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Research Article

FOURIER TRANSFORM INFRA-RED SPECTROSCOPY AND GCMS DETERMINATION OF FATTY ACIDS PRODUCING MICROALGAE (ANABAENA SP., NOSTOC SP. AND PHORMIDIUM SP.) ISOLATED FROM MARAKKANAM SALT PAN, TAMIL NADU, INDIA

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A B S T R A C T

The pure microalgal species such as *Anabaena* sp., *Nostoc* sp., and *Phormidium* sp., were collected and isolated from marakkanam salt pan (water samples), tamilnadu. The culture was harvested in BG11, microalgae can be used to produce energy in different process; it is one of the best effective methods, to convert the algal oil derivatives in to biodiesel. These selected potential microalgal species were subjected to fatty acids extraction and transesterification to determine the fatty acid compounds by GC-MS and FT-IR analysis for the production of biodiesel. The Palmitic acids, Pentadecanoic acid, Oleic acid, Octadecanoic acid, Eicosedenoic acid etc., were accumulated from the selected micro algal species. The FT-IR spectrum retrieved from the FAME of all the three microalgal strains strongly confirms the presence of hydroxyl group, alkenes group, alkanes group, alkynes group, esters and aromatic groups.

INTRODUCTION

Cyanobacteria are autotrophic and slowly growing photosynthetic bacteria, using solar light to convert Co2 from atmosphere into organic carbon. Chlorophylls, Carotenoids and phycobillins are micro algal pigments that harvest light in the process of photosynthesis. Chlorophylls are primary photosynthetic pigment that contain tetrapyrrole macro cycle rings and are present in various form (Chlorophyll type-a, b, c1, c2, d, f). Cyanobacteria are unicellular or multi-cellular organisms dwelling in different environment such as fresh water, marine water, and surface of moist rocks. Algae are categorized into microalgae and macro algae. The optimum temperature for micro algal growth is usually 20-30°C. Microalgae considered as a rich source of secondary metabolites with potential application in the various fields like pharmacological, medical, aqua culture, agriculture, cosmetics and bio-fuel. Today research is focused on improving synthesis and maximizing production of valuable compound in higher amounts, as a response to stress conditions such as high temperature, high salinity, nutrient starvation and also metal stress. The biodiesel are commonly obtained from various sources like vegetable oil, waste cooking oil and animal fats, fish oil, chicken oil, soybean, sunflower, rice bran, corn oil and algae.

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The microalgae are considered as a second generation feedstock for the production of biofuel, because of their rich oil contents and mass cultivation. Microalgae biomass contains three important components are carbohydrates, lipids and proteins. More amount of the natural oil made by microalgae is in the form of tricylglycerolmolecule. Edwards *et al*., 1998 reported that algae were one of the best sources of biodiesel. Fourier transform infrared (FTIR) spectroscopy helps in rapid determination of lipids and identification of target algae by characteristic of each algal species. In the present study algae growth rate, biomass densities, lipid content of the selected algae have been investigated. FTIR spectroscopy was used for the determination of characteristic spectral signatures of each alga. The transitions in the proteins, carbohydrates, lipids and phosphates were also monitored across the growth period. Lipids were extracted and fatty acid methyl ester (FAME) profiling was carried out using gas chromatography and mass spectrometry (GC-MS).The traditional taxonomic characteristics of micro algae indicated that morphological, biochemical properties used in its identification, the cell size and shape are variable and depends on the nutrition and environmental factors. The traditional identification, differentiation of microalgae is achieved by microscopic and spectroscopic studies.

MATERIALS AND METHODS

Sample Collection

The sample collection was made during winter season in the month of November 2017. 250 ml of water samples were collected from different places of Marakkanam salt pan and immediately water parameters (The temperature, PH, conductance, TDS, DO, salinity and ORP) were analyzed by field portable water analyzing YSI-Instrument. The micro algal samples were then isolated by serial dilution, spread plate and streak plate method for further studies. Based on morphological appearance cyanobacterial isolates were identified as *Anabaena* sp., *Nostoc* sp. and *Phormidium* sp. These isolated Cyanobacterial species were cultured in BG11 medium under 3000 lux light intensity with static condition, for 16 h under illumination and 8 h under darkness. cyanobacterial cultures were harvested approximately after a production period of 15 days and used for GCMS and FTIR analysis.

