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EXTRACTION OF OIL AND BIODIESEL PRODUCTION FROM BIOMASS OF IRAQI STRAIN MICROALGAE –OSCILLATORIA SP.

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Abstract
Biodiesel is made from numerous renewable sources. The current study isolated and cultured a new strain of Oscillatoria sp. on a BG11 medium. The pure culture was identified based on morphological feature. Following the identification of the taxa, the impact of temperature, pH, and light levels on algal fresh and dry weight was investigated. The best development rate was found at 28°C, pH 8, and 1800 lux, according to the findings.
The oil extracted using the soxhlet method and the findings of lipid concentration using HPLC revealed that Oscillatoria sp. contains seven different lipids, including (Triolein, Cholesteryl palmitate, Tripalmitin, Phytol, Dipalmitin, Triacylglycerol, and Moonolein. According to the American Society for Testing and Materials, biodiesel was tested using established procedures. For Oscillatoria sp., specific fuel parameters were: kinematic viscosity (3.45 mm2 sec-1), density (0.8897g cm-3), total sulfur content (0.0134%), and flash point (150°C).
Keywords: Biodiesel, Microalgae, Dry biomass, Transesterification, oil, Hexane

1. Introduction:

The primary producers are algae, converting water and carbon dioxide into carbohydrates and oxygen under light conditions 1). One of the basic parameters to monitor the performance of the algal production system is the estimation of biomass, one of the direct methods to determine the biomass is an estimation of fresh and dry weight2). Microalgae are extremely high in some fatty acids as compared to other biological sources such as polyunsaturated fatty acids (PUFA) and γ-linoleic acid (GLA) 3).

Microalgae has recently been investigated as a potential biodiesel source material, because to its high energy content, quick growth rate, low-cost culture method, and exceptional CO2 fixation and O2 addition capability. Research is now underway to advance microalgal biofuel technology 4).

Microalgae has recently been investigated as a potential biodiesel source material, because to its high energy content, quick growth rate, low-cost culture method, and exceptional CO2 fixation and O2 addition capability. Research is now underway to advance microalgal biofuel technology. Biodiesel is an environmentally friendly energy source of renewable energy. Biodiesel generates less carbon dioxide, less greenhouse gas, and contains less sulfur with carbon monoxide content5).

Biodiesel provides reduced air toxicity and 90-95 % of cancer risk 6-8). Due to the elevated viscosity of the oil, diesel engines are not fitted to operate on pure vegetable oil. The oil must, therefore, be modified to decrease its viscosity. Transesterification is used to change the oil viscosity 9) chemically. Transesterification is a process of three-stage reversible reaction in which the glycerol as a by product 10). Lipid content in dry biomass ranges from 20- 50 percent microalgae have significant development potential 11). Algal biodiesel yields are ten to twenty times higher than oleaginous seed or vegetable oil biodiesel yields. The study's goal was to extract oil from a newly identified algae strain and utilize it to make biodiesel.

2. Materials and Methods:

2.1. Isolation and identification of Microalgae:

Water samples with recognizable microalgae communities were collected from a variety of locations. In Awdalok-Koya/Iraq. Cultured on BG11 medium at pH 7.8, 28 °C and under light intensity 800Lux distributed for 18/8hrs light dark. Two weeks later, 25ml of the colonies from pure culture removed to BG11 broth incubated at pH 7.8 , 800 Lux, and 28 °C, as described by 12).

After dilution and plating on the BG11 medium, the samples were analyzed and viewed under a light microscope based on the morphological characteristics as described by 13,14).

2.3. Effect of environmental factors

The impact of different light intensity Luxury 600, 1200, 1800, 2400, and 3000, pH 6.5, 7, 7.5, 8, and temperatures 20, 25, 28, 30 and 35°C on the development of microalgae (by measuring fresh and dry weight) were evaluated 15-17).

2.4. Biodiesel production using microalgae:

2.4.1. Oil extraction for Biodiesel production:

The isolated microalga was powdered after drying. A sample of 50 g of dried microalgae was put in the Soxhlet thimble. The Soxhlet thimble placed on a flask including the extracting solvent (Hexane). After numerous rounds of progressively filling the solid material chamber with heated solvents, the requisite oil was focused on the distillation bottle19). The chamber containing the solid material slowly filled with warm solvents after many cycles, the required oil was focused on the distillation bottle. By using HPLC, the extracted oil were characterized.

