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Biofuel Production from Microalga *Nannochloropsis oculata* using Dairy Industry Waste Water

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Abstract : In view of ever increasing global requirement for energy, there has been sizeable interest in escalating renewable biologically produced fuel. Today's petroleum fuels are unsustainable because of depleting supplies and the contribution of fuels to the accumulation of carbon dioxide in the atmosphere. We need substitute transportation fuel to prevent carbon dioxide accumulation in the environment. Microalgae are one of the best producing oil crops which converts carbon-dioxide to biofuels as well as involved in bioremediation process. In this present study marine microalga *Nannochloropsis oculata* strain was collected from CMFRI Chennai and cultivated with f/2 medium enriched with dairy industry waste water for cultivation. The lipid extraction was studied using solvent. The functional components in lipids were studied using GC-MS and FT-IR. This study proved to be an efficient tool for useful utilization of Industrial effluents by microalgae nurturing will pave the way for phycoremediation which will pilot to the manufacturing of eco-friendly biofuel.
Keywords: *Nannochlorospsis oculta*, GC-MS, FT-IR, Dairy waste water.

1. Introduction

The use of gasoline based fuels has become more constrained due to the dilapidated supply of fossil fuels and the demand for diminution of green house gas emissions[1] which causes global warming and there is a urgent need for renewable source which will be an alternate source for fossil fuels to avert the looming oil crisis and also mounting oil prices there is an persist on for an alternate energy source have attracted contemporary and universal interest in biofuel derived from microalgae. Microalgae signify an astonishing diverse but highly focused group of microorganisms adapted to different habitat. Microalgae are novel aquatic micro-organisms which has higher fuel yield potential than the traditional fuel crops. Several microalgae species have high biomass production rate compared to terrestrial plant[2,3]. Microalgae are simple photosynthetic organisms which can survive in fresh water as well as sea water, the microalgae has the ability to store high amount of energy rich triacylglycerol (TAG), Microalgae based system can drastically decrease both organic matter and nutrients in dairy industry effluents at nominal energy costs. Photoautotrophic nurturing of Microalgae is broadly acknowledged as a more cost-effectively feasible technique for large scale algal biomass production. Waste water can be used as a sustainable development medium for the algal feed stock. The utilization of microalgae in management and recycling of waste water has fashioned enormous deal of significance because of excessive biomass production at affordable cost. Most of the algal species such as *Scenedesmus obliquous*[4] and *Chlorella pyrenoidosa* [5]can be used as a valuable implementation for waste water treatment. Use of microalgae can eradicate chief inorganic contaminants like nitrogen (50%) and phosphorous (90%) from waste water[6]. The

thriving application of algal biomass for biofuel manufacturing will basically depend on the development and harvesting of algal biomass at cheaper cost[7]. The current study is an effort to decrease the waste water pollution and produce the algal biomass from nutrient rich dairy industry waste water.

2. Materials and Methods

2.1 Sample Collection

The waste water samples were collected from dairy plant in kanchipuram, Tamil nadu, India. The industrial waste water was collected in aseptic bottles and stored at 4^0C before use

2.2 Collection of Microalgae

Microalgae *Nannochloropsis oculata* strain number CCMP525 was delivered Centre Marine Fisheries Research Institute, Chennai, Tamil nadu

