Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Biology

Elixir Appl. Biology 61 (2013) 16753-16756

ISSN: 2229-712X

Fourier transform infrared (FTIR) spectroscopy for the analysis of lipid from *chlorella vulgaris*

Vidyadharani.G and Dhandapani, R

Department of Microbiology, Periyar University, Salem-11.

ARTICLE INFO

Article history:

Received: 19 June 2013;

Received in revised form:

24 July 2013;

Accepted: 1 August 2013;

Keywords

Microalgae,

Chlorella,

Fourier transform infrared (FTIR) analysis,

Biomass,

Isolation.

ABSTRACT

Microalgae are considered to be the world's future energy reservoir and various aspects have been followed for optimizing the algae growth for large scale production of biodiesel. For the same, selection and screening of microalgae strain for its lipid productivity is much more important. Fourier transform infrared (FTIR) spectroscopy was used in this study for the analysis of lipid extract from natural isolate of *Chlorella vulgaris*. In order to evaluate the strain productivity the reference strain was obtained. Both the cultures were grown in a chemically-defined media under photoautotrophic culture conditions. Lipid extraction procedures were standardized for both strains based on Fourier transform infrared (FTIR) spectroscopy .The extracted lipid samples were analyzed. This study reveals that the natural isolate identified as *Chlorella vulgaris* strain is suitable for further optimization procedures and found with high percentage of residual lipid proportions. Also the results show that FTIR technique can be applied for determination of single cell biomass composition from phytoplankton communities.

1. Introduction

Algae plays an important key role in nutrient cycling and also in the sequestration of inorganic nutrients such as carbon, nitrogen and phosphorous into organic forms. In total, they accounts for 50% of the total planet productivity [1]. They are essential in the maintenance of aquatic food chain and play an important role in the models describing global climatic change [2].

Microalgae are a heterogeneous group of organisms with a host of characteristics that distinguish them, some of those being cell size, colour, inhabitation of aquatic environments, as well as being unicellular and quite often, photoautotrophic. They can also be prokaryotic or eukaryotic, and in evolutionary terms, they can be either ancient species or recent ones. This diversity creates the capability for microalgae to be a valuable source for a multitude products that, at some level, support the food (both human and animal nutrition), cosmetic, pharmaceutical and fuel industries [3].

Fourier transform infrared (FTIR) spectroscopy was proven to be a fundamental technique that can provide a unique and accurate insight into the *in situ* and *in vivo* changes in the chemical character of human and microbial cells at high spatial resolution [4]. The strength and frequency of infrared absorptions are determined primarily by factors that include symmetry, bond type and the masses of the atoms [5].

FTIR spectroscopy has been widely used to provide information on a range of vibrationally active functional groups including O–H, N–H, C=O, =C–H, –CH2, –CH3, C–O–C and >P=O in biological specimens [6]. This technique has been largely used with isolated macromolecules and molecular complexes such as nucleic acids, proteins, lipids [7], polysaccharide and tissue culture cells [8], Studies have undergone for whole organisms too including bacteria, marine algae ,higher plants, fungi and yeast [9].

The purpose of the present investigation is to find a suitable species for the application of large scale biodiesel feedstock production. The water samples for isolating *Chlorella vulgaris* were collected from Ayiloor panchayat, Namakkal district. FTIR for the lipid extract analysis of Ch V and *Chlorella vulgaris* reference strain is carried out. The aim of the present work is to specify the FTIR role in analyzing lipid samples extracted from both the natural isolate Ch V and *Chlorella vulgaris* reference strain.

2. Materials And Methods

2.1 Isolation of microalgae

The algal samples were collected from the freshwater pond located in Ayiloor Panchayat, Namakkal district (Latitude 11.2300° N and Longitude 78.1700° E.), Tamil Nadu, India. The samples are cultured in sterile BBM medium and subjected to purification by serial dilution. The individual colonies are microscopically observed for their morphological and cultural characteristics. Pure culture was obtained from single colony and the culture was established in both liquid and agar slants of BBM medium, incubated at 25 ± 1°C under 40µmols-1 illumination with 16:8 h light dark cycle.

