Trop J Nat Prod Res, February 2021; 5(2):238-242

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Identification of Saturated and Unsaturated Fatty Acids Produced by *Chlorella vulgaris* as a Potential Candidate for Biodiesel Production

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ARTICLE INFO

Article history:
Received 12 August 2020
Revised 14 January 2021
Accepted 08 February 2021
Published online 01 March 2021

ABSTRACT

Algae are ubiquitous and have found wide applications in the food industry and biotechnological approaches. Cyanobacteria and green algae have high protein and fatty acid contents, making them suitable for biodiesel production. The present study was aimed at identifying the saturated and unsaturated fatty acid contents of *Chlorella vulgaris* towards its use for biodiesel production. *C. vulgaris* was isolated from the local environment in Mosul city, Iraq. Pure cultures were made and incubated at different times (3, 5, 7, 9, 11, 13, 15, 17 days) at 25 °C. At the end of the incubation periods, the cultures were analyzed. Final pH, optical density, biomass, chlorophyll and protein contents were determined. Gas chromatographic analysis was performed on petroleum ether, chloroform or ethanol extracts of *C. vulgaris* to identify constituent fatty acids. The results obtained indicated that growth of the algal cultures, as measured by OD increased with an increase in incubation periods until a maximum (OD 0.85), after which the values declined. Similar observations were made for the amount of biomass, final pH, chlorophyll and protein contents. The gas chromatographic analysis of the three extracts indicated the presence of secondary metabolites which included oleic, stearic, undecanoic, butyric, arachidonic, linolenic, behenic, pentadecanoic, mystic, caproic, eicosapent, lingoceric, cisdocosanoic, linolic and nervonic acids. Our findings revealed the presence of diverse amounts of saturated and unsaturated fatty acids in *C. vulgaris*, making it a potential candidate for biodiesel production.

Keywords: Algae, Biodiesel, *Chlorella vulgaris*, Fatty acids, Gas chromatography.

Introduction

Algae are diverse group of aquatic organisms that have the ability to carry out photosynthesis. They possess numerous benefits and have found wide applications in the food industry and biotechnological approaches because they have the ability to store food. Also, they are used as sources of pigments, lipids, vitamins, proteins and for the production of specific secondary metabolites.[1,2] Some of these organisms contain high amounts of protein and are used as a source of food and alternative to food sources. Cyanobacteria, such as *Nostoc*, *Anabaena*, and *Spirulina*, as well as green algae like *Chlorella* have high protein content. Moreover, algae contain nutrient compounds that facilitate more absorption compared to the proteins of plants and animals.[3,4] Furthermore, *Chlorella* and *Spirulina* possess the ability to accumulate large amounts of gummlinootenic and essential lenololic acids.[5] Generally, unicellular algae are considered as an important source of active organic compounds that have large industrial benefits.[6] Fatty acids (either saturated or unsaturated) are carboxylic acids with a long aliphatic chain, often regarded as organic compound. Saturated fatty acid generates stable chemical compounds like palmitic and pyutoric acids. Unsaturated fatty acid is susceptible to oxidation and reduction reactions, which is an important compound for biodiesel production from algae. Generally, algae are considered as the third generation for biodiesel production after coal and fossil fuel.[7,8]

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Citation: Al-shahery YJ and Alasady IN. Identification of Saturated and Unsaturated Fatty Acids Produced by *Chlorella vulgaris* as a Potential Candidate for Biodiesel Production. Trop J Nat Prod Res. 2021; 5(2):238-242. doi.org/10.26538/tjnpr/v5i2.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Chlorella vulgaris is considered as an important source of saturated and unsaturated fatty acids, and is an essential compound in biodiesel production.[9] The aim of the present study was to identify the saturated and unsaturated fatty acids produced by *Chlorella vulgaris* towards a potential candidate for biodiesel production.

