Research Article

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GC-MS and FT-IR spectroscopic determination of Fattyacid Methyl Ester of 16 freshwater Microalgae, Isolated from Cement Industries of Tamil Nadu, India

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ABSTRACT

In this study Fourier transform infrared spectroscopy (FTIR) and GC-MS was used to identify and quantify lipids in the freshwater microalgae. Microalgae are renewable resource containing rich lipids in their body and has the potential to refill the partial energy demands in an eco-friendly way. The present study carried out to find indigenous microalga from Cement factories, Ariyalur district as a potent source for biodiesel. The lipid fractions were extracted from the biomass through different solvent extractions and the fractions were analyzed for biodiesel under FTIR spectroscopy and GC-MS. These results make better understanding of rapid metabolic responses in percentage of Saturated Fatty Acids (SFA), Poly Unsaturated Fatty Acid (PUFA) and Mono Unsaturated Fatty Acids (MUFA). Among, the total Sixteen Microalgal groups, eight Microalgal groups produced SFA in high percentage, seven groups had high yields of PUFA and only one group of microalgal contain MUFA.

Key words: Biofuel, biodiesel, Fattyacid Methyl Ester, FT-IR, GC-MS, Saturated fatty acid, MUFA and PUFA.

INTRODUCTION:

Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. The cost of biodiesel, however, is the main hurdle to commercialization of the product. The used cooking oil and algae are used as raw material, adaption of continuous transesterification process and recovery of high quality glycerol from biodiesel by-product (glycerol) are primary options to be considered to lower the cost of biodiesel (Fangrui *et al.*, 1999). The transesterification of natural triglycerides (eg:- oils and fats) is employed to obtain fatty acid methyl esters (FAME) which are key reagents in the chemical industry (Loupy *et al.*, 1993, Ahn *et al.*, 1982). The FAME are the raw materials for the production of long chain carboxylic acids, detergents, alternative fuels for diesel engines (Bio Diesel) and mono and triglycerides (Sonntag 1982). Lipid qualification and quantification can be carried out by several means including Fourier transform infrared micro-spectroscopy (FTIR) and Gas Chromatography (GC) with Mass Spectrometry (MS) (Medina *et al.*, 1998). The present study aims the comparison of fatty acids profiling of microalgae. Microalgal samples were collected from various environmental conditions from Cement factories, Ariyalur district. The lipid analyzed and compared by 16 microalgae FT-IR and GC-MS.

MATERIAL AND METHODS:

Collection Site

In the present study, sample collected from different places of Cement factories, nearby ponds, Rock ponds, Caves ponds from Ariyalur district (Latitude – 11.1503, Longitude – 79.0685) Fig (1). More than 16 microalgal strains were isolated and identified from 20 different sites. The temperature, pH, specific conductance, and water depth were recorded at each collection site by using YSI Multi-Parameter Water Quality Monitor (600XL). Most of the water samples had the temperatures ranged from 18°C to 45°C, pH ranged from 6.1 to 10.2.

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Fig 1. Fresh water Microalgae in Ariyalur Districts

Harvesting and Lipid extraction

Extraction of lipids from wet biomass was performed according to the procedure of Sharif Hossain & Salleh, (2008). Typically, the cells were harvested by centrifugation at 8000 rpm for 5 min, at 15˚C and washed once with distilled water. Biomass was macerated in mortar and pestle. The crushed algal cells were spread over a clean glass plate for air drying. The dried biomass were further broken into powder and mixed with citric acid for making algal beads, allow it for oven drying at 120˚C for 1 min. for evaporation of citric acid and water completely. Lipid presenting in algal cell contents were extracted using petroleum ether, catalyst such as NaOH and methanol. The dried biomass was soaked with petroleum ether (1:1 by vol) solvent in a beaker overnight. The yellow colored oil extracts were collected on top of the solution and mixed with catalyst (0.30 g NaOH and 2 ml of methanol) for transesterification process. After that the solution was kept for 16 h to settle the biodiesel and sediment layers clearly.

Fourier Transform Infra-Red Spectrometry (FT-IR)

The instrument model used in this studies was Perkin Elmer model spectrum-I Pc. Lipid fraction were evaporated on the Thalium bromide and FR-IR spectra (Resolution: 4 cm-1, Scan Number:3) were performed (Elumalai *et al.*, 2011).

Gas Chromatography And Mass Spectroscopic Studies (GC AND MS)

The collected Fatty acid Methyl Ester were processed with GC and MS (JEOL GC mate II) at IIT, Chennai, India. Lipid fraction was resuspended in n-hexane and applied to silica gel column chromatography. Aliphatic hydrocarbon fraction passes through the column fatty acid and carotenoid fractions were trapped. Passing through fraction was defined as hydrocarbon fraction, lipid components in hydrocarbon fraction were identified by GC/MS. The sample (1µl) was evaporated in a split less injector at 300°c. The results were compared with the petro based or Fossil fuel diesel and gasoline oils.

The methyl esters of fatty acids were quantified by a gas chromatograph (Agilant-JEOL GC AND MS). The column (HP5) was fused silica 50m x 0.25 mm I.D. Analysis conditions were 20 minutes at 100°C the 3°/ min to 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas. The weight percentages of fatty acids were approximated by the area of the detector response. The fatty acid methyl esters were identified by gas chromatography coupled with mass spectrometry (Tadashi 2009).

RESULTS

Lipid Extraction From Microalgae

Based on our laboratory experiments and experience of Sharif Hossain & Salleh method of extraction of lipid is ideal and precise. Several important factors during the lipid extraction such as effect of solvent, drying temperature and incubation period was investigated to find out which method give the best yield of lipids

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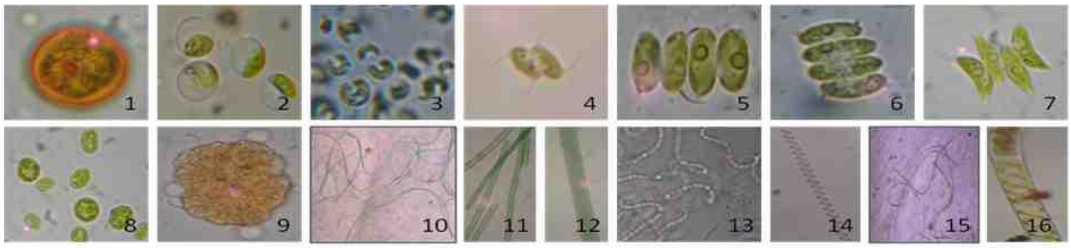

Fig 2. Chlorophyceae: *1.Chlorella vulgaris* Beijerinck, 2. *Chlorella mirabilis* Andreeva, 3. *Kirchneriella contorta* (schmidle) Bohlin, 4. *Scenedesmus quadricauda* Var. quadrispina (Chodat) G. M. Smith, 5. *Scenedesmus armatus* Var. dispar Var. Nov, 6. *Scenedesmus caudate*-aculeolatus Chodat, 7. *Scenedesmus dimorphus* (Turp.) Kuetz, 8. *Chlorococcum infusionum (Schrank) Meneghini*, 9. *Botryococcus braunii* Kuetzing, Cyanophycaeae: 10. *Phormidium corium* (C. Agardh) Gomont, 11. *Phormidium purpurascens* (Kutz.) Gom,12. *Oscillatoria princeps* Vaucher (Orign.), 13. *Nostoc verrucosum* Vaucher, 14. *Spirulina gigantean* Schmidle, 15. *Anabaena fertilissima* C.B.Rao, Zygnematophyceae: 16. *Spirogyra irregularis* Nageli.

Gas Chromatography and Mass Spectroscopy

Gas Chromatography is used to identify the chemical ingredients in the biodiesel. It was found in Fig (3 - 18) that there are different major esters in the Algal oil Methyl ester as shown in the table 1, 2 and 3. The chromatogram shows several compounds at various retention periods. Lipids were identified using Spectrum Data base SDBS software installed in GC-MS.

To compare the entire 16 microalgae Fig (2), mainly three types of fatty acids such as saturates, monoenes, polyenes found in extracts. The high content of Saturated Fatty Acids (SFA) were observed in *Spirulina gigantean* 100.02, *Oscillatoria princeps* 92.58, *Anabaena fertilissima* 91.53, *Spirogyra irregularis* 69.23, *Kirchneriella contorta* 55.38 and low content of SFA is *Scenedesmus quadricauda* 16.26, *Chlorella mirabilis* 26.23, *Botryococcus braunii* 26.66.

Mono Unsaturated Fatty Acids (MUFA) present in *Phormidium purpurascens* 52.29, *Spirogyra irregularis* 30.11, *Phormidium corium* 29.50, *Scenedesmus caudate* 26.91, *Scenedesmus armatus* 25 in high percentage and low content of MUFA is *Chlorella vulgaris* 4.45, *Anabaena fertilissima* 8.15, *Chlorella mirabilis* 10.18. *Spirulina gigantean, Chlorococcum infusionum, Oscillatoria princeps* did not have MUFA.