The purity of the culture is ensured by repeated plating and by regular observation under microscope. One of the seven isolates designated as Ch V was microscopically resembled the *Chlorella vulgaris* reference strain obtained from CAS in Botany, University of Madras, Chennai. This isolate is chosen from the seven isolates for the present study.

2.2 Biomass Estimation

2.2.1Dry and Wet biomass

10 ml of grown microalgal culture is centrifuged at 10,000 rpm for 10 min and the pellet was weighed . Both the algal cultures (Ch V, *Chlorella vulgaris*) were processed. For dry weight known volume of grown microalgal sample was washed with

Tele:

E-mail addresses: vidgkrt@gmail.com

0.5 M ammonium formate, filtered through filter paper and then allowed to pass through whatman No:1 paper. The air dried biomass is weighed and stored in a sterile container for further estimations. [Graph 1]

2.2.2Lipid extraction

14 days old well grown 10 ml of Ch V and *Chlorella vulgaris* were taken and the lipid samples were extracted by following the bligh and dyer method [11].

2.3 FTIR analysis

The lipid samples were processed using FTIR Perkin Elmer Model no-1400. 500 cm^{-1} to 3900cm^{-1} absorption spectra range are chosen for the present study.

3. Results:

3.1 Isolation of Microalgae

Microalgae existence is estimated to be more than 50,000 species, and among that only 25 thousand species were identified, and the remaining were yet to be explored [7]. In case of biodiesel from microalgae, there is a need for natural isolate which has the potential to reserve more amount of lipid. From the water samples collected from freshwater pond located in Ayiloor Panchayat, Namakkal district natural isolate of *Chlorella vulgaris* was isolated successfully [Fig:1]. Primary cultivation of the natural isolate of *Chlorella vulgaris* and the reference strain *Chlorella vulgaris* was carried out using Bold Basal Medium (BBM).[Fig : 2]

Fig:1 Microscopic observation of algal samples images of algal strain(400X magnification)

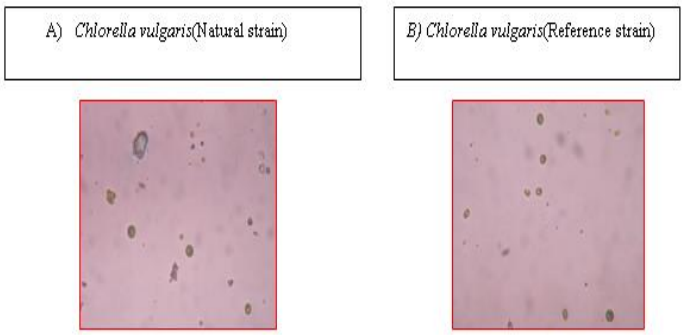
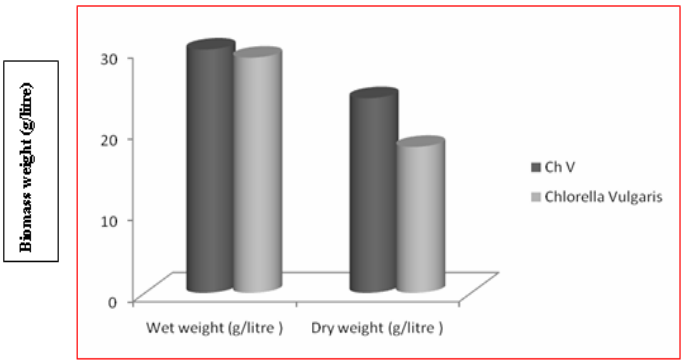


Fig:2 Figure showing the algal growth Primary cultivation
A) *Chlorella vulgaris* (Natural strain)
B)*Chlorella vulgaris* (Reference strain)

3.2 Biomass estimation

In order to evaluate the algal growth, initial parameters such as dry and wet weight were calculated [Fig:3]

Fig 3 : Dry Weight and Wet Weight of strains(g/litre)