2.4.2. Transesterification and biodiesel production:

The extracted oil evaporated under vacuum to release solutions of the solvent mixture using a rotary evaporator at temperature 40-45°C. A stainless steel stirrer was used to stir the mixture. A hot water tank was used to house the reactor. Methanol used to dissolve KOH, the ester layer was separated by gravity using separated funnel. The ester layer was repeatedly washed in a small amount of hot water then the physical properties of the raw materials, as well as biodiesel combines with petro-diesel, were evaluated utilizing a standard techniques devised by ASTM (American Society for Testing and Materials) 20).

Statistical analysis: Data analyzed by using Microsoft Excel Office 2010 and Prism 9.3.1 correlation matrix used to determine the relation between multi-variables, to compare the two groups, the significant correlation difference test was used.

3. Results and discussion:

3.1. Morphological charecteristics of Oscillatoria:

Filaments which were un branched, straight and the apical cell convex ,no calyptra seen was identified as Oscillatoria sp figure (1). Al-Shaheen and Sultan 21) Isolated and identified Oscillatoria tenuis from river in Basrah-Iraq.

Bao and co-authors 22) studied method for selecting local microalgae with high performance from various settings for use in nutraceuticals, functional foods, and animal feed in a short amount of time. In another study done by Sarwa and Verma 23) showed that 18s rRNA sequences had been deposited at GSPI Gene Bank and received Accession No. JX 519262.1 as (Acutodesmus obliquus strain PSV2).

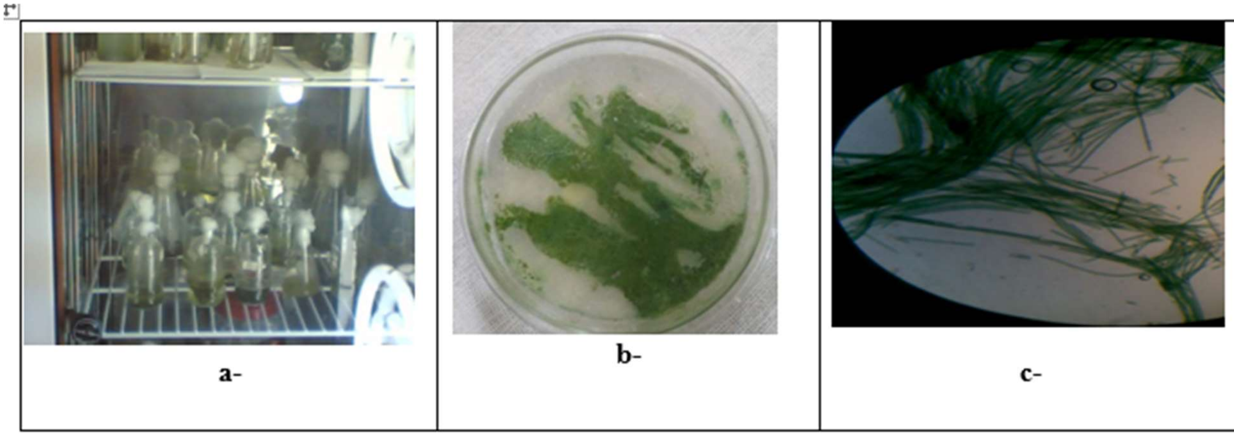

Figure (1) a- Microalgal culture b- pure plate of Oscillatoria sp.c-Morphology of Oscillatoria sp.

3.2. Impacts of environmental parameters on the microalgae growth rate:

Influence of different growth conditions were used to obtain optimum growth rate. Maximum fresh and dry weight showed at 28°C (43.83, 8.33 g/l) and the lowest obtained at 20 °C (19.5, 0.75 g/ml) respectively, figure (2). Microalgae grew well within the temperature range of 25-34°C, for around 14 days, these temperatures exhibit a brief exponential phase, as well as a linear phase and a stationary phase. Chlorophyll and cell dry weight increase when temperature increases from 20 to 30°C. The sluggish rate of growth might be related to an increase in respiration caused by a temperature rise above the species' optimal level this result is in consistence with the finding of 24-25)

The role of pH in the growth of microalgae Oscillatoria strains is shown in Figure (3), where the microalgae grow at all pH values. The best maximum fresh and dry was determined at pH 8 46.5, 8.6 g/ml for isolates, and similar results obtained by 26-27) reported that the most important factor of carbonaceous species relative quantities in water is pH. The availability of carbon from CO2 is reduced with higher pH, which inhibits algae growth. Carbonates are available to algae at higher pH (9-10).