The high content of Poly Unsatureated Fatty Acids (PUFA) noted in the follwing microaglae *Scenedesmus quadricauda* 73.27, *Chlorococcum infusionum* 70.68, *Chlorella mirabilis* 63.56, *Botryococcus braunii* 61.12, *Chlorella vulgaris* 40.67 and low in *Oscillatoria princeps* 7.02, *Phormidium purpurascens* 14.21, *Kirchneriella contorta* 22.04. Absence of PUFA in *Spirulina gigantean, Anabaena fertilissima, Spirogyra irregularis.*

Fig 3. GC of *Chlorella vulgaris* Beijerinck

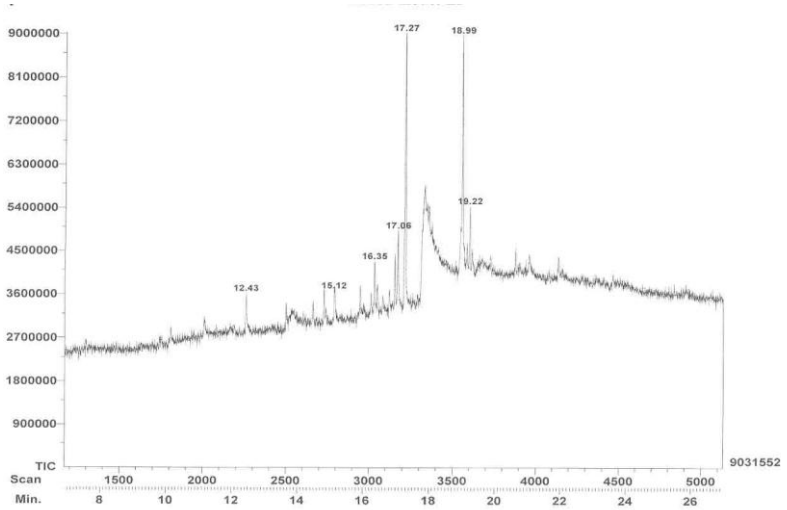

Fig 4. GC of *Chlorella mirabilis* Andreeva

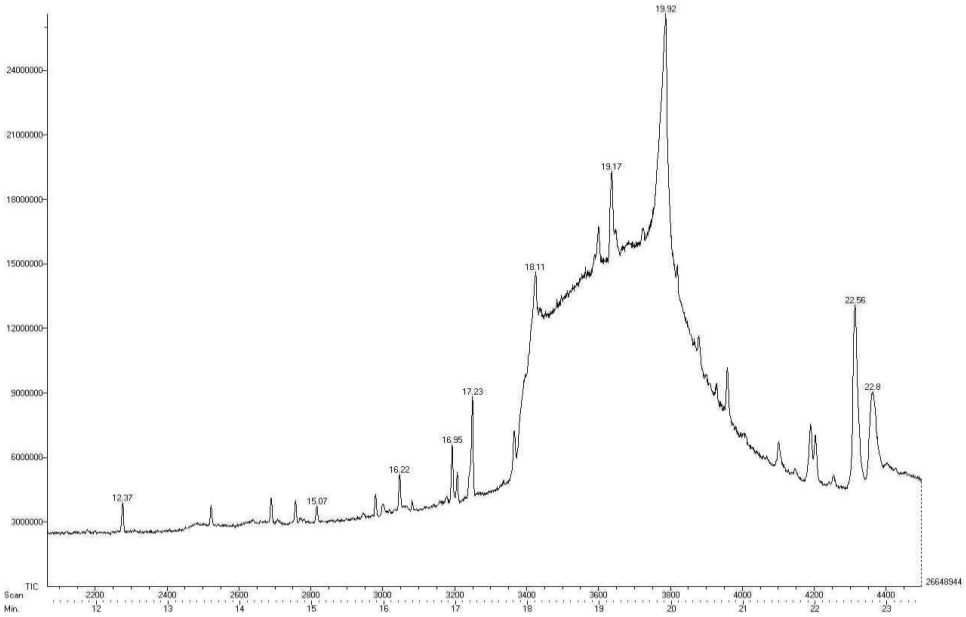

Fig 5. GC of *Kirchneriella contorta* (schmidle) Bohlin

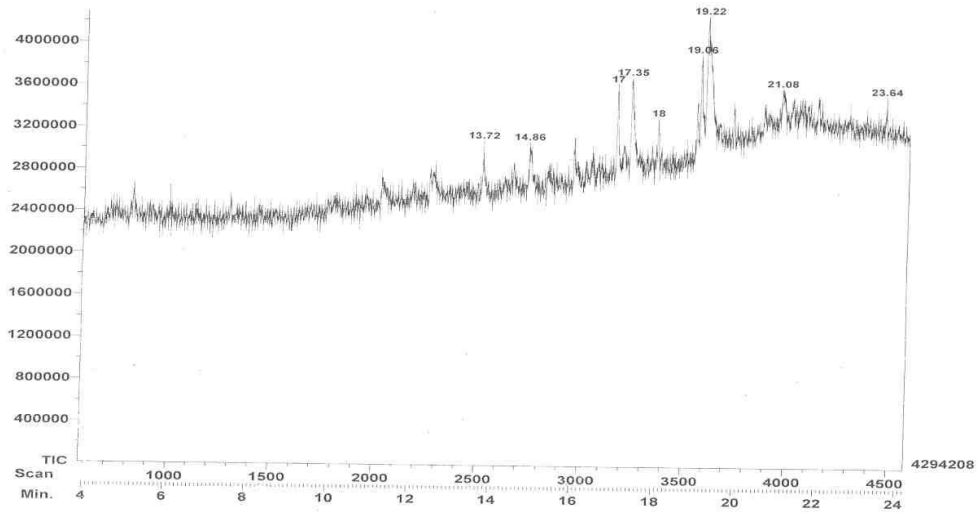

Fig 6. GC of *Scenedesmus quadricauda* Var. quadrispina (Chodat) G. M. Smith

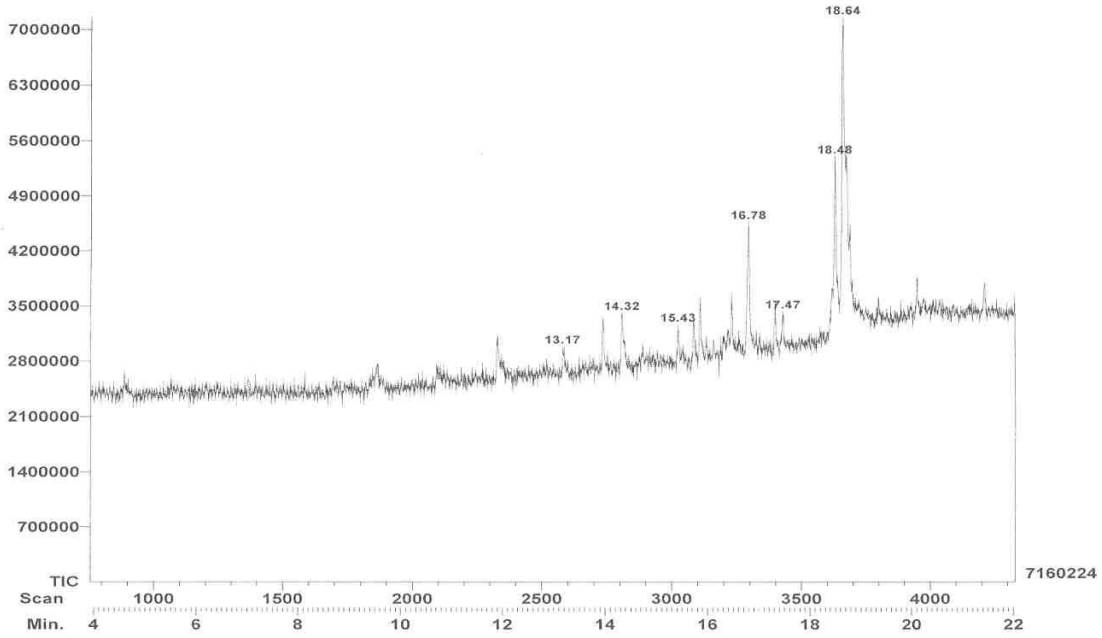

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Fig 7. GC of *Scenedesmus armatus* Var. dispar Var. Nov