Materials and Methods

Isolation of algae

Chlorella vulgaris Beijerinek used in this study was isolated from local environment in Mosul city, Al-Shallalat region, Iraq. Substrate was collected under specific conditions and cultured on Chu10 agar media in Petri dishes. A little drop of water was added to soil sample which was collected from the studied region and inoculated on agar media. The culture was incubated under continuous of photo period of 2,500 loxs at 28°C for 4-6 weeks, after which series of sub-cultures were made to obtain a pure culture of *C. vulgaris* as descried.[10]

Biomass production

To obtain an adequate weight of *Chlorella* biomass, photo bioreactor was fabricated. A conical flask with capacity of 1.5 L for cultivating algae at a high quantity was selected with additional Aeration system. A volume of 900 ml of culture media was added. An aliquot of 100 ml of inoculum was added to the medium. The conical flask was closed with cotton plug and covered with aluminum foil at axenic condition. The algal cultures were incubated at different time (3, 5, 7, 9, 11, 13, 15, 17 days) periods, illuminated with 12:12 photo period at 25°C to obtain optimal growth. At the end of each incubation period, *C. vulgaris* cultures were analyzed.

Analytical procedures

Different analytical procedures were performed on the experimental *C. vulgaris* cultures. There are a many parameters were determined

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

(Final pH, optical density for growth, biomass and chlorophyll content analyses). Also, protein content was estimated using Folin reagent and BSA was used as standard. These analytical procedures were carried out as described.[11,12]

Fractionation and identification of fatty acids

Fatty acids were fractionated by employing two techniques. The first method involved saponification of a raw petroleum ether chloroform or ethanol extract of *C. vulgaris*. Saponification was carried out by adding 10 mL of stable oil from *C. vulgaris* and 100 ml of 7.5 M KOH in a beaker. The solution was boiled for 90 min at 100 ^{o}C, after which it was cooled to ambient temperature. Then 100 ml of dH_2O was added to become emulsion. A separatory funnel (2x15 mL) was used for either removing non-saponified fatty acids or saponified solution and acidified with 20% H_2O to obtain a solution of pH 2. Fatty acids extracted by separatory funnel (2x25ml) were used to either obtain dissolved fatty acids or dried by magnesium sulfate hydrate which were filtered and made into solution that was concentrated by RVE. The second method used for fractionating fatty acids was esterification. This was achieved by preparation of acetyl chloride and 25 ml of methanol which were added to 0.5 ml separated fatty acids. The solution was placed in a water bath at 100^{o}C for 20 min, after which it was cooled to ambient temperature and used to perform Gas Chromatography (Shemaze model) technique.[13,14]

Gas chromatography analysis

The gas chromatographic analysis of petroleum ether, chloroform or ethanol extracts *C. vulgaris* were performed using

Results and Discussion

Identification of Chlorella vulgaris

The isolated colonies were characterized as having green colour. These colonies were examined for micro belly using compound microscope. This identification procedure has been previously reported.[12]

Effects of different incubation periods on growth, number of activities and biocompounds of Chlorella vulgaris

The results obtained for the effects of different incubation periods on growth, number of activities and biocompounds of *Chlorella vulgaris* are presented in Figure 1. Growth of *C. vulgaris* was measured by optical density (OD) of the culture. OD values increased with an increase in incubation period and the maximum OD (0.85) was obtained at the 15th day of incubation, after which the OD values started decreasing. The result suggested that the maximum growth of *C. vulgaris* was obtained at the 15th day of incubation (Figure 1A). A similar trend was observed for the amount of biomass (Figure 1B), final pH (Figure 1C), chlorophyll content (Figure 1D) and protein content (Figure 1E) with the highest recorded values of 500 mg/L, 8.5, 1.30 mg/L and 300 mg/L, respectively at the 15th day of incubation. Reports have shown that there is a direct effect of pH on photosynthesis and growth of algae. An increase in photosynthetic rate results in increased growth, which consequently increases the alkalinity of the growth medium, thereby increasing pH value.[10] Studies have shown that there is an association of protein content with the rate of daily growth and abundant weight of biomass in many algae.[15-17] Also, another study has reported high protein content for *C. vulgaris*.[10] Our results suggest that the best incubation period to obtain the highest growth and maximum amount of biomass of *C. vulgaris* is 15 days of incubation period.

