

3- Algae Biomass preparation

After good growth (7day culture) of *Cosmarium* algae in photobioreactor and collecting enough samples from *Spirogyra* and *Chara* algae, the isolates algae were washed in water and air dried. Algae samples were then dried in an oven at 40C° for 24-48 h and ground to powder by using a pestle with mortar. This dry biomass powder was stored in an airtight container until making oil extraction (Barker,2010).

4- Algae Oil Extraction

The Algae oil was extracted from 10 g DW with 200 ml of n-hexane (50- 60C°) in a Soxhlet extraction apparatus. Then, the solvent was removed using evaporating rotary and oven at 40C°. The crude algal oil was stored in glass vials until the next analysis for fatty acids **(Alfekaik & AL-Hilfi, 2006)**. Hence, the yield of algae oil

was extracted (oil yield = oil extract / dried algae biomass taken × 100) **(Karmakar *et al*., 2018).**

5-Fatty Acids Esterification

The sample was prepared according to the recommended method based on the esterification of triglycerides by reacting with hydroxide solution- methylated potassium (2N) prepared by dissolved (11.2g) potassium hydroxide (KOH) in 100ml methanol. The esterification process was achieved by taking 1 ml of the oil sample in a 10 ml- test tube, and heating in a water bath at a temperature of 50C° until being dissolved. Then, 5 ml of methylated potassium hydroxide solution was added, and the tube was shaken well for 5 minutes. Afterwards, 5 ml of hexane was added and the contents of the tube were mixed well until the materials appeared separated to two layers. The top layer contained the fatty acid methyl ester (FAME) in hexane, and the bottom layer contained the saponated materials released from the reaction **(AOAC, 2000).**

6-**Fatty Acids Identification and Determination by Gas Chromatography-Mass Spectrophotometer (GC-Mass)**

Algae fatty acids in each sample were detected by gas chromatography analysis. Thus, the process was done by using a Shimadzu QP2010 quadrupole gas chromatography-mass spectrophotometer (GC-Mass) instrument equipped with a carbowax (30 m X 0.25 mm ID (capillary column DB-MS 5); Colum Oven Temp 40 C°; Injection Temp. 280 C°; Injection mode split; Flow Control Mode- Linear Velocity; Pressure 49.5 K Pa; Total Flow 34 ml/min; Column Flow 1 ml/min; Linear Velocity 36.1 Cm/Sec purge Flow 3.5 ml/min; Split Ratio 30). The GC program is GS-MS-QP2010ultra: Ion Source Temp; 200 C°, Interface Temp; 280 C°, Solvent Cut Time; 3 min, Detector; Gain; Mode-Absolute, Detector Gain; 0.97 KV, Start Time; 3 min, End Time; 26 min, ACQ Mode-Scan Event Time; 0.5 Sec, Scan Speed; 1250.

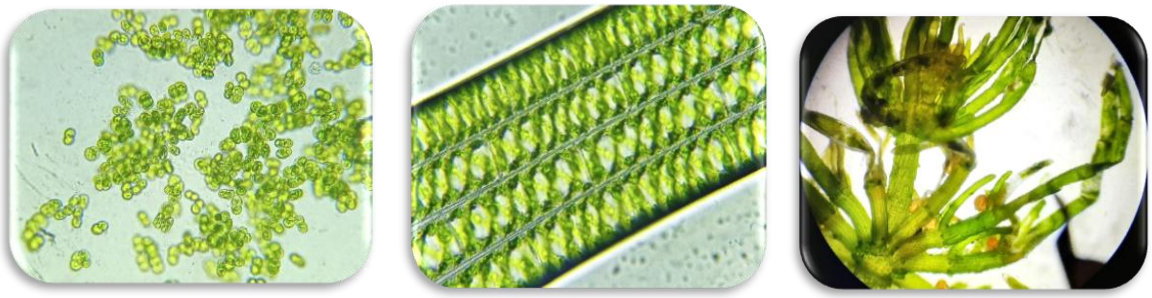
The fatty acid methyl ester was separated at constant pressure (49.5 KPa) and peaks were identified by comparing the mass spectra with the mass spectral database. The identification of compounds was based on the comparison of their mass spectra with NISI Library 2008**(Alfekaik & AL-Hilfi, 2006)**.