Fig 1 Map of Tamil Nadu and Marakkanam highlighting the sampling sites

Extraction of Fatty acids from Microalgae

The cells were obtained from 40ml medium containing culture flask, centrifuged at 8000X g for 5 min, at 15c and washed once with distilled water. The fresh culture cells were well homogenized using a mortar and pestle for 20 min at room temperature. Extraction of lipids from wet biomass was performed according to the procedure of Sharif Hossain & Salleh (2008). The algal cells were spread over a clean glass plate for air drying. The dried biomass was mixed with citric acid for making algal beads. Allow it for oven drying at 120c for 1 min. Fatty acid presenting in the algal cell contents were extract 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 the top of the solution and mixed with catalyst (0.30g NaOH and 2ml of methanol) for transesterification process. Then allow that the solution for 16h to settle their sediments clearly.

FTIR studies

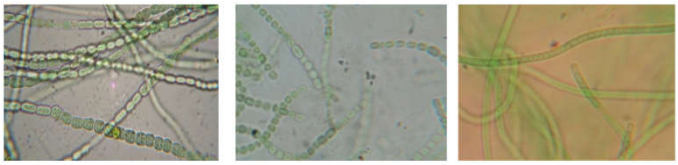
The Perkin Elmer Spectrum1 instrument which is used to record the dried algal biomass (FTIR instrument consists of globar and mercury vapor lamp as sources, an interferometer chamber comprising of KBr and mylar beam splitters followed by a sample chamber and detector. Entire region of 450-4000 cm^{-1} is covered by this instrument. The spectrometer works under purged conditions). Lipid compound fraction was dissolved in KBr or polyethylene pellets and the molecule are transfer to gaseous state, depending on the region of interest.

GC-MS studies

The collected fatty acid samples were processed with JEOL GCMATE II (GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI)). The sample was evaporated in a split less injector at 300°c. The fatty acids were quantified by a gas chromatography. The column (HP5) were fused silica 50m x 0.25 mm I.D. Analysis in 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 ester was identified by GCMS.

RESULTS AND DISCUSSION

The microalgae samples were observed under microscope, photographed and morphologically identified using micro algal such as *Anabaena* sp., *Nostoc* sp., and *Phormidium* sp., were found to be most dominant species in the collection sites.



1.) *Anabaena sp.* 2.) *Nostoc sp.* 3.) *Phormidium sp.*

Fig 2 Morphologically identified microalgae strains from salt pan.

Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR spectra are relation to specific groups. Each peak assigned a functional group. The molecular assignments of bands are based on the published data phytoplankton, bacteria and other biological materials. In this study *Anabaena* sp (1); *Nostoc* sp (2); *Phormedium* sp (3); protein spectra characterized by strong peaks (1) 3416 cm^{-1} protein (Amide I) and 1546 cm^{-1} (protein Amide I I); (2) 3419 cm^{-1} protein (Amide I) and 1639 cm^{-1} (protein Amide I I); 1654 cm^{-1} (amide I) and 1549 cm^{-1} (amide II). These bands were primarily due to C=O stretching and vibration and combination of N-H and C-H starching vibration in amide complexes. Lipid and Carbohydrates were characterized by strong vibration the C-H (1)2854 cm^{-1}; (2)2920 cm^{-1};(3) 2929 cm^{-1} ,C-O-C of polysaccharide at (1)1031 cm^{-1};(2) 1045 cm^{-1} (3) 1034 cm^{-1} respectively (Brandenburg, Seydel), while carbohydrates are the strongest absorbers between 1200 cm^{-1} and 1000cm-1.several other classes of compounds such as Nucleic acid have functional group with absorption bands in the same region of the spectrum. The strongest peaks (1) 1546 cm^{-1}; (2) 1639 cm^{-1} ;(3) 1549 cm^{-1} and (1) 1381 cm^{-1}; (2) 1384 cm^{-1}; (3) 1384 cm^{-1} shows that bending mode of methyl

group of protein. The 1243 peaks shows carboxylic acid present in the algae (Benning *et.al*.,). In this study, the close correlation between peaks and the existences of with band 2 suggested that lipid content very high and also Carbohydrates, Nucleic acid also present in *Nostoc* sp.

