Int. J. Chem. Sci.: 14(4), 2016, 3249-3255
ISSN 0972-768X
www.sadgurupublications.com

BIOFUEL PRODUCTION FROM MARINE MICROALGAE USING PAPER AND PULP INDUSTRY WASTE WATER

P. GURUMOORTHY[a*] and A. SARAVANAN[b]

[a]Department of Biotechnology, Arulmigu Meenakshi Amman College of Engineering, KANCHIPURAM (T.N.) INDIA
[b]Department of Chemical Engineering, Hindustan University, Padur, CHENNAI (T.N.) INDIA

ABSTRACT

Paper industry waste effluents are the chief consumers of water resources and these industries release enormous amount of bilge water to nearby water streams. The present investigation work delves into Paper and pulp industry waste effluents are used for the Biofuels production from Marine microalgae *Nannochloropsis oculata* was grown in artificial sea water medium enriched with pulp and paper industry waste water for cultivation, with the aim to achieve significant biomass and biofuels production. The lipid extraction was studied using solvent. The functional components in lipids were calculated using GC-MS and FT-IR. This study proved to be a competent tool for useful exploitation of Industrial effluents by microalgae fostering will pave the way for eco-friendly biofuels.

Key words: *Nannochloropsis oculata,* GC-MS and FT-IR, Pulp and paper industry, Biofuels.

INTRODUCTION

Escalating population and candid urbanization has formed stern problems of energy requirement. Due to a rapid hike in energy utilization, it is likely that there would be decline in oil reserves by 2050. Constant use of fossil fuels resulted in consequence on atmosphere by escalating green house gas emission leads to climatic changes. Therefore, there is a existing demand to find out the substitute eco friendly fuel against petro diesel. Global warming and energy crisis due to the ignition and exhaustion of conventional fossil fuel have called for substantial interests and efforts in exploring and budding economically and environmentally sustainable renewable fuels. Lipid derived biodiesel from microalgae, the third generation biofuels, provides a hopeful alternative to petroleum fuels that is potentially sustainable, carbon neutral, and can have an extensively smaller footprint in land area demand, compared to plant-based first and second generation biofuels.

[*]Author for correspondence; E-mail: biogp77@gmail.com; Ph.: +91 9585732613

Production of pulp and paper requires vast amounts of water. The water contains nutrients brought to the factory by means of the wood, and wastewater treatment plant is an essential part of a pulp/paper factory. Algae may offer a cost-effectively and environmentally sustainable way of utilizing the nutrients this was reported by Gurumoorthy and Saravanan[1]. However, the cultivation might become lucrative if the biomass contains a high-value component like a polyunsaturated fatty acid. *Nannochloropsis Oculata* is one of the potential genera of microalgae having higher, biomass and lipid productivities. It is also a significant genus which grows in marine water with some additional nutrients, hence with a lesser footprint on freshwater utility and nutrients for open mass development in the future was observed by Sukenik et al.[2-5] and Biondi et al.[6] Further, it is a competent carbon-fixing agent to combat global warming by sequestering CO_2, which is an invigorated attention besides fabrication of valuable and energy products through green processes, was reported by Rodolfi et al.[7]

In the present study *Nannochloropsis Oculata* was cultivated in artificial sea water medium enriched with the effluent from paper and pulp industry waste water. Our main objective is to use the waste water nutrients to increase the biomass and simultaneously improve the waste water management practices in the industry.

EXPERIMENTAL

Materials and methods

Cultivation of algae

The *Nannochloropsis oculata* strain CCMP525 was used for the experiments, the *Nannochloropsis oculata* was grown in 250 mL Erlenmeyer flask in artificial sea medium along with the waste effluent from the paper and pulp industry under pH 8.1 under continuous lightening and the algae were grown in batch mode and the algae culture was bubbled with air for 72 hrs and then with 0.3% CO_2 for 24 hrs.

Optical density measurements

The growth of *Nannochloropsis oculata* was monitored by the increase in optical density of the culture at 680 nm and by the accumulation of dry biomass.

Lipid extraction

Lipid were extracted from the cultures on the 10^{th} - 15^{th} day of growth algal cells were harvested by centrifugation at 11000 rpm for 10 min at room temperature isolated using modified Folch procedure[8]. Accurately weighed algal biomass were weighed and extracted with 200 mL of chloroform was added and kept in an ultrasonic bath for 20 min. The chloroform solvent was filtered through Whatman No. 1 paper and the filtered supernatant containing the extracted lipids were washed with water and was transferred to a separation funnel. The chloroform extracts of lipids was heated at 61^{o}C for evaporation of chloroform and suspended solids are removed, by adding 2 g of Noah in 110 mL methanol. 69 mL of lipid extract was trans-esterified. The lower organic phase was collected was transferred to a clean fresh pre-weighed vial. Conversion of total lipid content of the algal biomass was calculated using the conversion coefficient 0.9 as suggested by Shariff Hossain[9].