The most significant parameters influencing the rate of growth and biomass are light intensity and photoperiod 28). The higher fresh and dry biomass were recorded at 1800 lux.47.63, 6.6 g/ml As shown in Figure (4). Although the rate of growth increases with light intensity depending on the strain and culture temperature, algal growth is maximum with saturated light intensity and reduces with both rising and decreasing light intensity. Dai and his friends 29) concluded that the 16:8 h light/dark photoperiod shown to be acceptable for outdoor growing. When the development rate of algae is reduced owing to reaching the saturation point, raising the light intensity to 3000 lux has an effect on the cellular composition of algae.

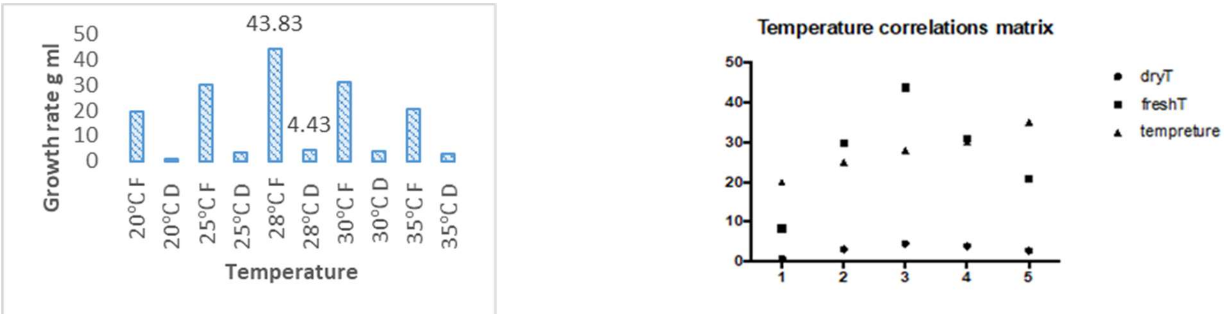

Figure (2): Effect of Temperature on A-fresh (F) and dry (D) weight of microalgae (g ml-1), Correlation matrix (F) r=o.5; p=0.3; (D)r=o.3; p=0.6

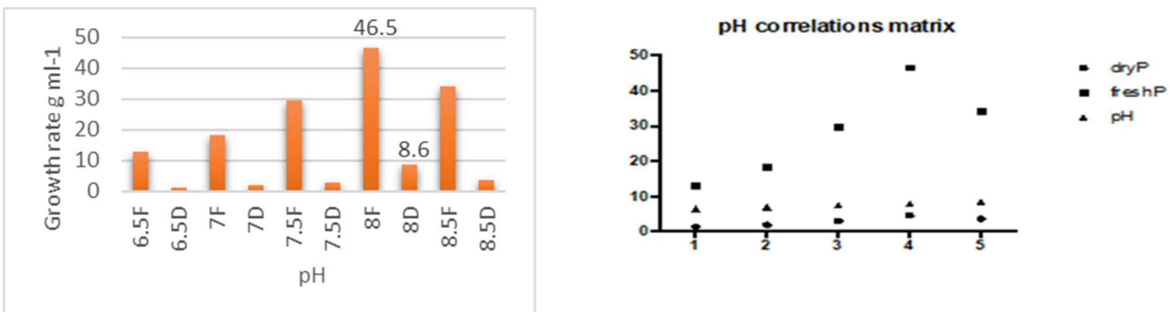

Figure (3): Effect of pH on fresh (F) and dry (D) weight of microalgae (g ml-1)), Correlation matrix (F) r=o.5; p=0.3; (D) r=o.5; p=0.3