This technique was carried at the GC-Mass Lab, College of Agriculture, University of Basrah, Iraq.

RESULTS AND DISCUSSION:

1. Algal sample

The pure algae samples were obtained from *Cosmarium* as microalga, and *Spirogyra* and *Chara* as macroalgae, all from division Chlorophyta (Green algae) (Fig.3)



A-*Cosmarium* **B-*Spirogyra*** **C-*Chara***

Fig. 3: Microscopic picture of algal isolates.

The morphological detection of algal samples was formed by using algae classification references **(Prescott, 1973; Wehr & Sheath, 2003).**

Results showed that, *Cosmarium* alga is free floating, solitary desmid that occurs in fresh water ponds intermingled with other algae. They are abundant in mucilaginous masses along the walls of tanks and reservoirs in winter. The genus includes over 800 species **(Vashishta, 2012)**. In the current study, algae were isolated from wells of the home tank. *Cosmarium* consist of a small, flat cell, and the unicell has a deep median constriction (Sinus) that divides the cell into two distinct symmetrical halves. The two semi-cells are joined by a narrow connecting portion (isthmus). The protoplast of the cell secretes mucilage through pores in the cell wall. This mucilage forms a mucilaginous coat external to the outer layer of the cell wall. The protoplast contains mostly large axial chloroplast having central pyrenoid, nucleus, one or more vacuoles, and cytoplasm **(Vashishta, 2012)** (Fig. 3A)

***Spirogyra*:**

One of the commonest algae of green algae abundant in spring is *Spirogyra*. It is bright green- colored, free- floating masses in the still water of fresh water ponds, lakes and ditches and also in slow flowing streams where they are called *Nadess*. Because of the slippery feel of the threads, it is often called the pond-scum or water silk. Its thallus consists of a long green cylindrical and unattached that often filament. Each cell is usually much longer than the broad. The cell protoplast includes plasma membrane, cytoplast, nucleus, one or more chloroplasts with pyrenoids and a large central vacuole, and spiral chloroplast which is a feature from which the genus takes its name **(Vashishta, 2012)** (Fig3. B.)

***Chara*:**

Is a submerged aquatic alga which grows attached to the soft mud at the bottom, along the margins of fresh water, pools, lakes and slow-flowing streams forming thick masses. The thallus is attached in the mud by multicellular rhizoids. It has a long, slender, flexuous upright branched main axis which is differentiated into a well-marked series of alternating short nodes and long internodes. From each node arise four types of appendages = branchlets (leaves), long branches (axillary branches), stipulates, and cortex. The nodal and unelongated intermodal young cells are small in size and have similar structure. The central nucleus is centrally located. The numerous small discoid chloroplasts lacking pyrenoids are distributed throughout the cytoplasm **(Vashishat, 2012)** (Fig. 3C)

2.**Algae Cultures and Biomass production**

The algae samples were cultured in simple photobioreactors. The *Cosmarium* algae growth was recorded as cell density throughout the first week of culture and second week as well (Fig. 4). The recorded results showed that, the algae were growing well in modified Chu10 medium and