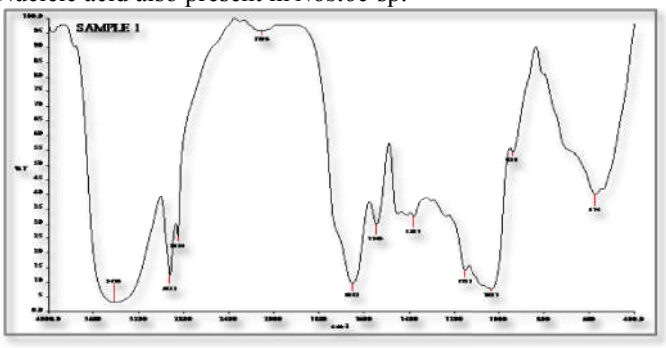


Fig 3 FTIR spectra of *Anabaena* sp

Table 1 Tentative assignment of bands found in FTIR spectra of *Anabaena* sp

S.No	Class of Compound	Absorption cm^{-1}	Intensity	Assignment
1	Carboxylic Acids; Amines; Amides	3416	S, broad	N – H Stretch; N – H symmetric and asym Stretch; O – H Stretch
2	Alkanes and Alkyls; Carboxylic Acids	2922	S	C – H stretch; O – H Stretch.
3	Carboxylic Acids	2854	S, broad	C – H stretch; O – H Stretch.
4	Alkynes	2106	S, sharp	C-H Stretch
5	Amides	1546	M –S	N-H Bend
6	Alkanes and Alkyls	1381	M –S	CH3C-H Bend
7	Alkyl Halides; Ethers;	1152	VS,S	C-F stretch, C-O Stretch
8	Alcohol	1031	M -S	C-O Stretch
9	Alkenes	938	M+S	C-H Bend
10	Alkyl Halides	574	S	C-Br Stretch

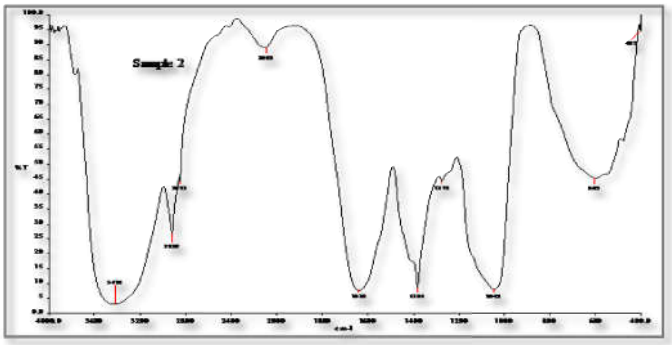


Fig. 4 FTIR spectra of *Nostoc* sp.

Table 2 Tentative assignment of bands found in FTIR spectra of *Nostoc* sp

S. No	Class of Compound	Absorption, cm-1	Intensity	Assignment
1	Carboxylic Acids; Amines; Amides	3419	S, broad	N – H Stretch; N – H symmetric and asym. Stretch; O – H Stretch
2	Alkanes and Alkyls; Carboxylic Acids	2920	S	C – H stretch; O – H Stretch.
3	Carboxylic Acids	2852	S, broad	C – H stretch; O – H Stretch.
4	Alkynes	1639	S, sharp	C= O stretch
5	Amides	1384	M –S	CH3 C- H bend
6	Alkanes and Alkyls	1275	M –S	C – F stretch; =C-O-C symmetric and asym. Stretch; O=C-O-C Stretch.
7	Alkyl Halides; Ethers;	1045	M-S	C – F stretch; C- O Stretch; =C-O-C symmetric and asym Stretch;
8	Alkyl halides	603	S	C – Br stretch.
9	Alkyl halides	405	S	C- I Stretch

