International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Precocious Study of Micro Algae for Biofuels of Aundha Region Dist. Hingoli

R. B. Borse[1], Pathan A. A.[2]

[1]Nagnath Art, Commerce and Science College Aundha (Nag.) Dist. Hingoli (MS), India

[2]Vasantrao Naik, Science College, Shahada Dist. Nandurbar-425409 (MS), India

Abstract: ***A survey of microalgae biodiversity in Aundha region, identified, investigated for the production of a number of different biofuels including biodiesel, dried biomass, bio-oil and biogas. High oil yielding species abundantly distributed naturally in this region. The species were cultured in BBM and BG-11 media to obtain pure clones and biofuels extracted by the Bligh and Dyer method. Microalgae are photosynthetic microorganisms that grow rapidly due to their simple structure. They can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner.***

Keywords: Biofuels, biodiesel, microalgae, environment and lipid

1. Introduction

Concerns about shortage of fossil fuels, increasing crude oil price, energy security and accelerated global warming have led to growing worldwide interests in renewable energy sources that is increasingly being used worldwide is biodiesel. India is one of the fastest growing economies in the world. Energy is a critical input for socio-economic development. The energy strategy of a country aims at efficiency and security and to provide access which being environment friendly and achievement of an optimum mix of primary resources for energy generation. Fossil fuels will continue to play a dominant role in the energy scenario in our country in the next few decades. However, conventional or fossil fuel resources are limited, non-renewable, polluting and, therefore, need to be used prudently. On the other hand, biofuels are indigenous, non-polluting and virtually inexhaustible. India is endowed with abundant renewable energy resources. Therefore, their use should be encouraged in every possible way.

Biofuels are derived from renewable bio-mass resources and, therefore, provide a strategic advantage to promote sustainable development and to supplement conventional energy sources in meeting the rapidly increasing requirements for transportation fuels associated with high economic growth, as well as in meeting the energy needs of India's vast rural population. Biofuels can increasingly satisfy these energy needs in an environmentally benign and cost effective manner while reducing dependence on import of fossil fuels and thereby providing a higher degree of National Energy Security.

The least environment friendly, bio degradable and non toxic fuel that has a low emission profile, making less harmful to the environment compared with petroleum-based fuel. Biodiesel does not contain sulfur or aromatics. Biodiesel usage results in a substantial reduction of carbon monoxide, unburned hydrocarbons and particulate matter. A U.S. Department of Energy study showed that the production and use of biodiesel resulted in a 78.5% reduction in carbon dioxide emissions compared to petroleum diesel. Additionally, biodiesel has a positive energy balance.

Current feeds tocks of commercial biodiesel are soybean, canola oil, animal fat, palm oil, corn oil, waste cooking oil, and Jatropha oil. Biodiesel may be directly used in existing diesel engines or mixed with petroleum diesel to create a biodiesel blend.

Microalgae, recognised as one of the oldest living organisms, are thallophytes (plants lacking roots, stems, and leaves) that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells (Brennan, L., 2010). While the mechanism of photosynthesis in these microorganisms is similar to that of higher plants, they are generally more efficient converters of solar energy because of their simple cellular structure. Recent advances in bio resource studies show that microalgae, a single-celled photosynthetic organism that contains a large amount of fatty acids, may be a promising source for biodiesel. Studies have shown that it gives the highest yield per hectare compared to oil crops (Table 1), has rapid growth rates, and could easily double their biomass within 24 hours.

Some microalgae grow quickly in mineral rich waters with doubling time as short as 3.5 hours. their dry biomass contains up to 70% lipid. This makes microalgae a more efficient oil producer than terrestrial plant sources. As an aquatic species, algae do not require land for cultivation and will not compete with agricultural commodities for growing space. In fact, algae cultivation facilities can be built on marginal land that has few uses. Water used in algal cultivation can be fresh water or saline with salt concentrations up to twice that of seawater. This means that algae need not compete with other users of fresh water. Algae have a greater capacity to absorb carbon dioxide (CO2) than land plants, and are not prone to photosynthetic inhibition under conditions of intense sunlight. After oil extraction from algae, the remaining biomass fraction can be used as a high protein feed for livestock. This gives further value to the process. It also reduces wastes.