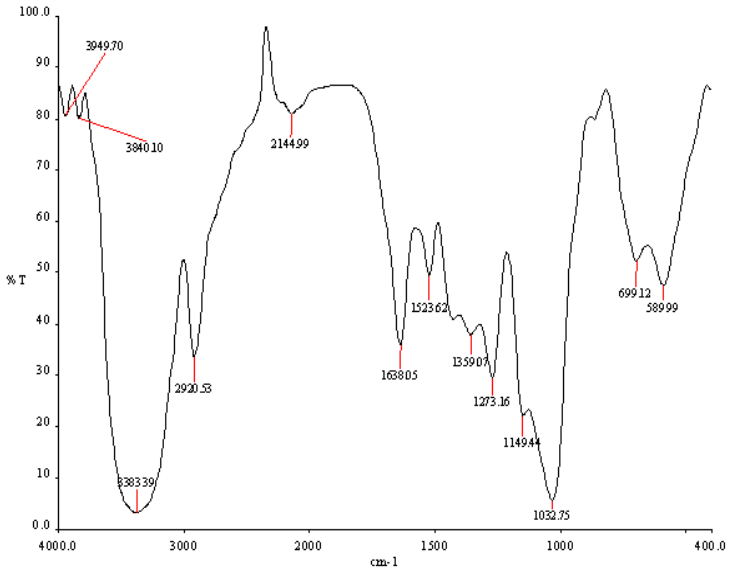


3.3 FTIR analysis

The lipid content were extracted by following Bligh and Dyer method and the samples were subjected to FTIR analysis to know the absorption spectra in the range of 2000 to 3000 cm^{-1} for the confirmation of lipid.

For the natural isolate *Chlorella vulgaris* the lipid samples were analyzed using FTIR and the results were produced in Figure 4 had 8 clear bands over the wave number range 4000 to 5000 cm-1. These bands were tentatively identified on the basis of reference standards [8] and published FTIR spectra in relation to specific molecular groups. The results were interpreted (Table-1). Absorption peak at 3949.70 cm^{-1} and 3840.10 cm^{-1} represented the presence of primary amines and very weak secondary amines. Peaks at 3383.39 cm^{-1} denoted the O-H stretch indicates the presence of strong alcohol group. C-H stretch appeared at 2920 cm-1 showed the presence of lipid substance strongly. 2144.99 cm^{-1}, $-C\equiv C-$ stretch denoted alkynes group, 1638.05 cm^{-1} and 1523.62 cm^{-1} showed c=c stretch that represents alkenes group. In particular 1273.16 cm-1 stretch showed the presence of strong acid. 1149.44 cm-1 stretch indicates the ether group, the remaining absorption range 589.99 cm-1, 699.12 cm-1 and 1032.75 cm-1 denoted the strong alkyl halide presence.

Fig-4 FTIR Spectral image of *Chlorella vulgaris* natural isolate



For the reference strain *Chlorella vulgaris* the absorption spectra results were shown in Figure 5. Absorption peak at 3831.34cm-1 and 3707.82cm-1 represented the presence of primary amines and very weak secondary amines. Peaks at3333.79cm-1 and 3234.03 cm^{-1} denoted the O-H stretch

indicates the presence of strong alcohol group. Asymmetric stretch appeared at 3052.51 cm-1 and 2669.95 cm-1 showed the presence of lipid substance strongly. Overtone was appeared at 1906.05 cm-1 showed carbonyl group, 1592.15 cm-1 and 1491.49 cm-1 stretches were due to less alkene group presence. In particular 1278.97 cm-1 stretch showed the presence of strong acid. 1127.29cm-1 stretch indicates the ether group, [Table-2] appeared peak at 824.65 cm-1 indicated the alkene group, the remaining absorption range 589.10 cm-1, 731.49 cm-1 denoted the strong alkyl halide presence.