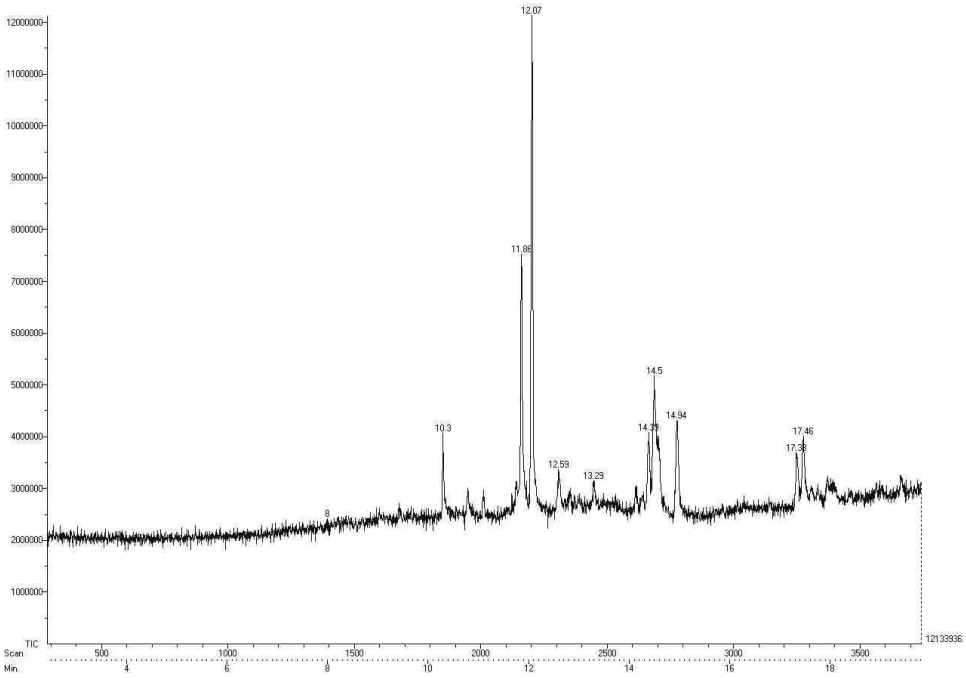

Fig 8. GC of *Scenedesmus caudate*-aculeolatus Chodat

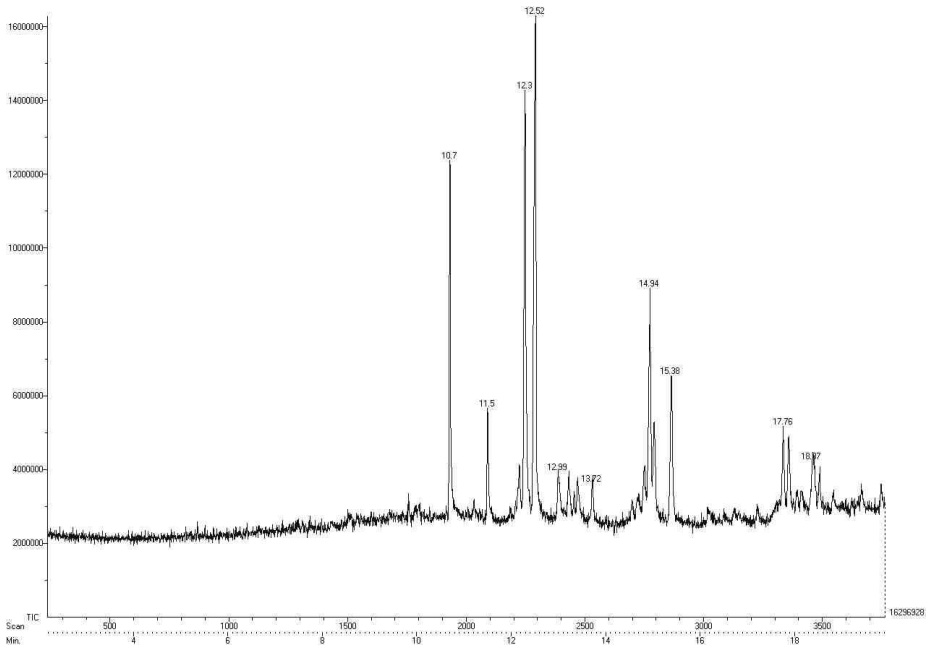

Fig 9. GC of *Scenedesmus dimorphus* (Turp.) Kuetz

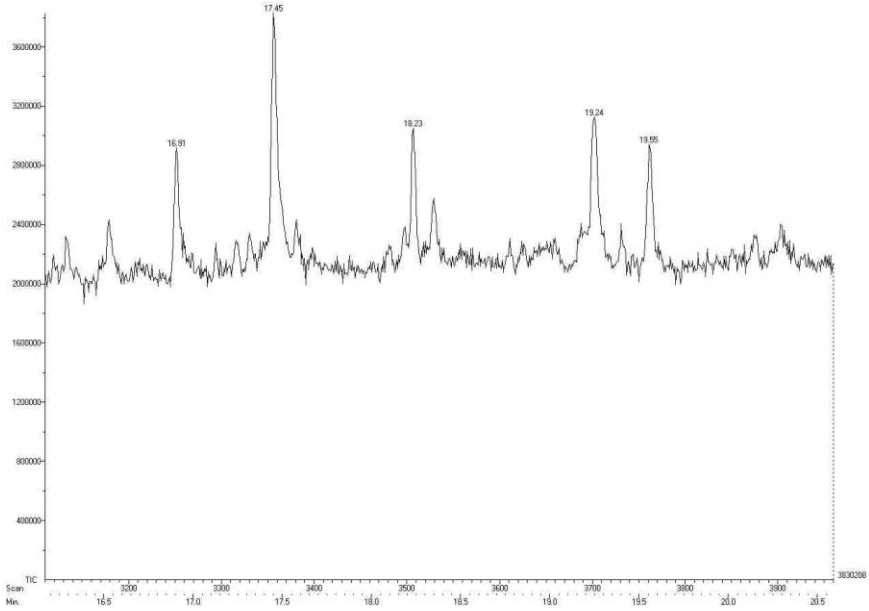

Fig 10. GC of *Chlorococcum humicolo* (Naegeli) Rabenhorst

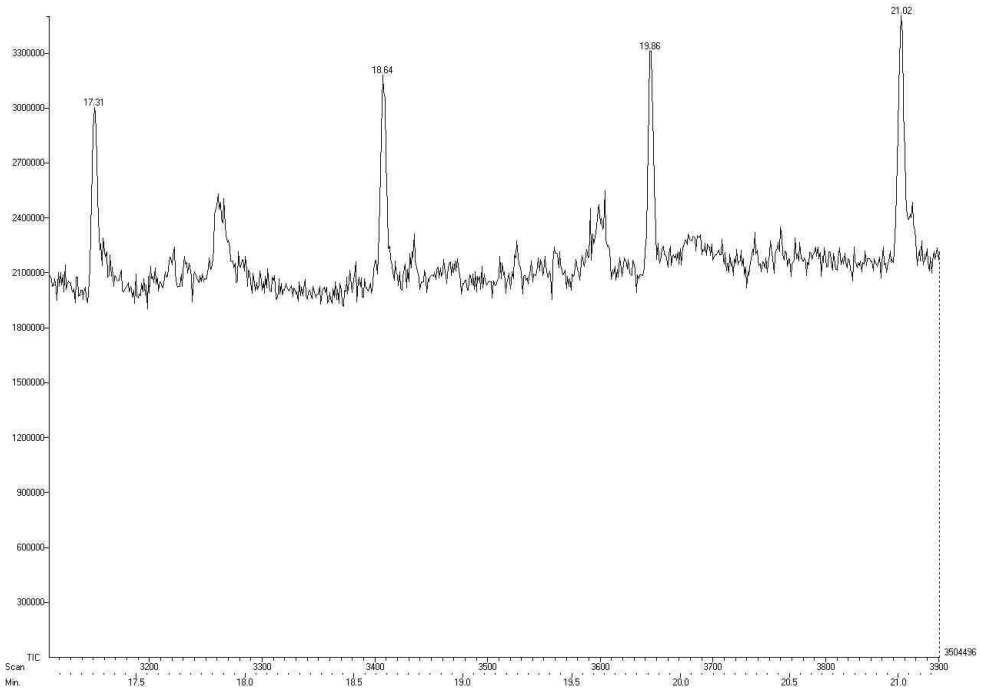

Fig 11. GC of *Botryococcus braunii* Kuetzing

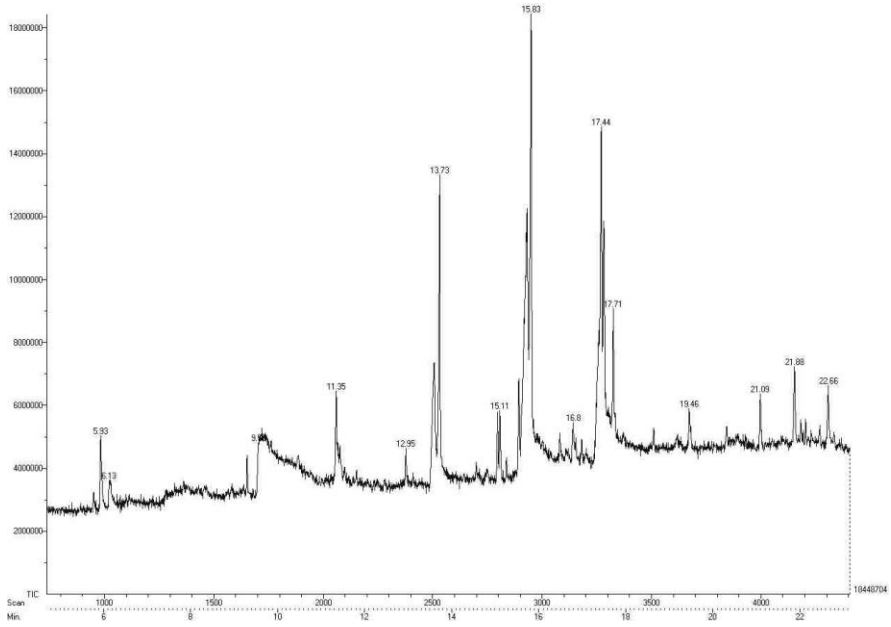

Fig 12. GC of *Phormidium corium* (C. Agardh) Gomont