Identification of fatty acids in Chlorella vulgaris using gas chromatography

Gas chromatography (GC) was used for identification of fatty acid components of *C. vulgaris*. The results of the GC revealed the cellular contents of *C. vulgaris* comprised saturated and unsaturated fatty acids (Table 1; Figures 3-5) which were similar to the standard fatty acids (Fig. 2) used in the separation device. It has been reported that fatty acids are the main substrate for biodiesel production. The results obtained in this study are in agreement with previous findings.[14,18,19] The major fatty acids were reported to be similar to microalgae and cyanobacteria.[20-22]

Table 1: Gas chromatographic separation of *Chlorella vulgaris* extracted with different solvents

Saturated fatty acids	Standard Retention time (min)	Petroleum ether extract of *C. vulgaris*		Chloroform extract of *C. vulgaris*		Ethanol extract of *C. vulgaris*	
		Conc.	Retention time	Conc.	Retention time	Retention time	Conc.
Butyric acid	2.283	77.341	2.155	121.346	2.224	-	-
Caproic acid	3.622	-	-	13.289	3.634	-	-
Undecanoic	6.472	28.866	6.433	-	-	-	-
Lauric	10.810	-	-	-	-	-	-
Myristic	13.233	-	-	37.601	13.399	-	-
Pentadecanoic	15.718	-	-	16.189	-	-	-
Palmatic	18.126	-	-	-	15.735	-	-
Stearic	20.519	28.743	20.600	16.496	20.600	-	-
Elaidic	21.250			-	-	-	-
Oleic	22.791	83.241	22.600	-	-	-	-
Linoleic	23.461			-	-	-	23.190
Arachidic	25.221	5.257	25.230	-	-	471.718	-
Linolenic	27.383			-	-	-	-
Linolenic	27.473	39	27.470	-	-	-	27.444
Heneicosanoic	29.737	-	-	-	-	263.632	-
Behenic	30.333	-	-	211.408	30.471	-	-
Erucic	31.480	-	-	-	-	-	-

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Arachidonic	33.670	14.6986	33.655	-	-	-	-
Tricosanoic	34.343	-	-	-	-	-	-
Cis,docosanoic	35.077	-	-	-	-	-	-
Cis,docosanoic	35.705	-	-	124.918	35.627	-	-
Lignoceric	37.327	-	-	7.294	37.309	-	-
Eicosapent	38.743	-	-	71.340	38.966	-	-
Nervonic	39.230	-	-	141.691	39.490	-	-
Docosahexanoic	40.689	-	-	-	-	-	-
Docosahexanoic	40.735	-	-	-	-	-	-