Table 1: Expected compounds in Ch V natural isolate with reference to the FTIR absorption spectrum

S. No.	Frequency in wavelength, in cm-1	Assignment of vibration
1.	3949.70 and 3840.10	-NH_2stretching vibration
2.	3383.39	-OH stretching vibration
3.	2920.53	-C-H stretching
4.	2144.99	$-C\equiv C-$ stretching
5.	1638.05	C=C stretching
6.	1032.75, 699.12 and 589.99	Alkyl stretching

Fig-5 FTIR spectral image for *Chlorella vulgaris* reference strain

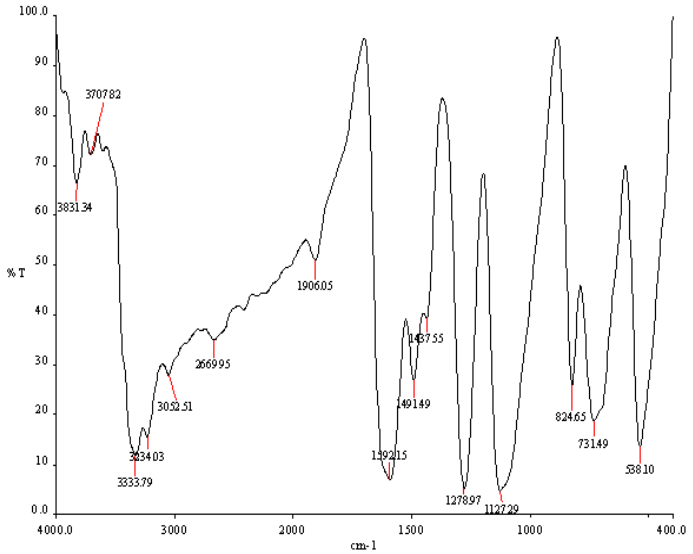


Table 2: Expected compounds in *Chlorella vulgaris* strain FTIR absorption spectrum

S. No.	Frequency in wavelength, in cm-1	Assignment of vibration
1.	3831.34 and 3707.82	-NH_2stretching vibration
2.	3333.79 and 3234.03	-OH stretching vibration
3.	3052.51	aromatic stretching
4.	2669.95 cm-1	-C-H stretching
5.	1906.05	C=O Overtone absorption
6.	1592.15, 1491.49 and 1437.55	C=C stretching
7.	731.49and 538.10	Alkyl stretching

Discussion

Isolation of microalgae with considerable lipid acquaintance is important for its application in bio-fuel sector. In the present study, water samples were collected from Ayialoor panchayat, Namakkal district. In total seven microalgae strains are isolated. From that *Chlorella vulgaris* microalga was selected for the present study. Maintenance of algal culture without contamination is much more important before the estimation processes. The reason for considering natural isolate of *Chlorella vulgaris* mainly due to its adequate lipid storing capacity up to 45% in Dry cell weight[12], also its adaptability to most of the industrial effluent systems. Bio-energy generation from Chlorella is a new facet in renewable energy research. Illman et al.2000 had studied calorific values of Chlorella strains grown in low nitrogen medium including four fresh water strains including Chlorella protothecoides, C. vulgaris, Chlorella emersonii and Chlorella sorokiniana and one marine strain namely Chlorella minutissima and suggested Chlorella strains may be suitable for diesel replacements. Later on Scragg et al.,2003 successfully used an emulsion consisting of transesterified rape seed oil, a surfactant and slurry of C. vulgaris in an unmodified single cylinder diesel engine. They also reported that high quality biodiesel production from heterotrophic microalgae C. protothecoides.[13,14]

Total dry and wet biomass are calculated for the Ch V strain after 21 days of incubation. In order to avoid contamination with other type of bacteria and fungi specific antibiotics were used, for carrying out error free estimation studies. The strain was subjected to phototrophic cultivation and biomass was estimated, lipid samples were extracted and FT-IR analysis for lipid samples was performed to find out the chemical composition of the extracted lipid. The FT-IR transmittance spectrum revealed the presence of Amine, Alcohol, aromatic, alkyne, alkene, acid, ether and alkyl halide groups in *Chlorella*. Ch V extract was found with the weak band centered on 2920 cm–1 due to the presence of asymmetric C–H stretching vibration. The observed bands around 2669 cm–1 corresponded to the symmetric C–H stretching vibration in the *Chlorella vulgaris*. The bands nearing the range of 3051cm-1 denoted the presence of acetic acid and its methyl ester derivatives and 3334 cm-1. 1592.15, 1491.49 and 1437.55cm-1 denoted C=C stretching pattern in *Chlorella vulgaris* [8].