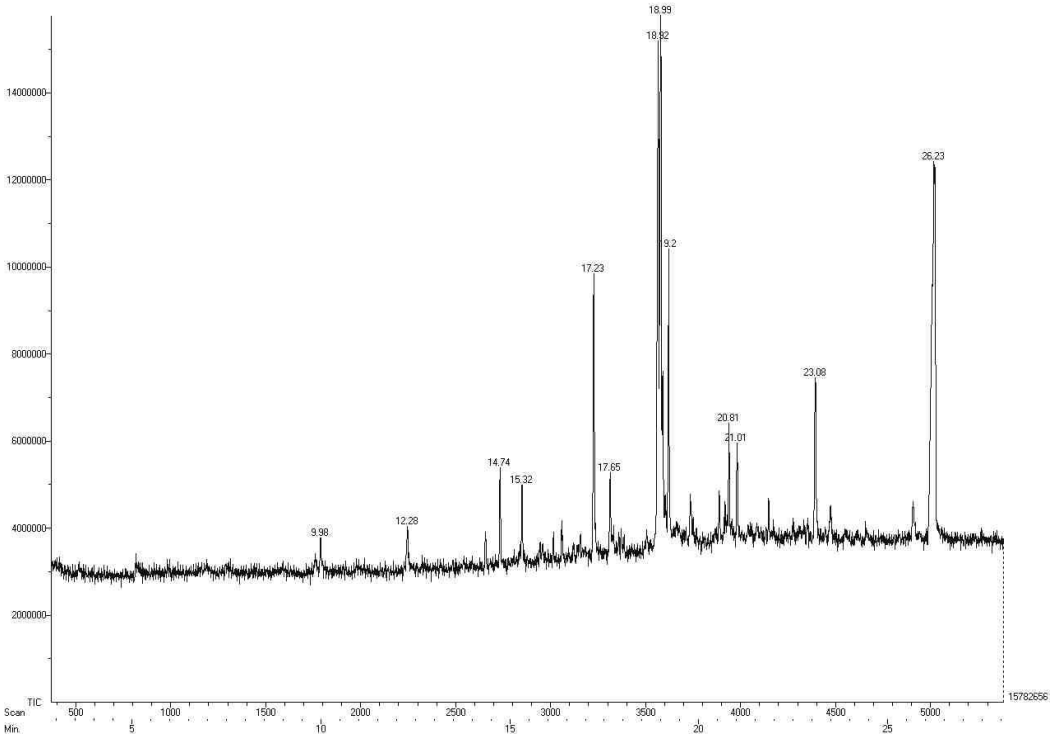

Fig 13. GC of *Phormidium purpurascens* (Kutz.) Gom

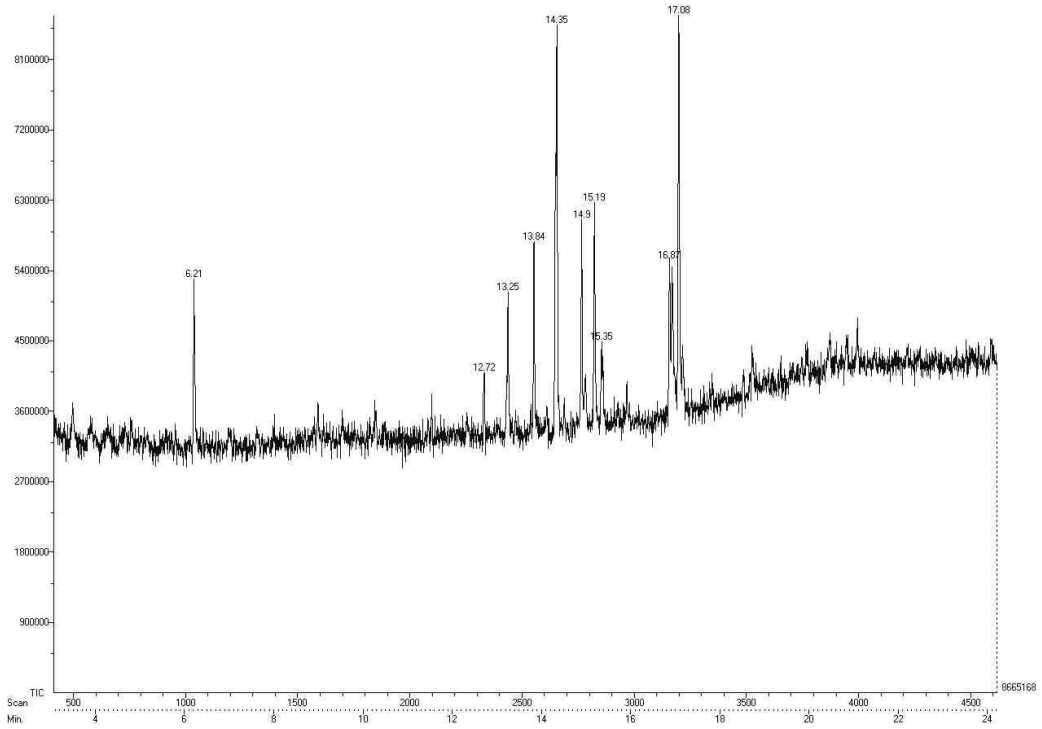

Fig 14. GC of *Oscillatoria princeps* Vaucher (Origin.)

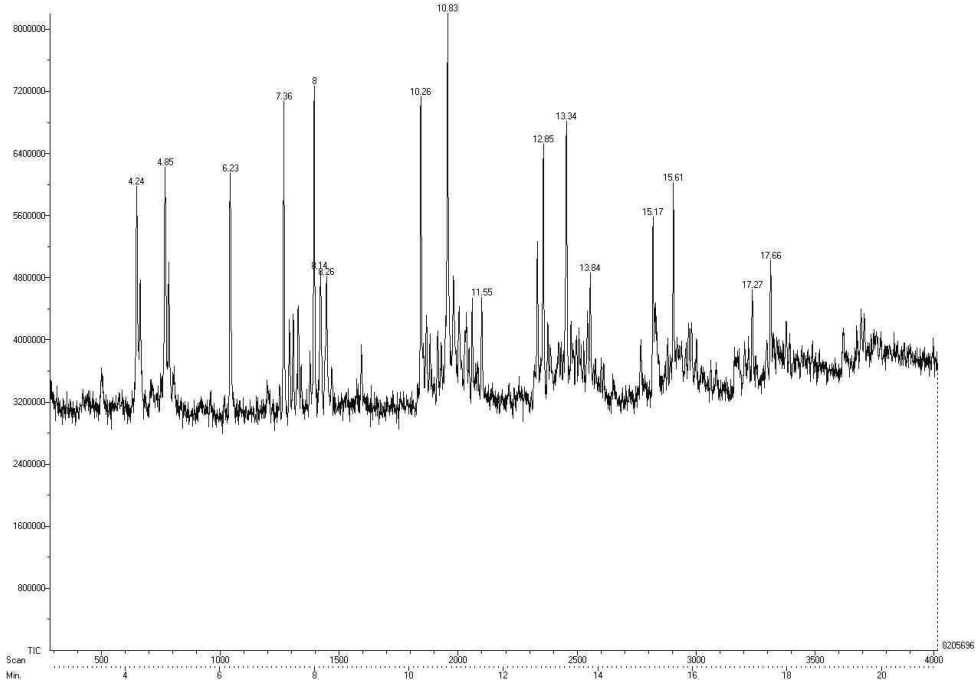

Fig 15. GC of *Nostoc verrucosum* Vaucher

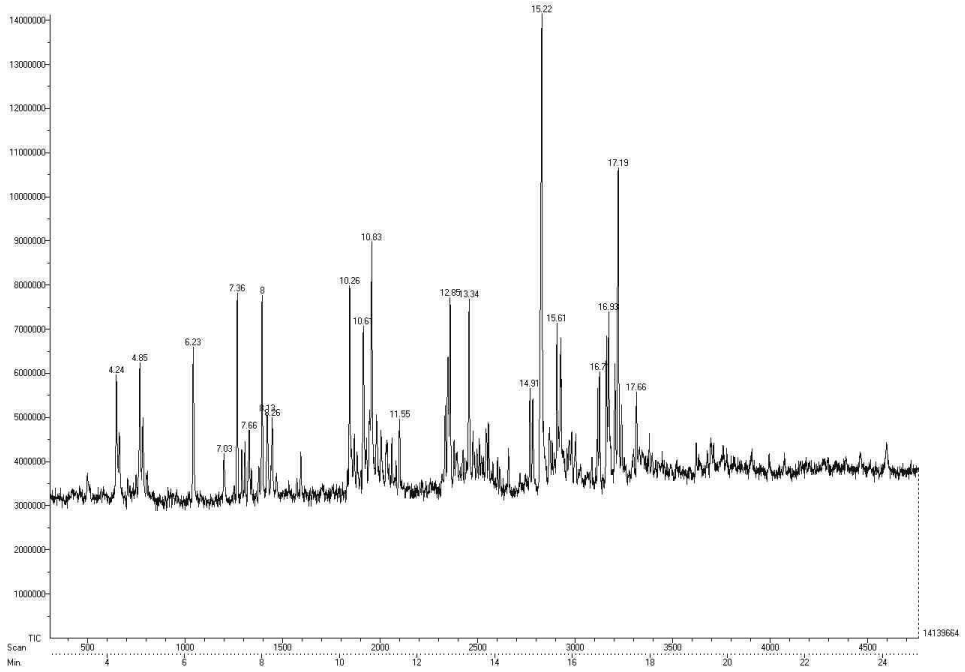

Fig 16. GC of *Spirulina gigantean* Schmidle

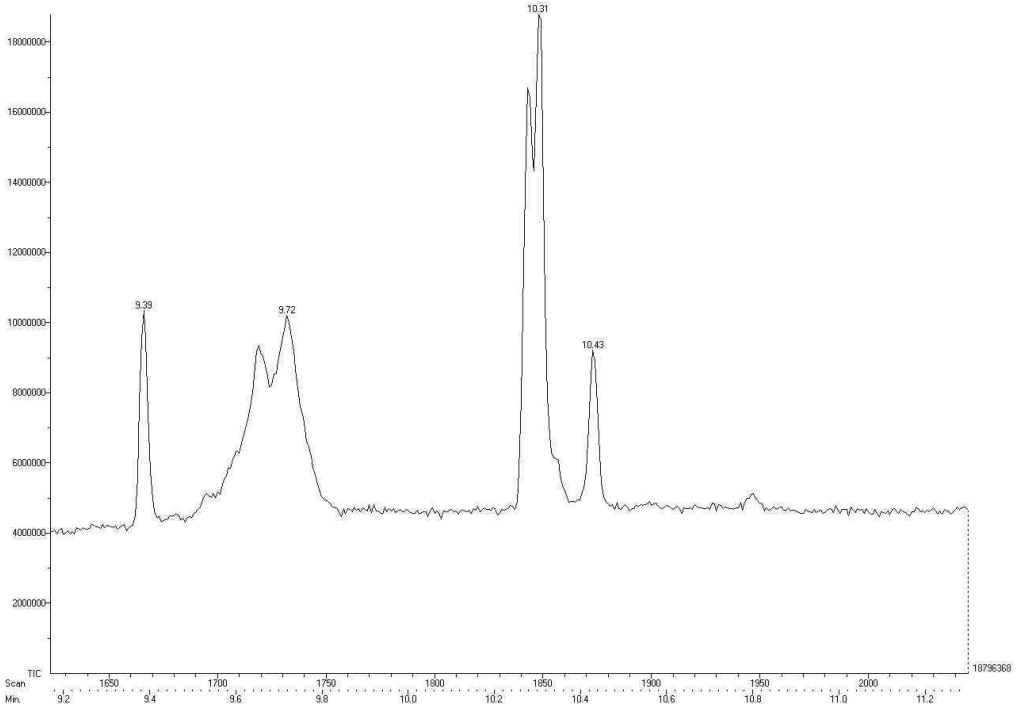

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Fig 17. GC of *Anabaena fertilissima* C.B.Rao