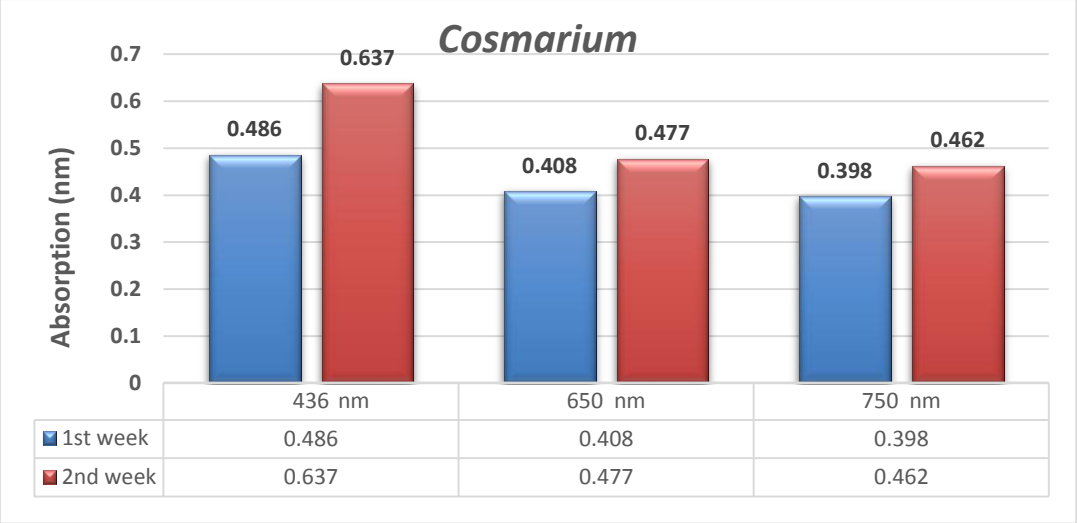


Fig. 4: *Cosmarium* culture growth.

cell density increased slightly in second week compared to the first week that may be referred to reaching a constant growth at a 14 days- period culture in a photobioreactor (at absorption length 650 and 750 nm), whereas at 436 nm, the difference between results of two periods (0.486 and 0.637 nm) was greater for first and second week, respectively. Thus, collecting *Cosmarium* biomass was done after 2 weeks- culture, while *Spirogyra* and *Chara* cultures were maintained and recultured continuously each two weeks. The biomass of algae was separated from the culture medium by decantation and centrifugation **(Francisco *et al.*, 2009).** After collection of fresh algae biomass, it was dried and the weight was found to be one-tenth of the primary weight with respect to the estimates of **Karmakar *et al.* (2018)**. *Spirogyra* and *Chara* macroalgae were collected from stream due to the formation of huge algal blooms. This process was done from stream because of the economic importance, easy availability, and non- purposed achievement. Collection was done using algae net and brought to the laboratory following the method of **Karmakar *et al.* (2018)** to obtain algal biomass.

3. Algal oil Extraction

The extraction of algal oil was achieved by using n-hexane that was referred to as the best organic solvent **(Karmakar *et al.*, 2018)**. Studies conducted on oil extraction from the algal species under study are rare, while there are many addressed other microalgae such Cyanobacteria and others from Chlorophyta (*Chlorella*, *Scenedesmus*, *Chlamydomonas*, etc.), Rhodophyta, Crysophyta, and Diatoms which are considered common raw biomass to this kind of work. This point, in turn, would enhance the valuable importance of the present study because of dwelling with important or common species that lacks attention beside its availability in huge quantity that has not been invested to benefit the human beings so far. Algae can be profoundly beneficial to human life as they are renewable, available, economic, easy work and rich resource for many compounds' isolation traditionally or in culture field biofertilizers to serve the huge pollution of earth life, as well as production of biofuel. However, limited concern has been paid to either *Cosmarium* as microalgae available in water and rave home culture, or *Spirogyar* and chara which represent macroalgae that have been avoided or neglected by researchers, while great attention was paid to other macroalgae genera such as *Polysiphonia* (Rhodoplayta), *laminaria*, and *Fucus*

(Phaeophyta). In this context, available sources should be benefited from and converted to best use for serving human beings as well stem use animals and earth ecosystem.

It is worth mentioning that, *Spirogyra* was used as feedstock for biodiesel production **(Ahmed *et al.*, 2012)** with hexane as extraction solvent (without culturing alga in laboratory). Reported results assessed that it was a good and potential resource for biodiesel production, while **Abdel- Aal *et al*. (2015)** referred to *Spirogyra* species as one of the most common freshwater green algae reported to have antimicrobial activities and fatty acids with esters that represented about 30 % of the total extract. The upper- mentioned results would encourage extracting bio-oil from that genus even that the study collected the alga from El Serw agriculture Canal Dakahlia in Egypt without culturing alga in laboratory. *Chara* alga has been rarely referred to in researches, as genus cultured in laboratory. Notably, it was scarcely reffered to as natural feedstock within the results concerning *Cosmarium*, *Spirogyra* and *Chara*. Table (1) represents some water parameters of *Spirogyra* and *Chara* habits (freshwater stream showing the algae collect region).