-: Absence of compound

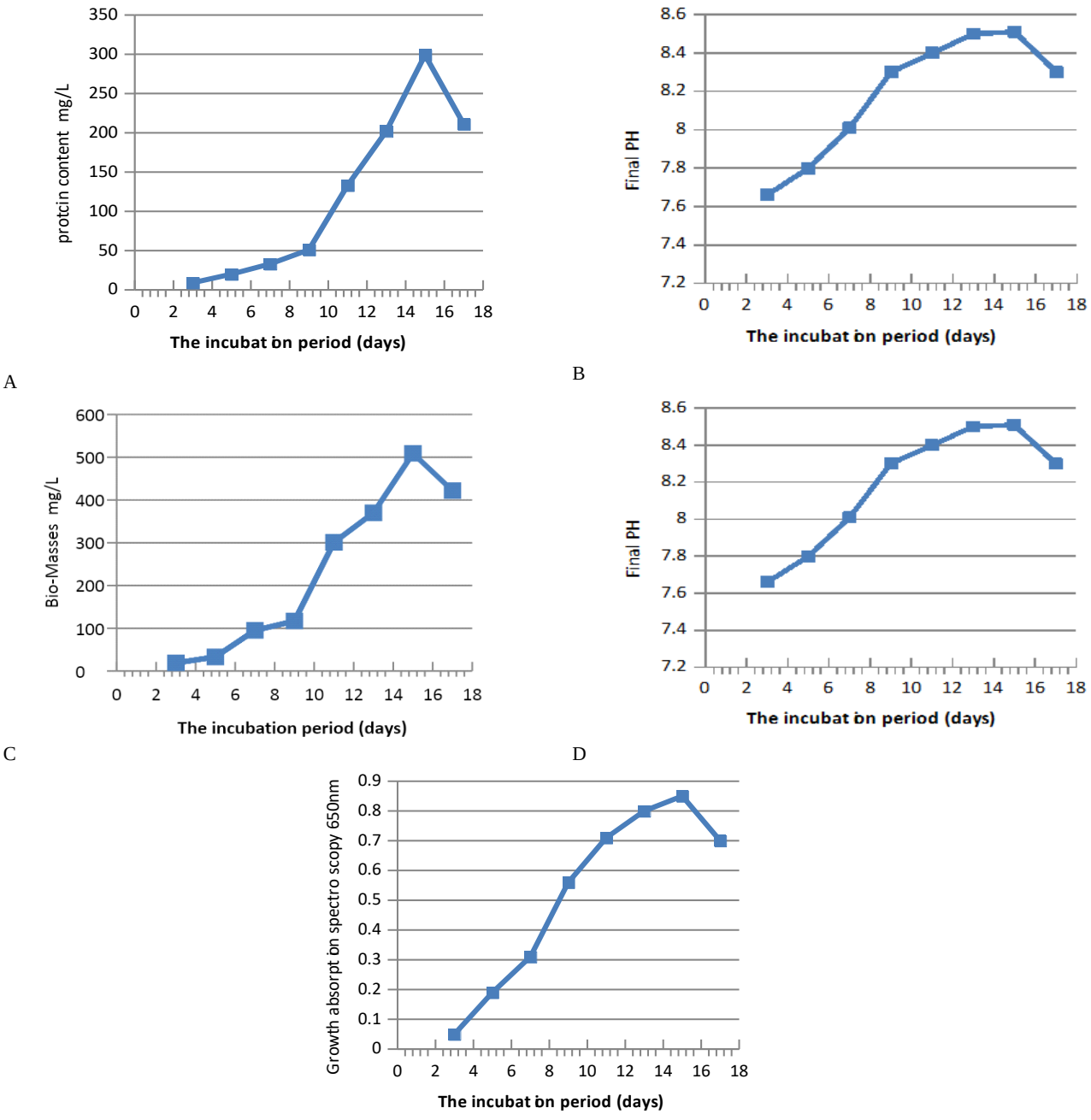

Figure 1: Effects of different incubation periods on growth, number of activities and bio-compounds of *Chlorella vulgaris*. **A:** Growth curve; **B:** Amount of biomass; **C:** Final pH; **D:** Chlorophyll content; **E:** Protein content

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

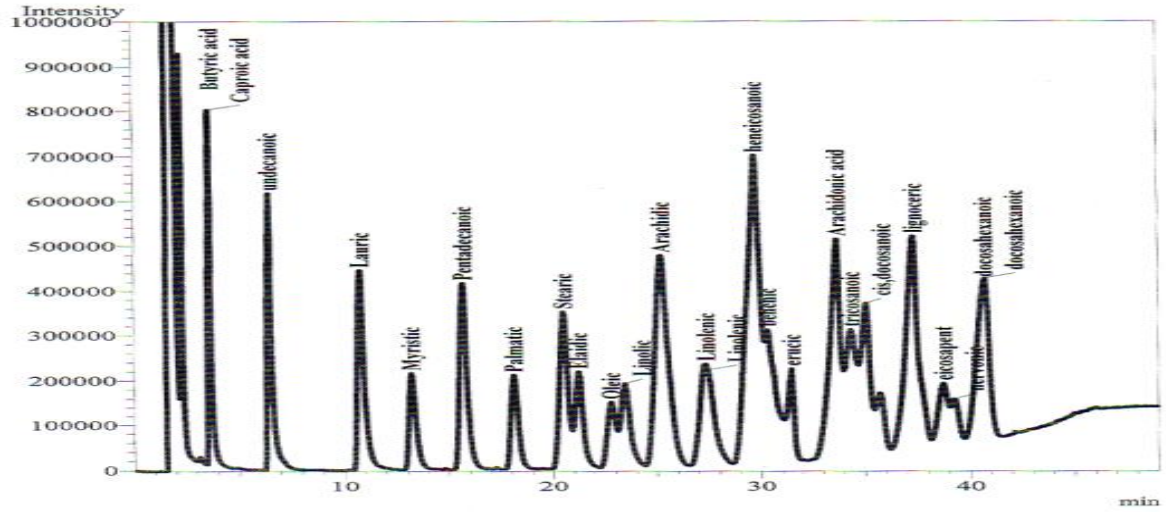

Figure 2: Gas chromatographic spectrum of standard used for fatty acid identification.