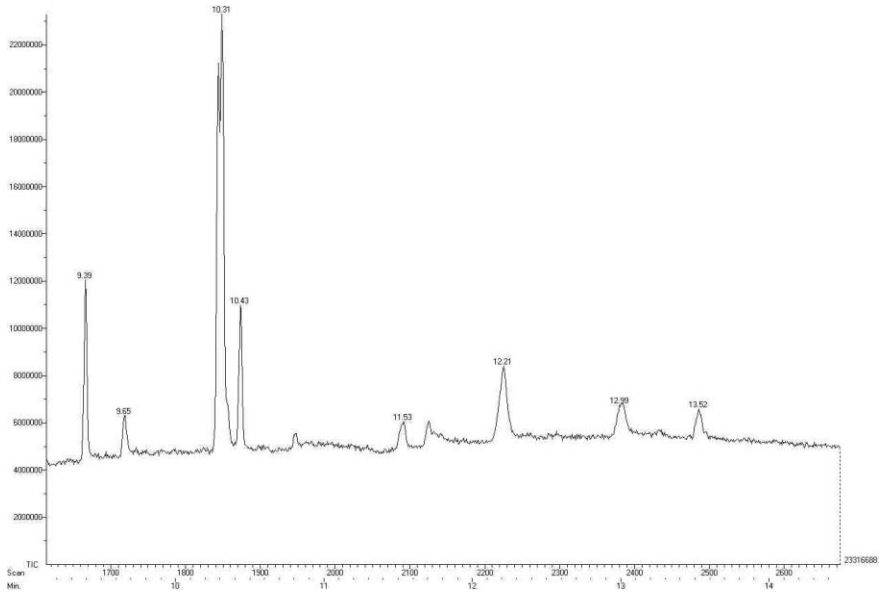

Table 1. Molecular weight and retention time of Saturated, Mono Unsaturated Fatty acids (MUFAs) and Poly Unsaturated Fatty acids (PUFAs) Fatty acids obtain from GC-MS of different Microalgae isolated from Cement Industy, Ariyalur District, Tamil Nadu, India.

Cetane Number	*Chlorella sp.*	*Chlorella sp.*	*Kirchneriella sp.*	*Scenedesmus sp.*	*Scenedesmus sp*	*Scenedesmus sp*
SFA						
13:0	3.81	ND	ND	1.56	15.58	ND
14:0	ND	10.30	2.29	ND	ND	21.98
15:0	7.61	ND	6.19	0.78	17.61	5.17
16:0	9.21	ND	0.69	10.38	ND	6.38
17:0	ND	7.76	44.63	3.54	ND	ND
18:0	34.21	8.17	1.58	ND	ND	ND
MUFA						
$14:1\Delta^{9}$	ND	ND	ND	ND	28.81	ND
$16:1\Delta^{11}$	ND	ND	15.84	ND	3.19	24.20
$17:1\Delta^{9}$	ND	6.69	6.71	ND	ND	2.71
$18:1\Delta^{11}$	4.46	3.49	ND	10.45	ND	ND
PUFA						
$16:2\Delta^{7,10}$	ND	ND	ND	ND	ND	26.63
$16:2\Delta^{9,12}$	34.48	17.05	ND	ND	ND	ND
$18:2\Delta^{6,11}$	ND	ND	9.69	ND	2.18	12.96
$16:3\Delta^{6,9,12}$	ND	ND	ND	ND	25.20	ND
$16:3\Delta^{9,12,15}$	ND	ND	ND	ND	1.89	ND
$17:3\Delta^{8,11,14}$	ND	16.93	12.35	ND	ND	ND
$18:2\Delta^{9,12}$	6.20	19.32	ND	63.13	ND	ND
$20:3\Delta^{11,14,17}$	ND	10.26	ND	ND	ND	ND
$20:4\Delta^{8,11,14,17}$	ND	ND	ND	10.14	ND	ND
$24:2\Delta^{15,18}$	ND	ND	ND	ND	3.45	ND
$24:3\Delta^{5,9,17}$	ND	ND	ND	ND	2.03	ND
Percentage of SFA	**54.86**	**26.23**	**55.38**	**16.26**	**33.19**	**33.53**
Percentage of MUFA	**4.45**	**10.18**	**22.55**	**10.45**	**25**	**26.91**
Percentage of PUFA	**40.67**	**63.56**	**22.04**	**73.27**	**34.75**	**39.59**
TFA	**99.98**	**99.97**	**99.97**	**99.98**	**92.94**	**100.03**

*ND: Not Detected

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Table 2. Molecular weight and retention time of Saturated, Mono Unsaturated Fatty acids (MUFAs) and Poly Unsaturated Fatty acids (PUFAs) Fatty acids obtain from GC-MS of different Microalgae isolated from Cement Industy, Ariyalur District, Tamil Nadu, India.

Cetane Number	*Scenedesmus sp.*	*Chlorococcum sp.*	*Botryococcus sp.*	*Phormidium sp.*	*Phormidium sp.*	*Oscillatoria sp.*
SFA						
06:0	ND	ND	ND	ND	ND	14
08:0	ND	ND	ND	ND	ND	15.12
09:0	ND	ND	ND	ND	11.11	13.08
10:0	18.6	ND	ND	ND	8.04	ND
11:0	ND	ND	ND	ND	17.02	26.09
12:0	ND	28.77	7.39	ND	ND	12.02
13:0	ND	ND	ND	4	ND	4.05
15:0	ND	ND	ND	ND	ND	8.22
16:0	ND	ND	19.27	12.02	ND	ND
17:0	14.15	ND	ND	ND	ND	ND
20:0	ND	ND	ND	4.02	ND	ND
21:0	ND	ND	ND	2.12	ND	ND
22:0	ND	ND	ND	9.24	ND	ND
24:0	ND	ND	ND	13.28	ND	ND
29:0	ND	ND	ND	3.28	ND	ND
MUFA						
$12{:}1\Delta^{9}$	ND	ND	ND	ND	22.42	ND
$14{:}1\Delta^{9}$	17.98	ND	ND	4.06	5.03	ND
$16{:}1\Delta^{11}$	ND	ND	ND	ND	24.84	ND
$18{:}1\Delta^{11}$	ND	ND	12.19	ND	ND	ND
$18{:}1\Delta^{9}$	ND	ND	ND	25.44	ND	ND
PUFA						
$16{:}2\Delta^{7,10}$	ND	ND	39.99	ND	ND	ND
$16{:}2\Delta^{9,12}$	ND	24.36	ND	ND	ND	7.02
$17{:}2\Delta^{9,12}$	34.94	24.99	ND	ND	ND	ND
$18{:}2\Delta^{7,12}$	ND	ND	ND	5.82	ND	ND
$18{:}2\Delta^{9,11}$	ND	ND	ND	20.08	14.21	ND
$18{:}2\Delta^{11,14}$	17.02	ND	ND	ND	ND	ND
$18{:}3\Delta^{3,9,12}$	ND	ND	6.80	ND	ND	ND
$22{:}2\Delta^{5,13}$	ND	21.33	ND	ND	ND	ND
$26{:}3\Delta^{5,9,19}$	ND	ND	14.33	ND	ND	ND
Percentage of SFA	**32.75**	**28.77**	**26.66**	**47.96**	**36.17**	**92.58**
Percentage of MUFA	**17.98**	**NIL**	**12.19**	**29.50**	**52.29**	**NIL**
Percentage of PUFA	**51.96**	**70.68**	**61.12**	**25.90**	**14.21**	**7.02**
TFA	**102.69**	**99.45**	**99.97**	**103.36**	**102.67**	**99.6**

*ND: Not Detected

Table 3. Molecular weight and retention time of Saturated, Mono Unsaturated Fatty acids (MUFAs) and Poly Unsaturated Fatty acids (PUFAs) Fatty acids obtain from GC-MS of different Microalgae isolated from Cement Industy, Ariyalur District, Tamil Nadu, India.

Cetane Number	*Nostoc sp.*	*Spirulina sp.*	*Anabaena sp.*	*Spirogyra sp.*
SFA				
06:0	11.07	ND	ND	ND
09:0	13.04	ND	ND	ND
10:0	4.25	ND	ND	ND
11:0	ND	35.88	ND	ND
12:0	12.08	ND	ND	43.22
13:0	6.25	37.42	24.22	26.01
14:0	5.22	ND	35.04	ND
15:0	ND	ND	22.14	ND
16:0	ND	26.72	24.22	ND
MUFA				
$14:1\Delta^{9}$	ND	ND	8.15	30.11
$16:1\Delta^{11}$	24.3	ND	ND	ND
PUFA				
$18:2\Delta^{9,11}$	11.05	ND	ND	ND
$18:3\Delta^{3,9,12}$	5.78	ND	ND	ND
$20:2\Delta^{11,14(n-6)}$	7.22	ND	ND	ND
Percentage of SFA	**51.91**	**100.02**	**91.53**	**69.23**
Percentage of MUFA	**24.03**	**NIL**	**8.15**	**30.11**
Percentage of PUFA	**24.05**	**NIL**	**NIL**	**NIL**
TFA	**99.99**	**100.02**	**99.68**	**99.34**