From recorded results (Table 2)

Table 2: Algal bio-oils properties

No.	Algae Species	Algae (DW) (gm)	Algae oil (gm)	Algae oil (%)	Unsaturated Fatty acids %	Saturated Fatty acids % (USFA)	% Total Fatty acids (SAFA)	USAFA/SAFA Ratio %	DU
1	*Cosmarium*	10	0.28	2.8	44.11	21.49	65.6	2.05	84.86
2	*Spirogyra*	10	0.79	7.9	19.66	12.41	32.07	58.1	27.74
3	*Chara*	10	0.14	1.4	7.76	13.82	21.58	0.56	9.68

Results in Table (2) shows that, from 10 gm of dry weight alga, the best genus in algal oil quantity production was *Spirogyra* (0.79g), followed by *Cosmarinm* (0.28 gm) and *Chara* (0.14 gm). The oil content of alga depends mainly on the type and division of the alga. There is great variation between macroalgae and microalgae contents, in addition to the big difference between members within each group (Ahmed *et al.,* 2012; Tabatabaei *et al*., 2015 ;Wacker *et al*., 2016 ;Li-Beisson *et al.,* 2019;Ganesan *et al.,* 2020). The extracted algal oils are viewed in Fig. (5).

Cosmarium. *Chara.* *Spirogyra*

Fig. 5: Extracted algal oil.

Depending on the recorded values in Table (2), results showed that the quality of algal oil is an important feature represented in unsaturated fatty acid percentage compared to saturated ones of the algal oils. Due to that feature, *Cosmarium* oil has proved to be the best of all other oils as shown in the successing paragraph after detection of fatty acids of oils.

4. Fatty Acids of Algal oil

After esterification of algal oil and production of free fatty acids, GC mass spectrophotometer was used for identifying the fatty acids in algal oil. The total percentage of fatty acids in oil was recorded with % unsaturated vs saturated fatty acids (Table 2). Analysis revealed that, the quality of algal oil was the best algal resource for the production of algal biodiesel having the best features. Results showed that, *Cosmarium* oil was superior to other oils in concern to the total fatty acids percentage (%) reaching a value of 65.6%, whereas others recorded 32.07 and 21.58% for *Spirogyra* and *Chara* oil, respectively. Thus, *Cosmarium* could be regarded as the best algal sample to produce biodiesel. The content and quality of fatty acids are important more than algal oil yield (%). This agrees with most references that emphasized considering microalgae being the best algae for biodiesel production while macroalgae was not widely used for that. Apparently, microalgae are much faster and easier to grow. It is good to mention that, algae are an economical choice for biodiesel production, because of their availability and low cost. Further research should be done on macroalgae and microalgae to compare the ratio of biodiesel production and chemical analysis **(Chakraborty & Bhattachavya, 2010**). The algal oils after transesterification were set to be prepared for analysis by GC-Mas. Variations of the components included in oils are shown in Fig. (6).

A. GC-Mass instrument B. Algae oils after estrification

Fig.6: Identification of algal fatty acids by GC-MS.

Cosmarinm oil recorded the highest total fatty acids % (65.6) (Table 3; Fig.7) and were distributed as: (21.49 90) saturated fatty acids (SAFA), (3.36 %) monounsaturated fatty acids (MUFA), and (40.75%) polyunsaturated fatty acids. Those types of fatty acids with their percentages indicate the unsaturated/saturated fatty acids ratio (2.05) (Table 2) which represents the highest amount of unsaturated content of oil. This is proved when the application of algal oils in GC Mass analysis. This equivalent of Degree of Unsaturation (Table 2) **(Francisco *et al.*, 2010).**

Table 3: Data of GC-MS analysis for cosmarium fatty acids.