Actual lipid content (g) = Total lipid (g) * Conversion efficiency (0.9)

$$\text{Total Volume of pure bio oil (mL)} = \frac{\text{Actual lipid content (g)}}{\text{Density of pure bio oil (g/mL}^{-1})}$$

Biodiesel characterisation

GCMS and FTIR analysis were employed to study the Transesterification reaction. JOEL GC-MATE II data system equipped with a double focussing and high resolution impact helium gas was used as carrier. The time range was 60 to 600 ionizations. The scan range of FTIR spectrometry was found to be MIR 500-3500 cm^{-1} and the resolution was 1.0 cm^{-1}.

RESULTS AND DISCUSSION

Effect of paper and pulp waste water on microalgae growth

Algal growth was monitored in terms of increasing optical density at 680 nm at different concentrations (10%, 25%, 50%, 75% and 100%) of paper and pulp industry waste water showed the algal growth increased with increasing concentrations of waste water fraction (10 = 80%, v/v), the microalgae utilizes all the nutrients present in the paper and pulp industry waste water, the concentration played significant role in increasing the biomass production the paper pulp industry waste water lacked the important nutrients such as nitrogen and phosphorous we need to add trace element to compensate the loss of trace elements. The high ammonium and manganese concentrations present in paper and pulp waste water would be toxic to algae therefore the waste water needs to be diluted to obtain a suitable growth medium. The *Nannochloropsis oculata* is a marine species and therefore its ability to grow in paper and pulp industry waste water based medium shows great plasticity in the osmo-tolerance of the algae which is stimulated by the 10% - 80% concentration of paper and pulp industry waste water. The results showed significant algal growth starts and continued till 12th day of the treatment. However the lower the concentration of waste water does not support algal growth due to low levels of nutrients.

Biomass and bio oil extraction

Algal biomass was calculated in terms of fresh weight and dry weight obtained from 80% concentration of paper and pulp industry waste water after the 10th and 15th day of experiments, the results showed better biomass yield of *Nannochloropsis oculata* on the 10th day was (f.wt. 7.7 g/L) and on the 15th day was (f.wt 6.3 g/L) is shown in the Table 1. A comparison of the bio oil yield revealed higher biodiesel yield on10th day might be due to exponential phase of cell growth and optimum biomass and on the 15th day of the study cleared showed lower levels of biodiesel yield due to cell growth entering the stationary phase.

Table 1: Showing the yeld of biodiesel on 10th and 15th day culture

Sample	Fresh weight	Moisture content	Weight of dry mass	Lipid content
Batch 10th day	7.8	52	3.9	42%
Batch 15th day	6.4	51	3.5	40%

GCMS and FTIR

The biodiesel samples were injected to GCMS and Table 2 corresponding monoglycerides, triglyceride and methyl esters peak values were obtained Fig. 1.

Table 2: Composition of FAME in *Nannochloropsis Oculata*

Peak No.	Retention time (min)	Description of the ester	Name of the acid	Chemical formulae	Scan	Ions
1	15.00	Tridecanoic acid 12 methyl ester	Pentadecanoic acid	$C_{15}H_{30}O_2$	558	1716
2	17.52	Pentadecanoic acid 14 methyl ester	Margaric acid	$C_{17}H_{34}O_2$	660	3262
3	19.42	10 Octadecanoic acid methyl ester	Nonadecylic acid	$C_{19}H_{36}O_2$	746	3223
4	21.03	Mono(2,2,6,6-tetramethyl-4-piperidinyl) ester	Decanedioic acid	$C_{19}H_{24}NO_4$	799	2535