*ND: Not Detected

Fig 18. GC of *Spirogyra irregularis* Nageli

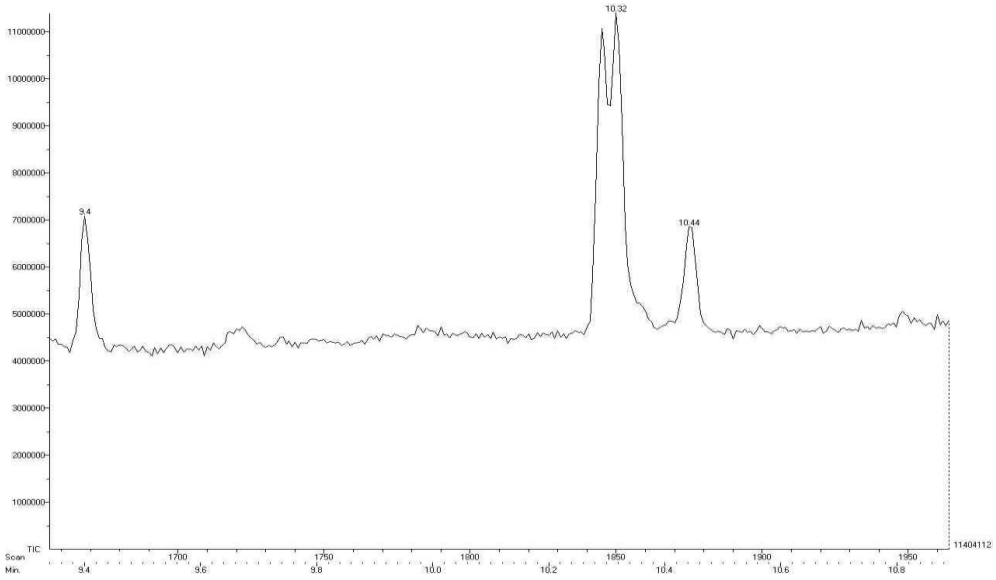


Fig 19. FT-IR of *Chlorella vulgaris* Beijerinck

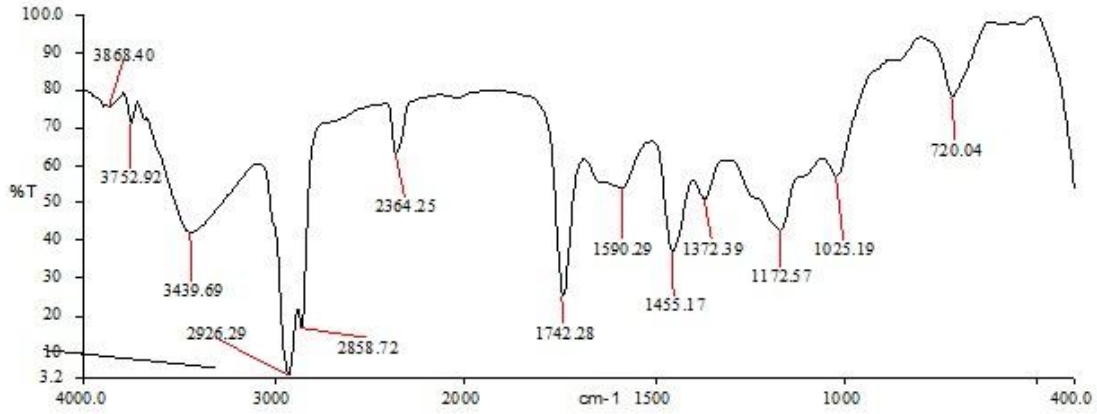


Fig 20. FT-IR of *Chlorella mirabilis* Andreeva

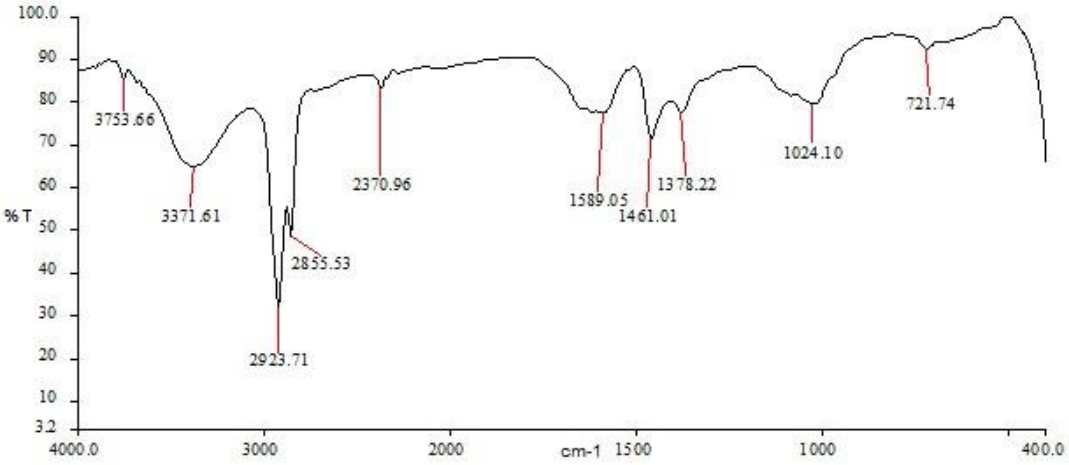


Fig 21. FT-IR of *Kirchneriella contorta* (schmidle) Bohlin

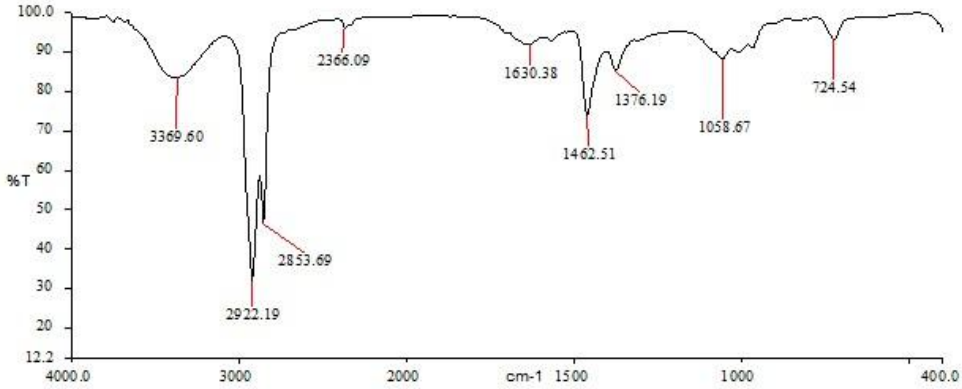


Fig 22. FT-IR of *Scenedesmus quadricauda* Var. quadrispina (Chodat) G. M. Smith

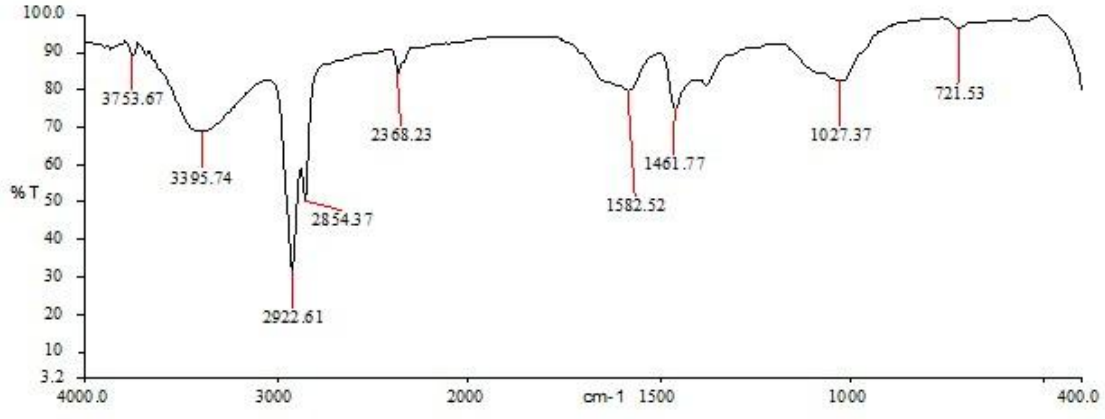


Fig 23. FT-IR of *Scenedesmus armatus* Var. dispar Var. Nov

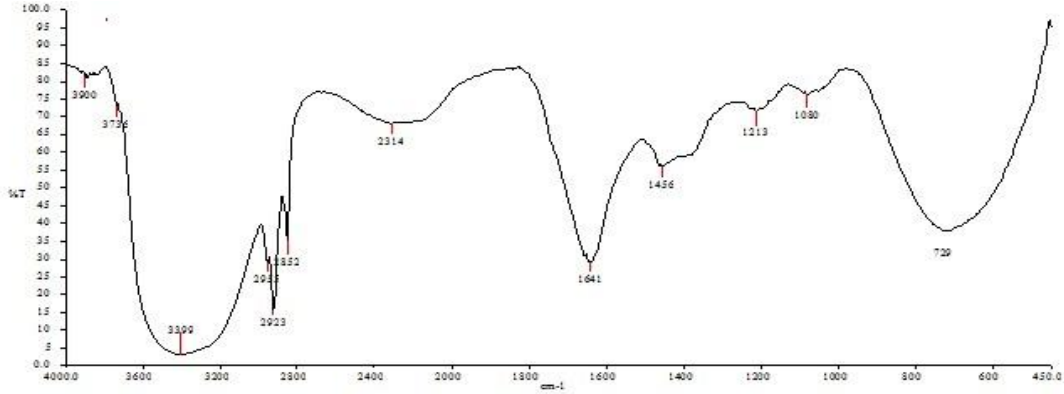