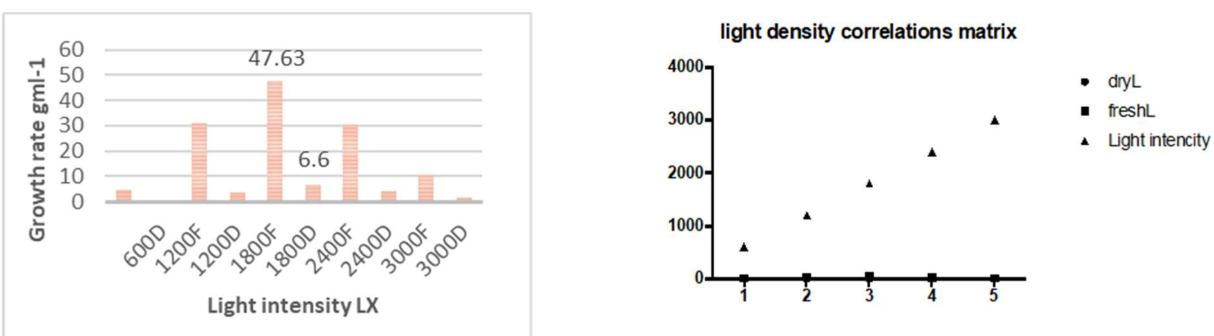

Figure (4): Effect of light intensity on fresh (F) and dry (D) weight of microalgae (g ml-1) Correlation matrix (F) r=o.5; p=0.3; (D) r=o.5; p=0.3.

3.3. Biodiesel production

3.3.1. Oil extraction

Production of biodiesel from Oscillatoria sp. included two main steps, the initial step included extraction and characterization of oil that will be converted to biodiesel by transesterification process. In the present study, 20 ml of oil extracted from 50 gr biomass (40%) (Table 2 and Figure 5 a,b&c) showed oil extraction and characterization to determine the content of microalgae oil and compression of some oils characteristics such as (viscosity, density, total sulfur content, flash point, and water content) that extracted from Oscillatoria sp., Rahman and co-authors 30) extracted lipid from algae for biodiesel production and discovered that high amount of oil obtained with longer contact period, and a smaller algal biomass size gas chromatography mass spectrometry. The functional groups of algae and the composition of oil were revealed by Yuvarani and co-authors 31). Basic fuel parameters were also evaluated to define the algal oil's oil quality using ASTM, AOCS, and IS/ISO standards.

Figure (5): Extracted oil from Oscillatoria sp. a-Soxhlet system b-before c-after filtration

Table (2): Physicochemical properties of Microalgae oils

No.	Characteristics	Unit	Test method	*Oscillatoria* sp. oil
1	Kinematic Viscosity @40℃	$mm^2\ sec^{-1}$	ASTM D445	17.52

2	Density @15.5℃	g cm-³	ASTM D1298	0.9909
3	Total Sulfur Content	mass%	ASTM D4294	0.0126
4	Flash point	℃	ASTM D92	301
5	Water content	vol%	ASTM D6304	0.00

3.3.1. Determination of Lipid content by HPLC:

Results of lipid concentration showed that there are 7 type of lipids in Oscillatoria sp. which included (Triolein , Cholesteryl palimiate, Tripalmitin, Phytol, Dipalmitin, Triacylglycerol and Moonolein) table(3). The major type Moonolein and Dipalmitin were recorded in Oscillatoria sp while the minor type was Triolein. The chromatography peak and its retention time with standard are shown in figure (6).

Table (3): Rt. (min.) of different lipid Standard and Oscillatoria sp.

Lipid	Rt. Standard	Rt *Oscillatoria*
Triolein	1.19	1.19
Cholesteryl palimatate	2.12	2.12
Tripalmitin	3.96	3.94
Phytol	4.88	4.96
Dipalmitin	6.13	6.11
Triaceylglycerol	7.30	7.27