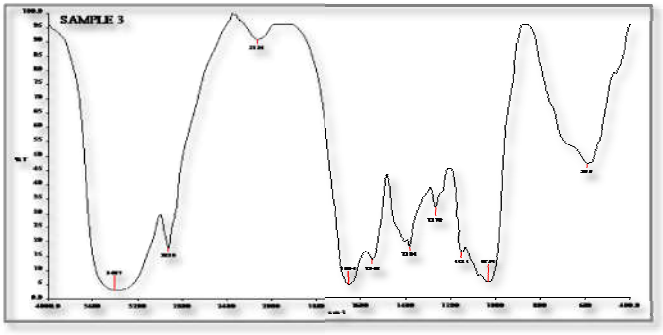


Fig 5 FTIR spectra of *phormidium* sp

Table 3 Tentative assignment of bands found in FTIR spectra of *phormidium* sp

S.No	Class of Compound	Absorption, cm-1	Intensity	Assignment
1	Carboxylic Acids; Amines; Amides	3407	S, broad	N-H Stretch; N-H sym stretch; O-H stretch.
2	Alkanes and Alkyls; Carboxylic Acids	2929	S	C-H Stretch; O-H Stretch.
3	Alkynes	2134	S, broad	C=C Stretch
4	Amides	1654	S, broad	C=O Stretch
5	Amides	1549	M –S	N-H Bend
6	Alkanes and Alkyls	1384	M –S	CH3C-H Bend
7	Alkyl Halides; Ethers;	1270	VS,S	C-F stretch, C-O Stretch
8	Alcohol	1151	M –S	C-O Stretch
9	Alkenes	1034	VS	C-F Bend
10	Alkyl Halides	574	S	C-Br Stretch

GCMS

Gas chromatography is used to identify the biochemical components; there are different types of fatty acids in the microalgae cells. The chromatograms show several compounds at various retention periods. The spectrum data base software installed in GC-MS. Mainly three types of fatty acids such as saturates, monoenes, polyenes found in extracts. The high content of saturated fatty acids (SAF) were observed in *Anabaena* sp (1) **(** Pentadecanoic acid, 13-methyl-, methyl ester -15.93 Retention time ,Palmitic acid -16.55 Retention time); *Nostoc* sp(2 **)(** Cetylic acid- 17.45 Retention time , Oleic acid 19.1 Retention time); *Phormidium* sp (3) (Palmitic acid -16.57 Retention time, Octadecanoic acid -18.33 Retention time). Mono unsaturated fatty acids (MUSA) present in *Anabaena* sp(1) (6,11- Eicosedenoic acid, methyl ester - 20.12 Retention time) *Nostoc sp*(2) (16-Octadecenoic acid, methyl ester - 18.45 Retention time).The high content of poly unsaturated fatty acid (PUFA) noted in the following microalgae *Anabeana* sp (1)(Octadec-9-enoic acid -18.13 Retention time)(3) *Phormidium* sp (Methoxyacetic acid, octadecyl ester -21.57 Retention time).

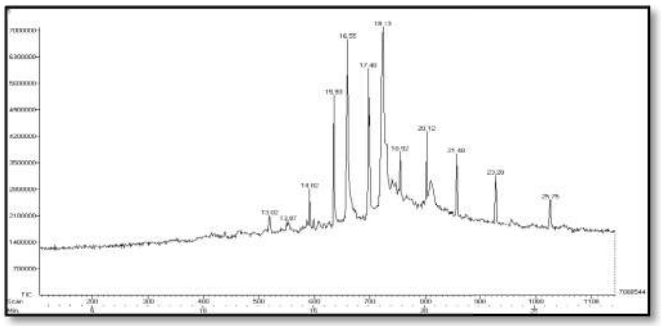


Fig 6 The GC spectrum of *Anabaena sp.*

Table 4 Molecular weight and retention time of saturated fatty acids, Mono unsaturated fatty acids and poly unsaturated fatty acids obtain from GC-MS of *Anabaena sp.*