In total, the Ch V strain was found with strong acid concentration and a peak was observed within 2000 to 3000 cm-1 for lipid confirmation range. According to the results Ch V was selected for further lipid analysis and optimization studies. Algae strains were screened for the accumulation of significant quantities of lipids in provided growth conditions. Many methods are available for lipid analysis including nile red flurescence microscopy analysis, thin layer chromatography (TLC), fourier transform infra red spectroscopy (FTIR) and so on. FTIR based lipid analysis was much more preferable due to its technical benefits such as sensitivity, high throughput means to assess carbon allocation changes o select an efficient method [6]. Else other methods that were mentioned have some drawbacks either due to technical challenge, time consuming, or have poor efficiency or sensitivity. FT-IR played a pivotal role in determining the chemical composition of phytoplanktonic community to make use of it in the bio- energy field.

References

1. Shelly K, Heraud P, Beardall J (2002). Nitrogen limitation in Dunaliella tertiolecta (Chlorophyceae) leads to increased susceptibility to damage by UV-B radiation but also increased repair capacity. J. Phycol. 38: 1-8

2. Heraud P, Wood BR, Beardall J, McNaughton D (1999). Physiological change in microalgae monitored using FTIR spectroscopy. In: Greve J, Pupples GJ and Otto C (ed.) Spectroscopy of Biological Molecules: New Directions, Dordrecht: Kluwer Academic Publishers. 449-450.

3. Guerin M, Huntley M E and Olaizola M. 2003. Haematococcus astaxanthin:applications for human health and nutrition.TRENDS in Biotechnology Vol.21 No.5

4. Naumann D. (1997) FT-IR and FT-Raman spectroscopy in medical microbiology. In: P Carmona, R Navarro, A Hernanz

(eds): Spectroscopy of biological molecules: modern trends. Dordrecht: Kluwer, 433-434.
5. McMillan P. F. And Hofmeister A. M. (1988) Infrared and Raman Spectroscopy. In:Spectroscopic Methods in Mineralogy and Geology. Reviews in Mineralogy 18, Chapter 4. Pp. 99-160, ed. H. C. Hawthorne, Mineralogical Society of America, BookCrafters, Inc, chelsea, Michigan.
6. Dean AP, Sigee DC (2006). Molecular heterogeneity in Aphanizomenon flos-aquae and Anabaena flos-aquae (Cyanophyta): a synchrotron- based Fourier-transform infrared study of lake micropopulations. Eur. J. Phycol. 41(2): 201-212.
7. Lewis RN, McElhaney RN (1996). Fourier transform infrared spectroscopy in the study of hydrated lipids and lipid bilayer membranes. In: Mantsch HH and Chapman D (ed.) Infrared Spectroscopy of Biomolecules, Wiley: Chichester. 159-202.
8. Brian C Smith,(1996), Fundamentals of Fourier Transform Infrared Spectroscopy, Second Edition, CRC PressINC,
9. Wennign M, Seiler H, Scherer S (2002). Fourier-Transform infrared microspectroscopy, a novel and rapid tool for identification of yeasts. Appl. Environ. Microbiol. 68(10): 4717-4721.
10. Parvin M, Zannat M N and Habib M A B. 2007. Two Important Techniques for Isolation of Microalgae. Asian Fisheries Science 20(2007):117-124.
11. Bligh,E.G. and Dyer,W.J. 1959. A rapid method for total lipid extraction and purification. Can.J.Biochem.Physiol. 37:911-917.
12. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306.
13. Illman AM, Scragg AH, Shales SW. Increase in Chlorella strains calorific values
when grown in low nitrogen medium. Enzyme Microb Technol 2000;27:631–5.
14. Scragg AH, Morrison J, Shales SW. The use of a fuel containing Chlorella vulgaris in a diesel engine. Enzyme Microb Technol 2003;33:884–9.