Fig 24. FT-IR *Scenedesmus caudate*-aculeolatus Chodat

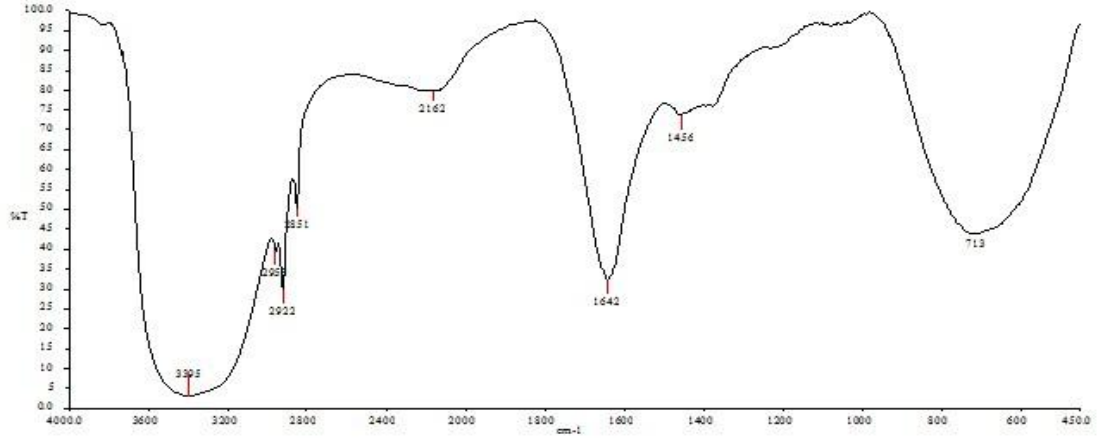


Fig 25. FT-IR of *Scenedesmus dimorphus* (Turp.) Kuetz

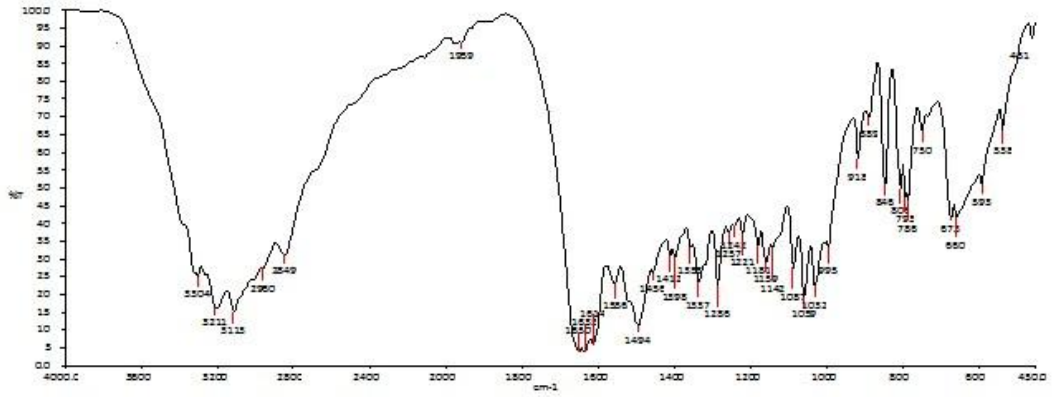


Fig 26. FT-IR of *Chlorococcum humicolo* (Naegeli) Rabenhorst

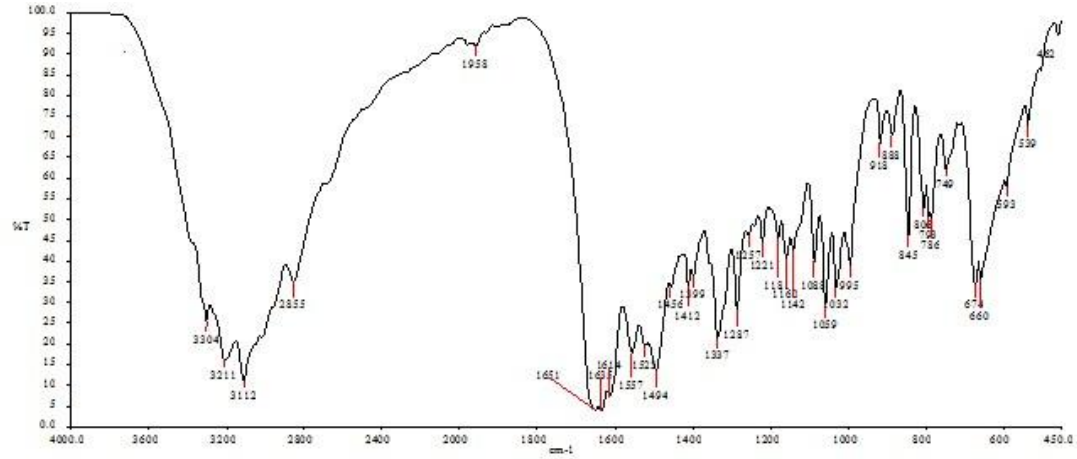


Fig 27. FT-IR of *Botryococcus braunii* Kuetzing

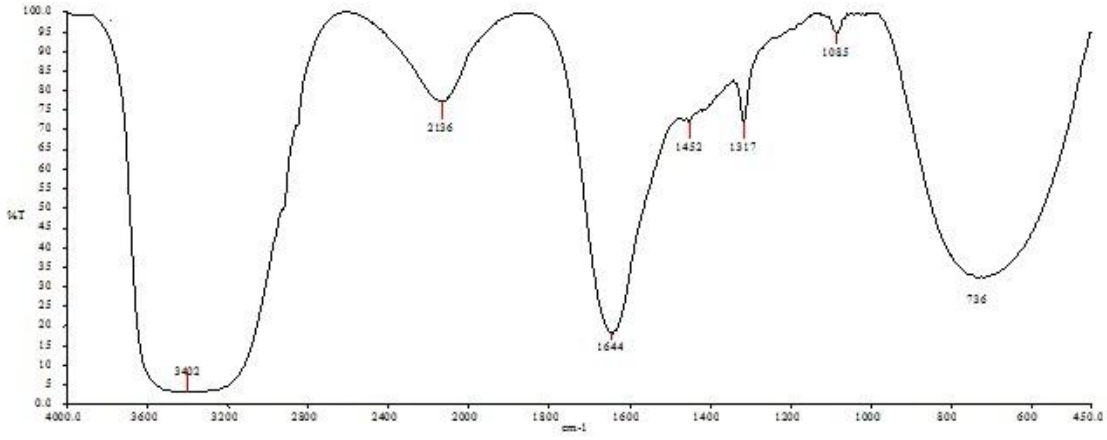


Fig 28. FT-IR of *Phormidium corium* (C. Agardh) Gomont

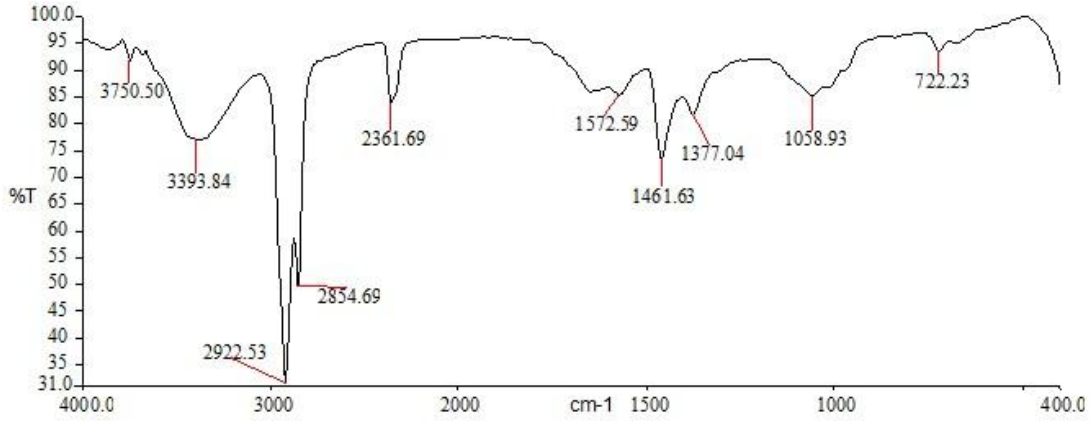


Fig 29. FT-IR of *Phormidium purpurascens* (Kutz.) Gom

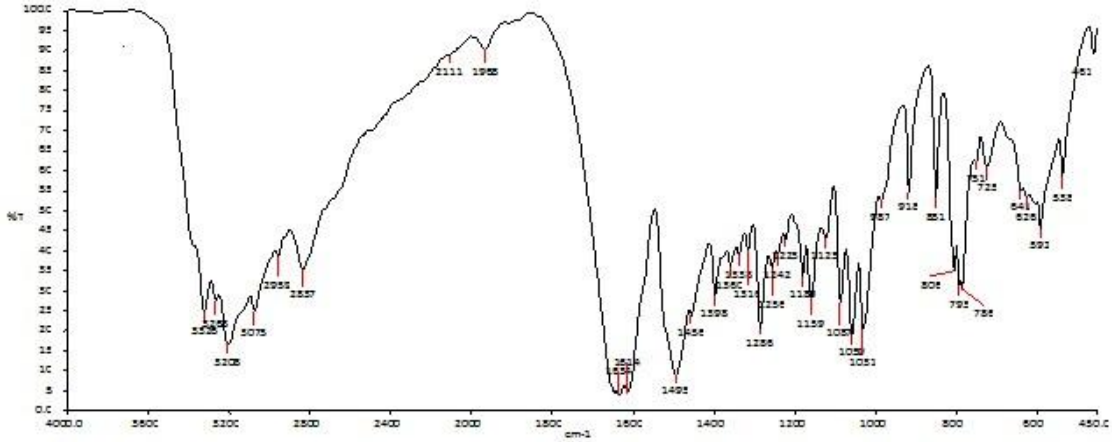


Fig 30. FT-IR of *Oscillatoria princeps* Vaucher (Orign.)