Retention time	Compound	Chemical formula	Molecular weight
13.02	Cyclo hexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α)-	$C_{10}H_{20}O$	156.2652
13.87	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.329
15.93	Pentadecanoic acid, 13-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.450
16.55	Palmitic acid	$C_{16}H_{32}O_2$	256.43
18.13	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.468
18.92	Pyrimidine 5 ethyl 2-(-4(ethyl cyclo hexyl) phenyl)-	$C_{25}H_{36}N_2O$	294.433
20.12	6,11- Eicosedenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.541

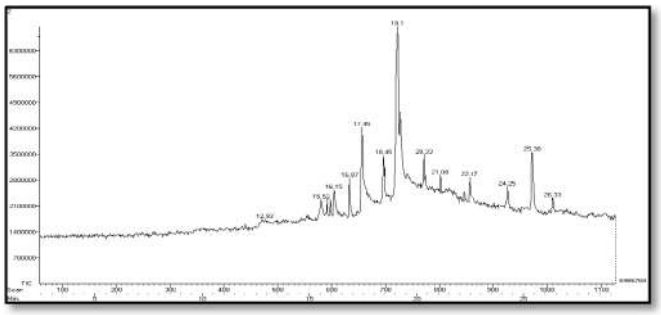


Fig 7 The GC spectrum of *Nostoc* sp.

Table 5 Molecular weight and retention time of saturated fatty acids, Mono unsaturated fatty acids and poly unsaturated fatty acids obtain from GC-MS of *Nostoc* sp.

Retention time	Compound	Chemical formula	Molecular weight
12.93	Allyl (2-methylphenyl) sulfide	$C_{10}H_{12}S$	164.266
15.53	Quinolin , 5-nitro-, 1-oxide	$C_9H_6N_2O_3$	190.158
16.15	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}$	206.281
16.87	Flavone	$C_{15}H_{10}O_2$	222.243
17.45	Cetylic acid	$C_{16}H_{32}O_2$	256.43
18.45	16-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.495
19.1	Oleic acid –	$C_{18}H_{34}O_2$	282.468
22.47	Tricosan-12-ol	$C_{23}H_{48}O$	340.636
24.25	Quinazolin-4(3H)-one,3(3-methoxyphenyl)-2-(2-phenylethenyl)-	$C_{20}H_{14}N_2O$	298.345

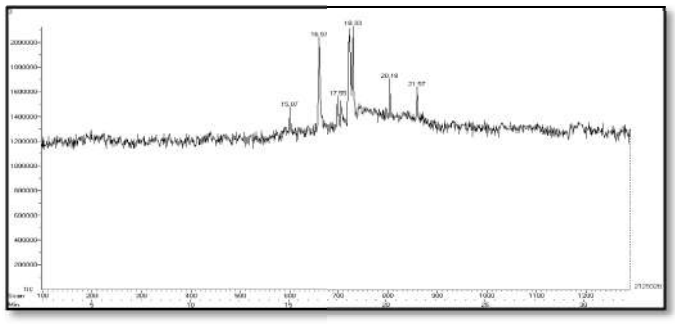


Fig 8 The GC spectrum of *Phormidium* sp

Table 6 Molecular weight and retention time of saturated fatty acids, Mono unsaturated fatty acids and poly unsaturated fatty acids obtain from GC-MS of *Phormidium* sp

Retention time	Compound	Chemical formula
15.07	Flavone	$C_{15}H_{10}O_2$
16.57	Palmitic acid	$C_{16}H_{32}O_2$
18.33	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$
20.18	Nonadecane-2,4-dione	$C_{19}H_{36}O_2$
21.57	Methoxyacetic acid, octadecyl ester	$C_{21}H_{42}O_3$

CONCLUSION

Microalgae are play an important role in commercial production of biodiesel and the research work confirm that tricylglycerolmolecule obtained from microalgae. The simple and reliable authentic methods are used in extraction and purification of fatty acids from the microalgae. The lipid derivatives are characterized by GC- MS and FT-IR. The selected three strains have good application in the field of biofuel. The regular transesterication process is the best choices to reduce the production cost. The improving of high quality fatty acid can be achieved via genetic and metabolic engineering.

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