2.3 Biomass production and Lipid extraction

Indoor algal stock cultures were maintained in special air conditioner room. Stock cultures were kept in 2 liter culture flasks. 100 ml of *Nannochloropsis oculata* was inoculated with seawater and enriched by dairy industrial effluent was kept in a 250 ml Erlenmeyer flask and kept in the shaker for 30 minutes for a period of 10 days and the optical density was measured on daily basis at 540 nm. The lipid extraction was carried out using partial polar and non polar solvents; the solvents used are petroleum ether, Acetone, Methanol and Acetonitrile. The above solvents were taken in triplicates for lipid extraction and the extraction was carried out using Bligh and dyer[8] methods. The dairy industrial effluent harvested on the 8^{th} day was centrifuged at 10,000 rpm for 20 minutes at 5^0C. The supernatant was discarded and pellet was dried in oven for 2 hrs at 70^0C. Petroleum ether was added in equal ratio to biomass and transfer to a separating funnel and leaves it for 24 hrs. After 24 hrs the layers were separated and the lower organic lipid layer was transferred to a fresh vial. The algal lipid was subjected to trans esterification process dried algal biomass of (12.5mg) was placed in a glass test tube and mixed with 1ml of methanol and 0.25 gram of NaOH. The reaction mixture was heated at 90°C for 40 min and the samples were mixed well during heating. After the reaction was completed, the tubes were allowed to cool at room temperature. Samples were centrifuged at 3000 rpm for 10 min. To accelerate phase separation and the sample's which contains fatty acid and glycerol which separated after saponification process; The Organic layer that contained biodiesel (FAME) was collected. After the manual shaking the biomass solution was kept for 16 h to settle the biodiesel and sediment layers clearly, the algal oil in the upper layer was separated and the rest of the components like glycerol and other pigments settle down at the bottom[9] Conversion of total lipid content in the algal biomass was calculated using the equation given below (Yanqun Li, 2008).

$$P_{lipid} gL^{-1} day^{-1} = C_{lipid}(g/g) * DCW(g/L) \div Time\ (day)$$

Gas chromatography and Mass Spectrometry

The separated lipids were subjected to GCMS studies; the study was carried out in JEOL GCMATE II GC-MS instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI). The lipid fraction was re-suspended in n-Hexane and applied to silica gel column chromatography. Aliphatic hydrocarbon tiny proportion passes throughout the column fatty acids and carotenoid fractions were trapped and passed through this 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 recorded and compared with the Fossil diesel and gasoline oils were the methyl esters of fatty acids were quantified by a gas chromatograph[10]. The column (HP5) was fused silica 50m x 0.25 mm I.D with 30 m length. Analysis conditions were 20 minutes at 50°C the 3°/ min to 235°C for column temperature, 250°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 recognized by gas chromatography coupled with mass spectrometry.

2.4 FTIR Spectrometry analysis

Fourier Transform Infra-Red spectrometer of Perkin Elmer model spectrum-IPC was used. The thallium

bromide pellets are taken for correction of background spectrum[11]; The FTIR spectra are recorded over a range of wave number from 4000 to 450 cm^{-1}. Each sample was analyzed in triplicates

3. Results and Discussion

3.1 Algal growth on dairy waste water and diminution of effluence load

Algal growth was examined in terms of increase in the optical density at 540 nm at different concentrations of dairy waste water. The results showed algal growth increased with the concentration of dairy waste water fraction (10-65% v/v). The waste water dependent growth of algae depends on the growth response of F/2 medium combined with dairy waste water; the growth of algae is stimulated by 25% - 65% concentration of dairy waste water. The results showed that the algal growth continued up to 16^{th} day of treatment, the lower concentration of the dairy waste water does not support the algae growth due to low level of nutrients in the supplied medium. The algal cells used the nutrients present in the waste water for their growth resulted in gradual increase in algal biomass subsequently there is gradual diminution of reasonable load of nutrients, the algal cells where precise on the 4^{th}, 9^{th} , and 14^{th} day of alga growth. The 9^{th} day of alga growth showed marked diminishing in the level of nitrates, fluoride, phosphate and ammonia concentration from 46.7 to 4.3 mg/L. However on the 14^{th} day there was a preferential removal of nitrate over nitrite up to 9^{th} day These attributes make microalgae an efficient and eco-friendly tool to remediate the waste water at low cost

3.2 Algal biomass and algal oil production

The algal biomass was measured with respect to fresh weight and dry weight derived 65% concentration of waste water after 9^{th} and 14^{th} day of experiment showed better algal biomass of *Nannochloropsis Oculta* on the 9^{th} day (f.wt 7.9 g/l) and on the 14^{th} day (f.wt.6.4 g/l), a higher biomass yield on 9^{th} day is due to the exponential phase of cell growth and the cell biomass observed on the 14^{th} day showed that the cell growth is entering in the stationary phase showed in (Table 1).