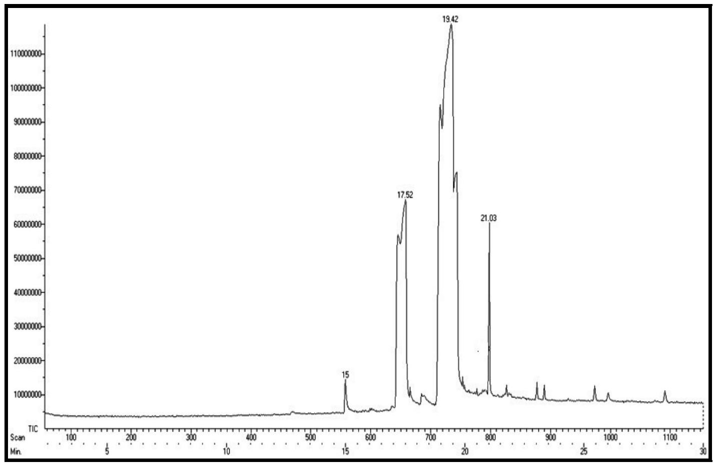

Fig. 1: GCMS Spectrum of *Nannochloropsis Oculata*

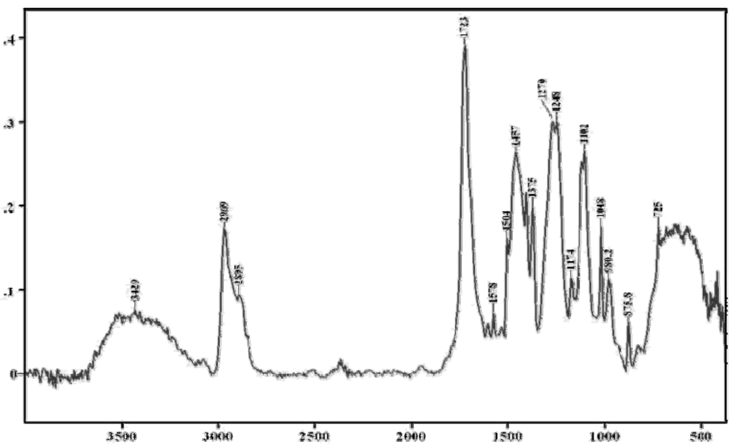

Fig. 2: FTIR Spectrum of *Nannochloropsis Oculata*

The FTIR analysis showed strong signals were identified between wave numbers 1102 cm^{-1} and 1723 cm^{-1}, which confirmed the presence of fatty acid methyl esters. The group of carboxylic acid compounds are found between 2895 cm^{-1} and 2969 and also aliphatic chloro and fluoro compounds were found between 725 cm^{-1} and 1018 cm^{-1}. The presence of alcohols with OH and CO stretch were also seen Fig. 2.

CONCLUSION

This study demonstrates that the paper and pulp industry waste water can be successfully utilized as nutrient resource and cultivation of *Nannochloropsis oculata* was promising in paper and pulp industry waste and the resulting algal biomass on the 10th day of treatment exhibiting 42% (w/w) lipid found to be prospective source of biodiesel which is apparent from the GCMS and FTIR analysis. These results suggested that the pulp and paper industry waste water can be utilized for the biofuels production and phycoremediation.

Conflict of interests

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

REFERENCES

1. P. Gurumoorthy and A. Saravanan, Biofuel Production from Microalga Nannochloropsis Oculata using Dairy Industry Waste Water, Int. J. ChemTech. Res., **9(5)**, 346-351 (2016).
2. A. Sukenik, O. Zmorab and Y. Carmeli, Biochemical Quality of Marine Unicellular Algae with Special Emphasis on Lipid Composition, II. Nannochloropsis Sp. Aquaculture, **117**, 313-326, Elsevier Science Publishers B.V., Amsterdam (1993).
3. A. Sukenik, Ecophysiological Considerations in the Optimization of Eicosapentaenoic Acid Production by Nannochloropsis Sp. (Eustigmatophyceae), Bioresour. Technol., **35**, 263-269 (1991).
4. A. Sukenik and Y. Carmeli, Lipid Biosynthesis and Fatty Acid Composition in Nannochloropsis Sp. (Eustigmatophyceae) Grown in a Light-Dark Cycle, J. Phycol., **26**, 463-469 (1990).
5. A. Sukenik, Y. Carmeli and T. Berner, Regulation of Fatty Acid Composition by Irradiance Level in the Eustigmatophyte Nannochloropsis sp. J. Phycol., **25**, 686-692 (1989).
6. N. Biondi, N. Bassi, G. C. Zittelli, D. De Faveri, A. Giovannini, L. Rodolfi, C. Allevi, C. Macrì and M. R. Tredici, Nannochloropsis sp. F & M-M24: Oil Production, Effect of Mixing on Productivity and Growth in an Industrial Wastewater, Environ. Prog. Sustain. Energy, **32**, 846-853 (2013).
7. L. Rodolfi, G. C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini et al., Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor, Biotechnol. Bioengg., **102(1)**, 100-112 (2008).

8. J. Folch, M. Lees and G. H. Sloane Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, J. Biol. Chem., **226**, 497-509 (1957).

9. A. B. M. Shariff Hossain, A. Saleh, A. N. Boyce, P. Chowdhury and M. Naqiuddin, Biodiesel Fuel Production from Algae as Renewable Energy, Am. J. Biochem. Biotechnol., **4**, 250-254 (2008).

Revised : 28.11.2016 *Accepted : 30.11.2016*