No.	Name of fatty acid	Chemical formula	R. Time	number of strings	saturated acids %Area	Unsaturated acids %Area
1	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	15.561	C16:00	2.25	
2	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	15.099	C17:00	13.21	
3	Hexadecenoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	12.777	C18:00	0.42	
4	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	17.634	C22:00	0.91	
5	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	17.216	C15:00	1.63	
6	Heptadecanoic acid, 10-methyl-, methyl ester	$C_{19}H_{38}O_2$	20.967	C19:00	0.55	
7	Tetrahydro abietic acid	$C_{20}H_{34}O_2$	24.210	C20:00	2.52	
8	11-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	14.858	C19:1		0.55
9	cis-Vaccenic acid	$C_{18}H_{34}O_2$	17.395	C18:1		2.81
10	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	14.714	C19:2		16.91
11	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	$C_{19}H_{32}O_2$	14.773	C19:3		4.77
12	5,8,11-Heptadecatrienoic acid, methyl ester	$C_{18}H_{30}O_2$	16.695	C18:3		0.35
13	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	16.940	C21:3		12.08
14	Arachidonic acid	$C_{20}H_{32}O_2$	16.375	C20:4		3.35
15	cis-4,7,10,13,16,19-Docosahexanoic acid	$C_{22}H_{32}O_2$	18.544	C22:6		3.29

∑ fatty acids	**21.49**	**44.11**

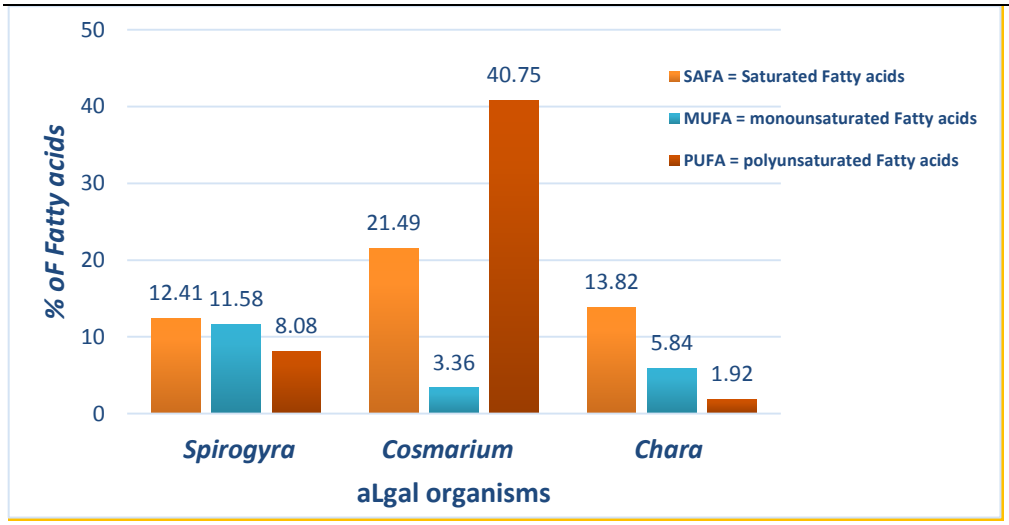


Fig. 7: Relative amounts of PUFA, MUFA and SAFA con

The degree of unsaturation (DU) of the *Cosmarium* oil was 84.86% where as others had a very low degree (27.74 and 9.68%) for *Spirogyra* and *Chara* oils, respectively.

GC-MS analysis showed all fatty acids in algal oil samples depicting their variance in Table (3). Du = MUFA + (2 X PUFA