Table 1 Algal biomass on the 9^{th} and 10^{th} day

Sample	Fresh Weight (g)	Moisture Content %	Weight of Dry biomass (g)	Biomass
Batch 9^{th} day	7.9	53	4.8	3.3
Batch 14^{th} day	6.4	50	4.1	2.1

3.3 FTIR analysis of biofuels

The FTIR spectrum analysis of biodiesel, the transmittance peak appeared in various distinct regions. The main characteristics of IR spectra of carboxylic compounds is the strong stretching bonds C=O stretching absorption band in the region of 1320 -1000 cm^{-1}, in the case of esters the bands appeared in the region of 1760 – 1400 cm^{-1}, C-H stretching absorption band is found in the region of 1370 – 1350 cm^{-1} shows methyl groups , In microalgae the stretching of C-H and O-H bands showed in the region of 3400 cm^{-1} indicated the carboxylic acids. As many algal species have been found to grow rapidly and produce substantial amounts of lipids and are thus belongs to as microalgae (Sanniyasi Elumalai, 2014). Figure.1 shows the FTIR analysis of biodiesel.

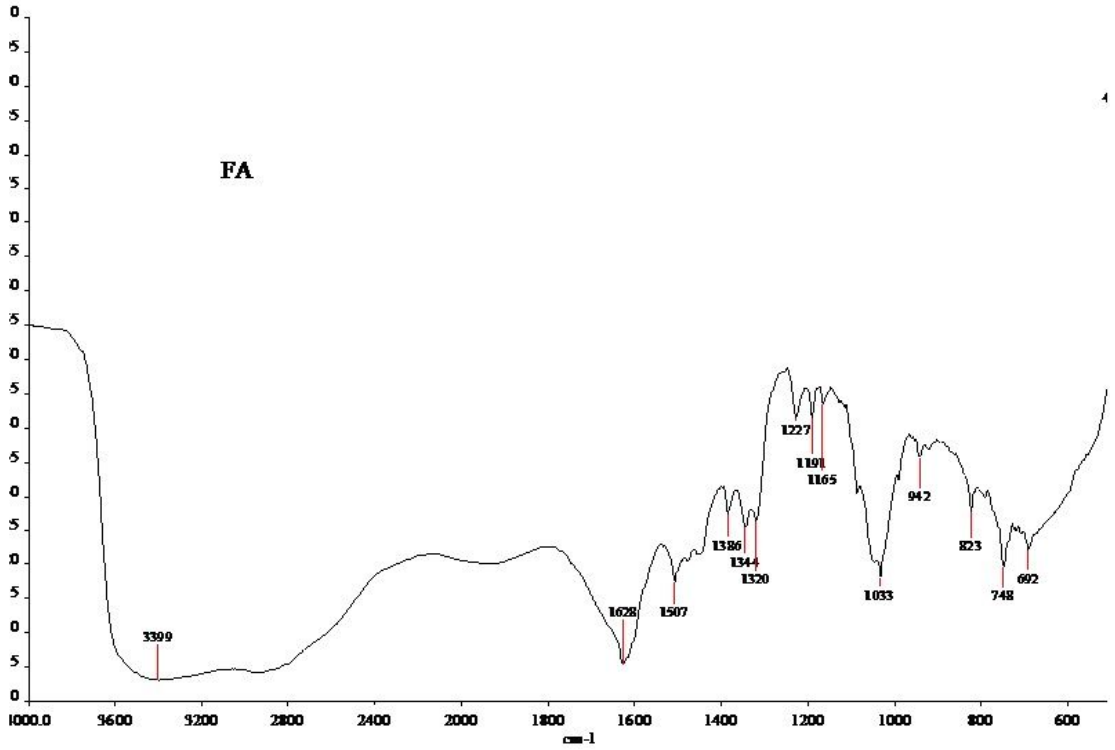

Fig 1. Shows the Peak Values of FTIR spectra analysis

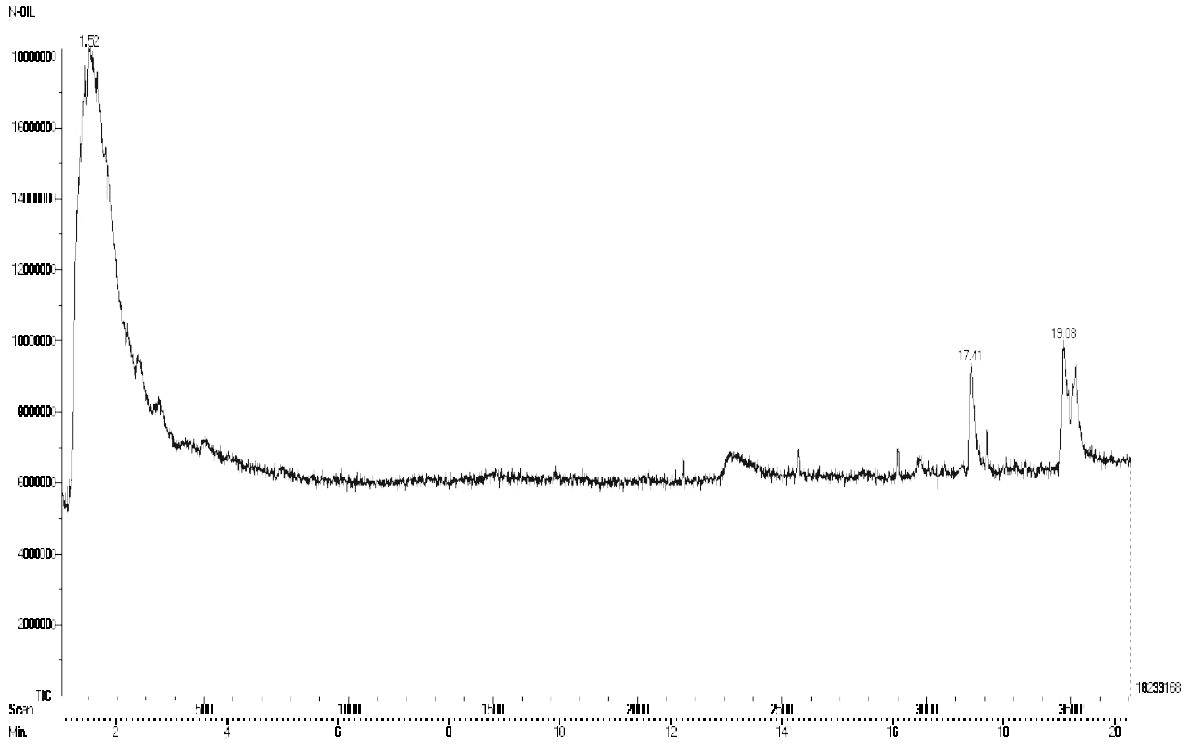

Fig 2. GCMS spectrum of Algal FAME

3.4 Gas Chromatography Mass Spectroscopic Analysis