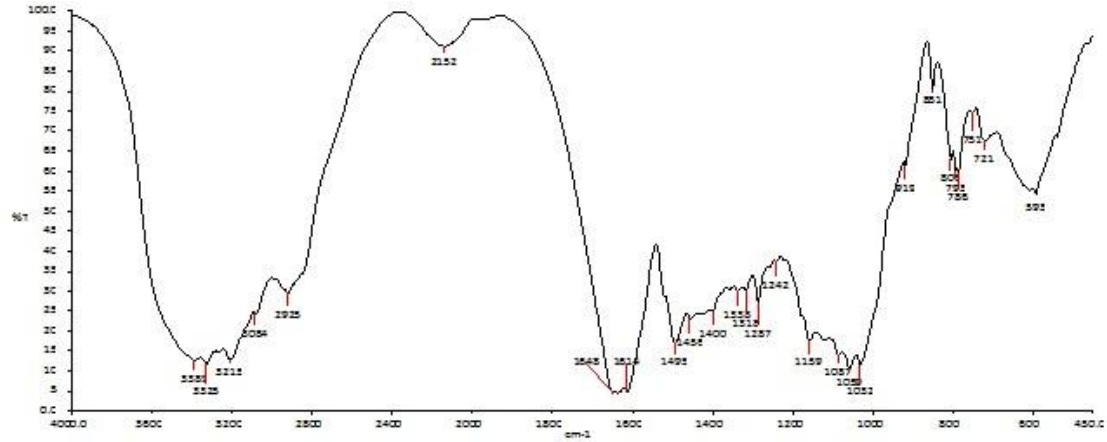


Fig 31. FT-IR of *Nostoc verrucosum* Vaucher

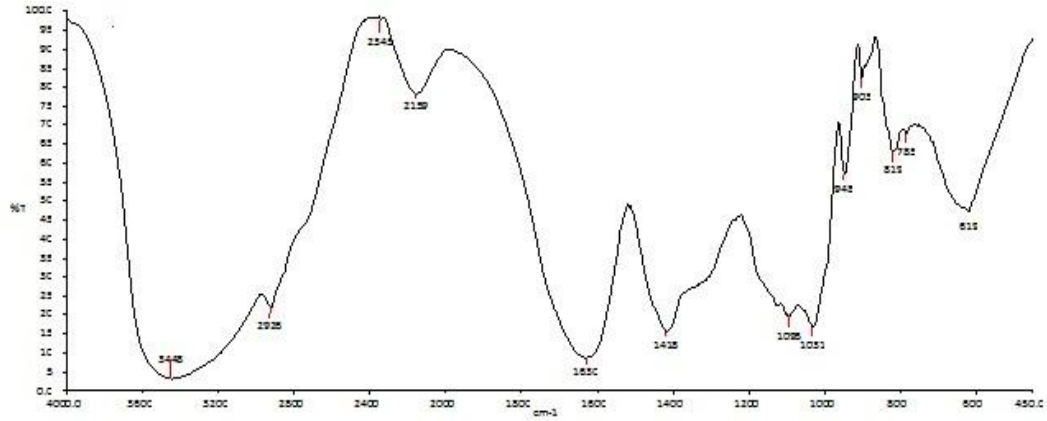


Fig 32. FT-IR of *Spirulina gigantean* Schmidle

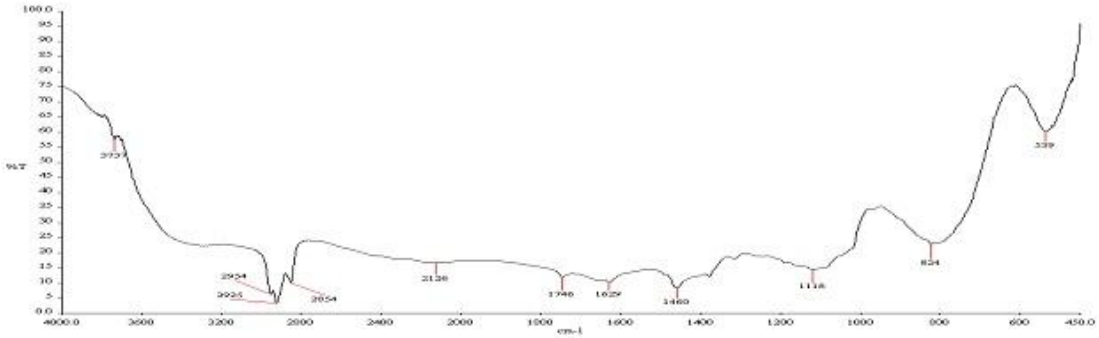


Fig 33. FT-IR of *Anabaena fertilissima* C.B.Rao

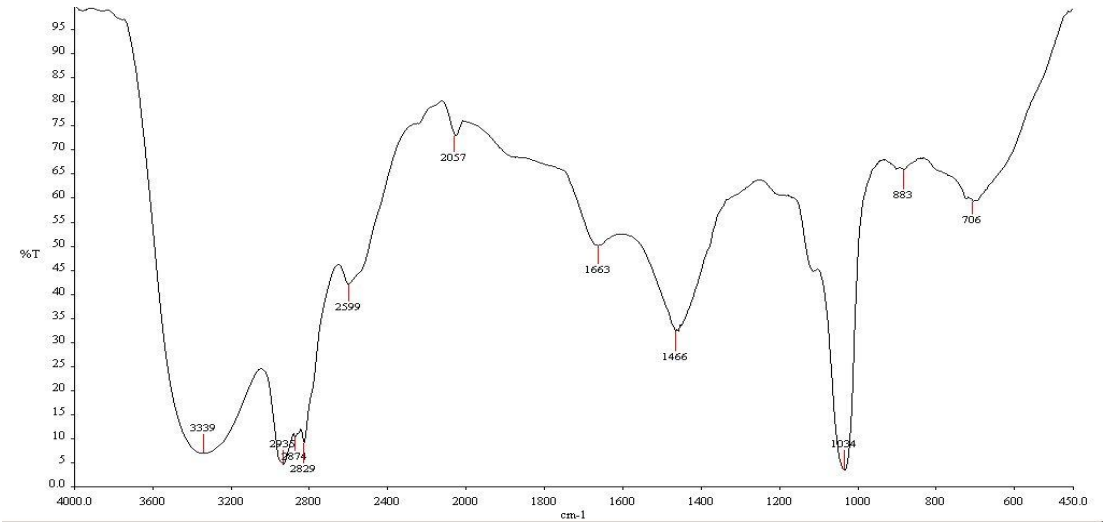
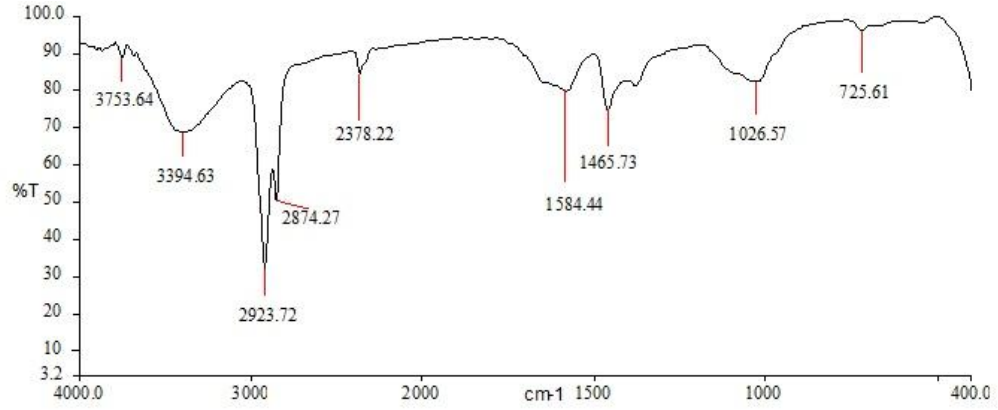


Fig 34. FT-IR of *Spirogyra irregularis* Nageli



FT-IR Spectroscopy

Five volatile products can be found in the spectrum of all microalgal lipid extracts. All collected extractions give bands at 706 and 3339 cm-1, so all are cis-isomer, as expected from alga lipid because trans isomers produce a strong band at 970 cm-1 and a weak band at 3012 cm-1 while cis-isomers gave medium nearby 720 and 3012 cm-1 bands. An analysis of the IR spectrum showed (Fig. 19 - 34) the main composition stage, reveals the existence of the absorption bands characteristic of these five different bonds:

• C═O: Carbonylic compounds (aldehydes, acids, etc.) are the strong C═O stretching absorption band in the region of 1870–1540 cm^{-1}. If esters, this band appears in the 1750–1735 cm^{-1}.

• C─O─C (Ethers): These stretching vibrations produce a strong band in the 1200–900 cm^{-1} region.

• C─H: absorption bands characteristic of the vibrations of C─H bonds, as an example, 2960 and 2875 cm^{-1} correspond to the asymmetric and symmetric vibrational modes of methyl groups, respectively, and 2929 and 2850 cm^{-1} correspond to the asymmetric and symmetric vibrational modes of methylene groups, respectively.

• CO_2: they produce strong bands in between 2800-2000 cm-1 as well as in 700 cm^{-1} region.

. • H_2O: the adsorption bands of water can be observed in the range of 1800-1200 cm^{-1}.

As many algal species have been found to grow rapidly and produce substantial amounts of TAG or oil and are thus referred to as oleaginous algae. It has long been postulated that algae could be employed as a cell factories to produce oils and other lipids for bio-fuel and other biomaterials (Benemann 1982).

There is an increase in total lipids stationary phase of algal cells or cells maintained under various stress conditions consisted primarily of neutral lipids, mainly TAGs. This was due to the shift in lipid metabolism from membrane lipid synthesis to the storage of neutral lipids. De novo biosynthesis and conversion of certain existing membrane polar lipids into triacylglycerols may contribute to the overall increase in TAG. As a result, TAGs may account for as much as 80% of the total lipid content in the cell (Klyachko-Gurvich 1974)

CONCLUSION:

The algal oil is extracted from 16 microalgae. Biomass by Sharif Hossain & Salleh extraction method using methanol and petroleum ether solvents. The chemical structures of various fatty acids were identified using GC-MS and FT-IR studies. Eight fatty acids were identified ranging between C13 to C24, in which Oleic acid, Stearic Acid and Linoleic Acid were found to be the main constituent for biodiesel production. From Sixteen Microalgal groups, eight Microalgae (*Chlorella vulgaris, Kirchneriella contorta, Phormidium corium, Oscillatoria princeps, Nostoc verrucosum, Spirulina gigantean, Anabaena fertilissima and Spirogyra irregularis*) produced SFA, seven groups (*Chlorella mirabilis, Scenedesmus quadricauda, Scenedesmus armatus, Scenedesmus caudate, Scenedesmus dimorphus, Chlorococcum infusionum* and *Botryococcus braunii*) yields of PUFA and only one microalgae

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(*Phormidium purpurascens*) had MUFA in high percentage.

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