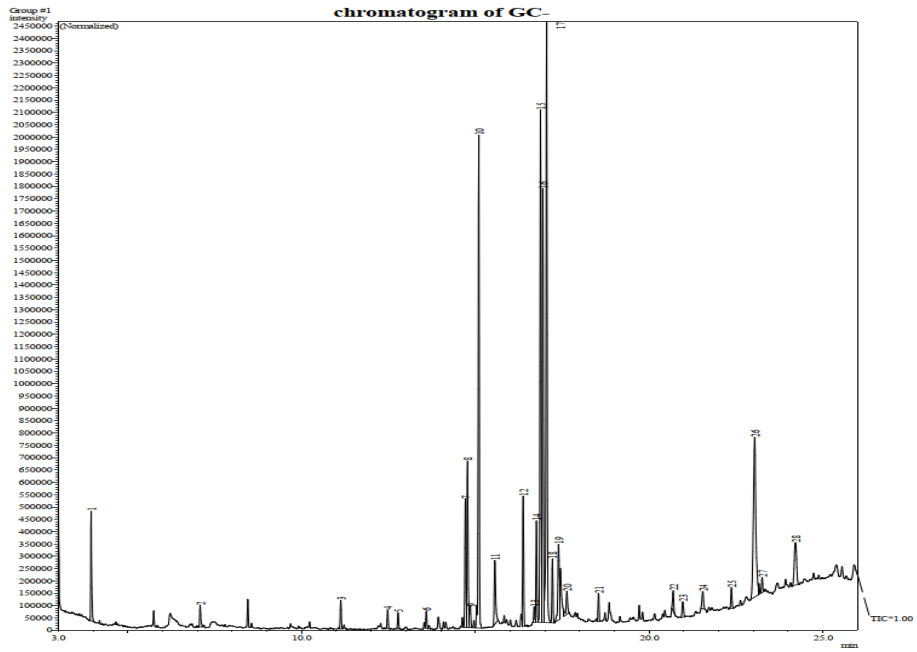


Fig.8: GC-MS analysis for Fatty acids of Cosmarium oil.

From the results above, the types of fatty acids in *Cosmarium* oil reached 12 types and most of them were unsaturated acids (44.11%) (Upon high to low

concentration) (C19:2 , C21:3 , C19:3 , C20:4 , C22:6 , and C18:1), whereas C18:3 recorded minimum percentage (0.35%), the saturated fatty acids (21.49%) were distributed on C17:0 , C20:0 , C16:0 , C15:0 and others below 1% (C22:0 , C19:0, and C18:0). This kind of variation of fatty acids with percentage gave value to *Cosmaruim* oils with respect to an important feature of the highest degree of unsaturation (DU). Very limited references attained analyses of *Cosmarium* oil to its fatty acid one of them refer to *Cosmarium* own high total fatty acid content (> 200 mg / g dry weight), among which *C. crenatum* had the highest average of total fatty acids (308.1 mg / g dry weight) **(Stamenkovic *et al.*, 2018**). That result agrees with the present results (280 mg /g dry weight) in (Table 2). Additionally, the previous authors recorded a high amount of linoleic (18:2) and palmitic (16:00) acid in *Cosmarium* oil that agrees partially with the current results assessing that linoleic acid was absent with good presence for palmitic acid. That could be attributed to algal species or the effect of any culture condition as many researchers referred to it. It was noticed that, with low nitrogen levels, chlorophyceae contained high percentages of total lipids (45% of the biomass) and that 70% of these are mainly 16:0 and 18:1. However, in high nitrogen levels, the percentage of total lipids dropped to about 20% containing polyunsaturated C16 and C18 fatty acid **(Becker, 2008).** Thus, maybe for that reasons the present data agree with the first cast due to the presence of C16:0 (2.25%) and C18:0 (2.81%) with the absence of C18:2 and two concentration of C18:3 (0.35%). In their study, **Stamenkovic *et al*. (2018)** noted that, the types and concentration of fatty acids, especially palmitic (16:0) and linoleic acid (18:2), depend on the species of *Cosmarium* and the stage of growth (Early logarithmic phase (0 days of culture), late log- phase (14 days of culture), and late stationary phase (24 days from culture). Another study by **Wackere *et al*. (2016)**, the authors reported that, the light acclimation in algae changed the concentration of exactly those PUFA. Higher concentration of C16:0, C16:3, C18:1, C18:2 and C18:3 was observed in *Cosmarium botrytis* among other Chlorophyta in the same study, and recorded a low concentration of C16:1, C20:4, C20:5, and C22:0 with no detection of C20:0, C20:1, C20:3, C22:6, and C24:0. In comparison, the present data recorded other fatty acids, among which some were not detected in the previous study such as C20:0, C22:6 and appearance of C19:0-3, C20:4, C21:3.

Fatty acids of *Spirogyra* according to GC-MS data are presented in Fig. (9) and Table (4).