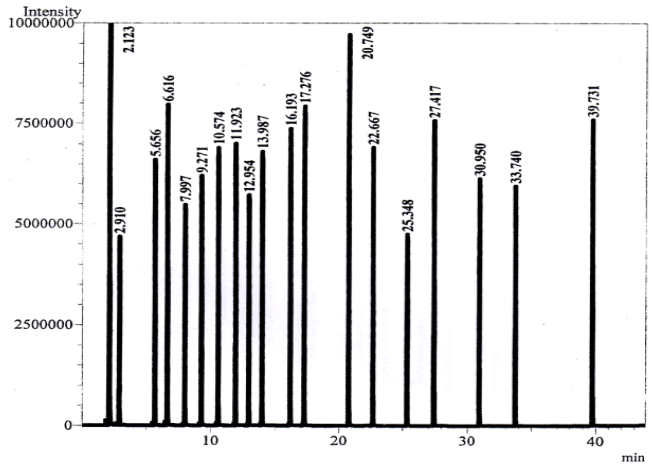

Figure 3: Gas chromatographic spectrum of fatty acids from petroleum ether extract of *Chlorella*

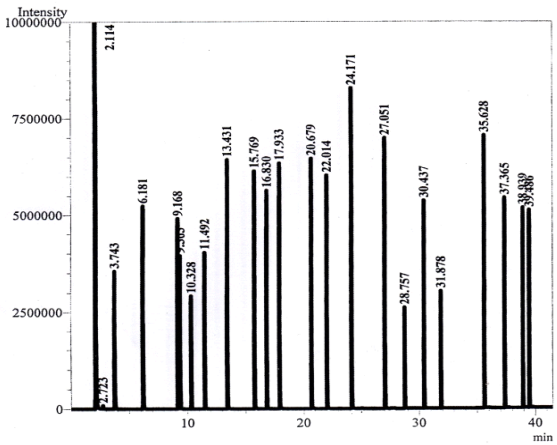

Figure 4: Gas chromatographic spectrum of fatty acids from chloroform extract of *Chlorella vulgaris*.

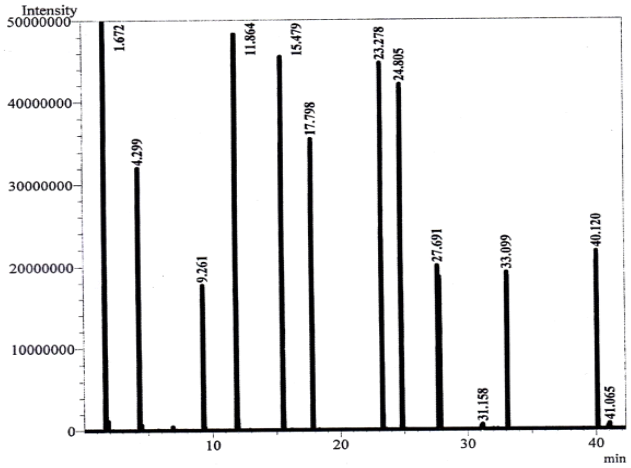

Figure 5: Gas chromatographic spectrum of fatty acids from ethanol extract of *Chlorella vulgaris*.

Conclusion

To the best of our knowledge, this study is the first research in Iraq to identify the fatty acid contents of algae at the local level. Our findings revealed the presence of high level of essential fatty acids in *Chlorella vulgaris* extracts, thereby making it a potential candidate for biodiesel production.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Acknowledgments

The authors express their profound gratitude and appreciations to the President of the University of Mosul, Mosul, Iraq for the facilities such as laboratories and equipment provided for conducting the research.

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