GCMS was employed to study the Algal FAME using JEOL GC MATE II data system was used the time range was 60 to 600 ionizations and it generated three different peaks the (Fig 2) Shows the GCMS peak values and their respective retention time, (Table 2) Shows the composition of fatty acid methyl esters in microalgae *Nannochloropsis Oculta*

Table 2 Composition of fatty acid methyl esters present in Algal FAME

Peak No	Retention Time (min)	Description of the ester	Chemical formula	Scan	Ions
1	1.52	2-Propenoic acid 4-Methylpentyl ester	$C_9H_{18}0_2$	102	2761
2	17.41	Dodecanoic acid, 10-methyl ester, methyl ester	$C_{14}H_{28}0_2$	3153	2410
3	19.08	16-Octadecenoic acid, methyl ester	$C_{19}H_{36}0_2$	3474	2419

4. Conclusion

This study demonstrate with the aim of dairy industry waste water can be utilized as nutrient medium for algal biomass production, during the growth *N.Oculta* on dairy waste water showed promising signs in the reduction of Nitrogen and Phosphorous which are the main foundation for water contamination, the resulting biomass on the 9^{th} day yield 41% (w/w) lipid found to be budding source of bio-fuels is evident from the results of FTIR and GCMS. The present study reveals that this alga can be used for treating waste water and as well as for the production of biofuels.

Conflict of interests

The authors certify that there is no conflict of interests with any financial organization regarding the material discussed in the paper.

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