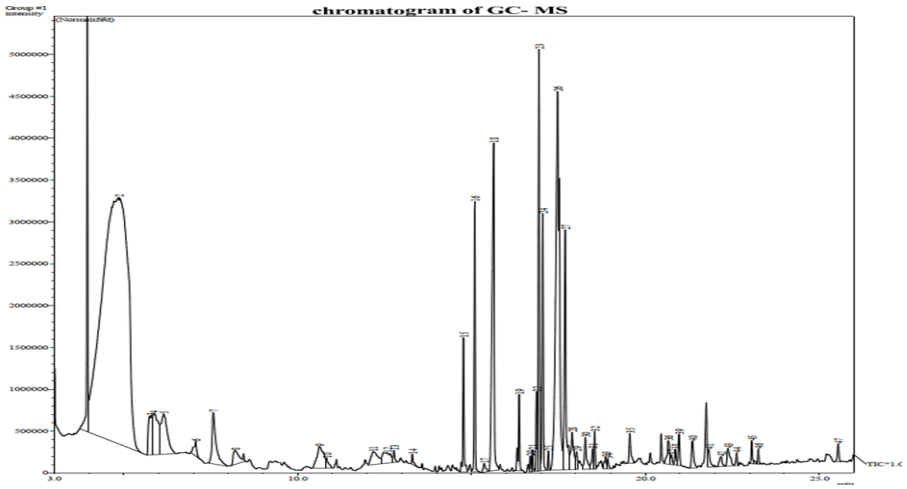


Fig.9: GC=mass analysis for fatty acids of *Spirogyra* oil.

Table 4: Data of GC-MS analysis of *Spirogyra* fatty acids.

No.	Name of fatty acid	Chemical formula	R. Time	The number of strings	saturated acids %Area	Unsaturated acids %Area
1	Dodecanoic acid	$C_{12}H_{25}O_2$	10.833	C12:00	0.20	
2	Tridecanoic acid	$C_{13}H_{26}O_2$	13.297	C13:00	0.14	
3	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	12.771	C15:00	0.15	
4	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	15.638	C16:00	5.44	
5	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	15.097	C17:00	2.22	
6	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	17.212	C19:00	0.16	
7	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	17.697	C22:00	3.13	
8	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	20.860	C21:00	0.19	
9	Eicosanoic acid	$C_{20}H_{40}O_2$	19.553	C20:00	0.41	
10	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	22.624	C25:00	0.14	
11	Tetra decanoic acid, 3,3a,4,6a,7,8,9,10,10a,10b-decahydro-3a,10a-dihydroxy-5-(hydroxy)	$C_{31}H_{50}O_6$	22.174	C31:00	0.23	
12	9-Hexadecenoic acid	$C16H_{30}O_2$	17.477	C16:1		10.70
13	17-Octadecynoic acid	$C_{18}H_{32}O_2$	21.352	C18:1		0.46
14	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	22.375	C21:1		0.42
15	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	18.274	C18:2		0.55
16	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	16.867	C19:2		0.72
17	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	$C_{19}H_{32}O_2$	18.920	C19:3		O.11
18	5,8,11-Heptadecatrienoic acid	$C_{18}H_{30}O_2$	16.692	C18:3		0.14
19	Arachidonic acid	$C_{20}H_{32}O_2$	16.371	C20:4		0.83
20	11,13-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	18.858	C21:2		0.19
21	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	16.941	C21:3		4.80
22	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	18.481	C21:4		0.24
23	cis-4,7,10,13,16,19-Docosahexanoic acid	$C_{22}H_{32}O_2$	18.543	C22:6		0.50
	∑ fatty acids				**12.41**	**19.66**

GC-MS analysis for *Spirogyra* showed 23 types of fatty acids contributed between saturated acids (12.14%) and unsaturated acids (19.66%).

The highest percentage form of SAFA was by C16:0, C22:0, and C17:0 as 5.44, 3.13, and 2.22%, respectively, whereas the highest form of USFA was by C16:1 and C21:3, recording 10.7 and 4.8%, respectively for . *Chara sp*. Respectively, while 0.69% and 4.24% of *Spirogyra sp*. Oil Respectively

Spirogyra recorded C20:4, C22:1, C22:2, while *Chara* had the same fatty acids in *Spirogyra* with lower quantity for all comparisons with *Spirogyra* **(Trifa *et al.*, 2013)**. However, the current study revealed that, *Spirogyra* and *Chara* recorded more productivity of algal oil (79% and 14%) respectively, whereas the fatty acids were more when hexane was used as a solvent.