Moonolein	8.12	8.20

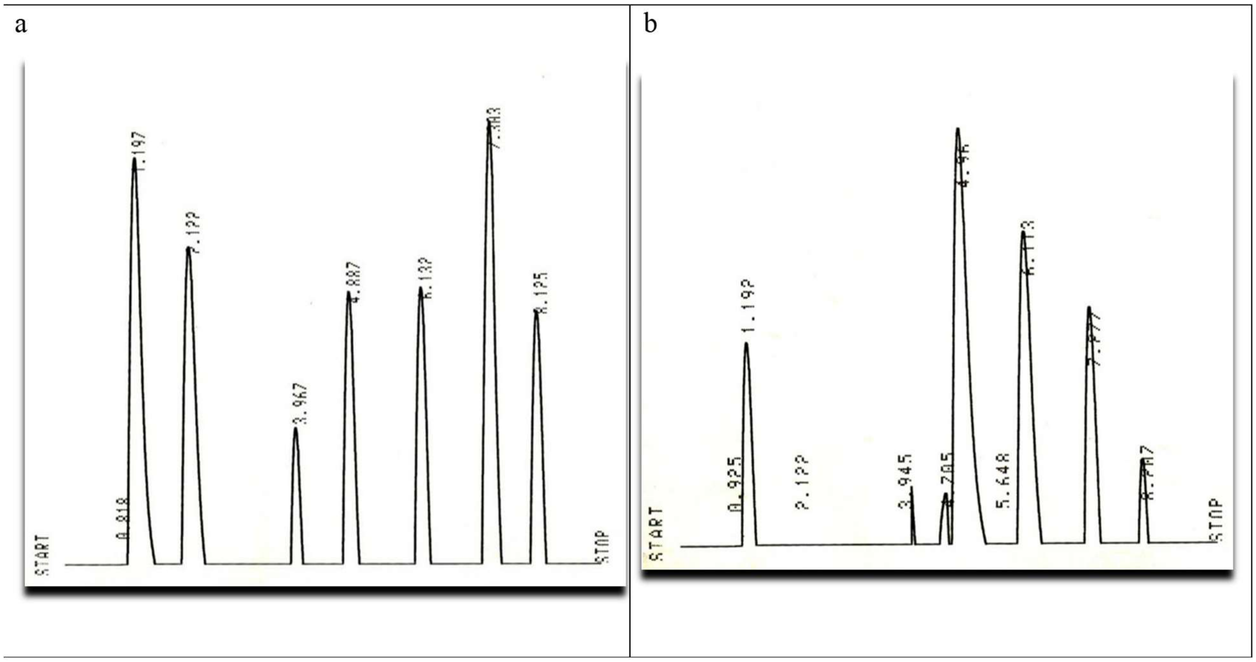

Figure (6) Chromatogram of Rt. (min.) of different standards of Lipid

Microalgal lipids have well-defined fatty acid forms, with the vast majority of fatty acids are saturated and monounsaturated fatty acids. 32, 33) In study done by Patnaik et al 34) they found that 57 % of fatty acid methyl ester produced by Oscillatoria sp. which comprises high-quality biodiesel fatty acids. The ability to handle a large number of samples quickly and simply is a key aspect of this technology. It is, however, less sensitive when compared to other current techniques.

3.3.3. Biodiesel production and characterization by Transesterification process

The process of transesterification of microalgae was carried out under the temperature 60 °C, for 120 min. Viscosity is important method that used to measure changing of extracted oil to methyl esters. The viscosity of the produced biodiesel from microalgae was determined to be 3.45 mm2 sec -1 for Oscillatoria sp., which is much lower than the viscosity of crude microalgae oil, which was 24.52 mm2 sec -1 for Oscillatoria sp., and the density of microalgae oil were greater than the density of microalgae biodiesel (Table 3). The result is agreed with studies done by 35), they reported that the viscosity and density of produced biodiesel of crude Spirulina maxima oil was 0.864 kg/m3 and 4.47 mm2 /s. Bagchi and co-authors 36) conducted a sharp decrease in the viscosity of methyl esters of vegetable oil following transesterification.

Table (3): Physicochemical properties of biodiesel from microalgae oils

No.	Characteristics	Unit	Test method	*Oscillatoria* sp. biodiesel
1	Kinematic Viscosity @40℃	mm^2 sec^{-1}	ASTM D445	3.45
2	Density @15.5℃	g cm^{-3}	ASTM D1298	0.8897
3	Total Sulfur Content	mass%	ASTM D4294	0.0134
4	Flash point	℃	ASTM D92	150
5	Water content	vol%	ASTM D6304	0.00

The flashpoint of the produced microalgae oil was 301˚C for Oscillatoria sp. and while in biodiesel decreased to 150 ˚C Zahan and Kano 37) conducted that the flashpoint for some of plant oils ranged between 312-325 ˚C , respectively, and reduced in vegetable biodiesel to 155, 150, 158, and 165 ˚C respectively. These characteristics of biodiesel from microalgae oils were compared with The American Society for Testing and Materials (ASTM) developed standard methods; this is an agreement with The obtained results (Table 3)demonstrated that the properties of biodiesel from microalgae oils are near to that obtained by 38).

4. Conclusion:

Oscillatoria sp. was effectively employed as a primal matter in the manufacturing of biodiesel. Oil extraction and transesterification were two processes in the biodiesel production. The fatty acid methyl ester (FAME) product will be improved in the future by optimizing the transesterification process by studding the effect of temperature, different mediums to enhance the rate of oil production.

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