Lipids produced by microalgae include neutral lipids, polar lipids, wax esters, sterols and hydrocarbons as well as phenyl derivatives such as tocopherols, carotenoids, terpense, quinines and pyrrole derivatives such as chlorophylls. Lipids produced by microalgae can be classified into two categories, storage lipids (non-polar lipids) and structural lipids (polar lipids). Storage lipids are mainly in the form of TAG (triglyceride) made of predominate saturated FAs and some unsaturated FAs which can transesterified to produce biodiesel. On the other hand, structural lipids have a high content of polyunsaturated fatty acids (PUFAs). TAGs are mostly synthesized in the light stored in the cytosolic lipid bodies, and then reutilized for polar lipid synthesis in the dark **(Sharma *et al.*, 2012).** The extent of unsaturation of microalgae oil and its content of fatty acids with more than double can be reduced easily by partial catalytic hydrogenation of the oil **(Baker, 2010)**. Thus, algae oil is the best choice to produce biodiesel in addition to other features such as being ecofriendly renewable source besides of being fast and easily growing. Fatty acids of *Chara* according to GC-MS data are shown in Fig. (10) and Table (5).

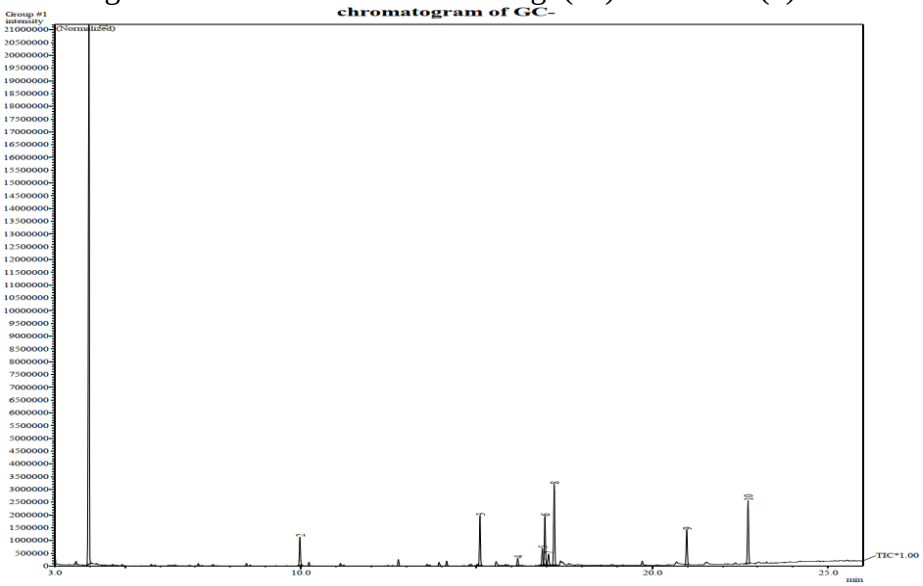


Fig.10: GC=mass analysis of fatty acids of *Chara.*

Table 5: Data of GC-MS analysis for *Chara* fatty acids.

No.	Name of fatty acid	Chemical formula	R. Time	The number of strings	saturated acids %Area	Unsaturated acids %Area
1	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	15.099	C17:00	5.40	
2	Phthalic acid, decyl methyl ester	$C_{19}H_{28}O_4$	16.171	C19:00	0.87	
3	Terephthalic acid, di(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	22.725	C24:00	7.55	
4	11-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	16.947	C19:1		5.84
5	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	16.874	C19:2		1.92
	∑ fatty acids				**13.82**	**7.76**

CONCLUSION

The production of biodiesel from algae (microalgae and macroalgae) can be a good alternative fuel, which serves toward the benefit of the human beings. In this context, it reduces the environmental pollution by producing eco-friendly fuel via investing ecofriendly safe organisms (algae). Additionally, it solves the problem of solid wastes produced by fossil fuel, introducing a clean economic algae biodiesel that is most favored due to the results of the current study. Hence, the algae/feedstock should be studied with respect to its oil productivity and the fatty acid content with its types, to reach the optimal conditions for best and optimal biodiesel production. Algae can utilize CO_2 for their growth and can minimize pollution level resulting in carbon credit for a country. The algal growth in many water forms in the country can be benefited from in using the huge biomass to produce biodiesel due to the presence of ready raw material that provides a high rate of process cost.

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