2. Materials and Methods

Algal samples collected and transported to the laboratory for analysis. Algal species identification, composition and abundance was quantified under an inverted microscope at 100x and 150x magnification using standard keys of Prescott.

Dominant algae species were isolated and inoculated in 100 ml Bristol's media in 75 cm^2 vented culture flasks and maintained under 12h light: 12h dark photoperiod at 26^0C. Algal biomass was observed at 680 nm using DU 640B spectrophotometer for exponential growth. After fourteen to fifteen days, the algal cells were harvested by centrifugation (6000 rpm, 6 min) and screened for lipid/oil content by a procedure paper chromatography. Briefly 200 ml of the algal culture was centrifuged to form an algae pellet. Lipids were extracted by adding 50ml of chloroform/methanol and the extract placed in an orbital shaker overnight at room temperature. The mixture was then filtered through glass wool and the filtrate transferred into separating funnel. Resultant residue was recovered and lipids re-extracted with 50 ml of chloroform/methanol. The combined filtrate was washed by addition of 0.2 volumes of 0.5% sodium chloride, shaken well and left to stand for 1 hour for phase separation. The lower phase (chloroform phase) containing lipids, was recovered and placed in a dry, pre-weighed volumetric flask. Chloroform was evaporated in an oven at 55^0C The percentage lipid content was determined as,

$$\text{Lipid content }\% = \frac{\text{weight of lipid in grams}}{weight\ of\ sample\ in\ grams} * 100$$

The culture media was prepared by chemicals.

Bristol's Medium for Algae (mg/liter)	
NaNO3	250 mg
K_2HPO_4	75 mg
KH_2PO_4	175 mg
$CaCl_2$	25 mg
NaCl	25 mg
$MgSO_4{\cdot}7H_2O$	75 mg
$FeCl_3$	0.3 mg
$MnSO_4{\cdot}4H_2O$	0.3 mg
$ZnSO_4{\cdot}7H_2O$	0.2 mg
H_3BO_3	0.2mg
$CuSO_4{\cdot}5H_2O$	0.06 mg

3. Results and Discussion

The result of four microalgae species with high lipid content abundantly distributed in the Aundha region lakes. There are four species named as *Scenedesmus spp*, *Chlorella spp*, *Euglena acus*, and *Nitzschiza spp*. These all four species successfully cultured in laboratory, grown in Bristol's medium under 12:12h dark: light cycles. The peak lipid content ranged from 1.5 – 11.6% of algal biomass . *Chlorella* species showed the highest oil yields (12.7%) followed by *Euglena acus* (6.78%), *Scenedesmus acuminatus* (1.47%). and *Nitzschia* (3.62%). Previous study done on *Chlorella protothecoides* spp under nitrogen limitation showed a 46.1% lipid content (Xiufeng et al., 2007). However, (Rodolfi et al., 2009.) observed that nitrogen deprivation did not have a great impact on fresh water microalgae species like *Chlorella* and *Scenedesmus* spp which had lipid content of 19.6% and 21.1%, respectively. Similar results have been observed from algae grown in municipal waste waters in California, USA (Mulbry, et al., 2008). In comparison, total lipid content of pure *Scenedesmus* and *Chlorella* cultures have been reported to range from 12-45% (Thompson, G A., 1996). Nonetheless, much higher algal lipid productivities were envisioned than observed in this study.

The lipid extracts were separated by thin layer chromatography, at least four bands appeared. The Retardation factors of the bands indicated the presence of triacylglycerols, free fatty acids, diacylglycerol and monoacylglycerol, when compared with the standard rfs values of neutral lipids.

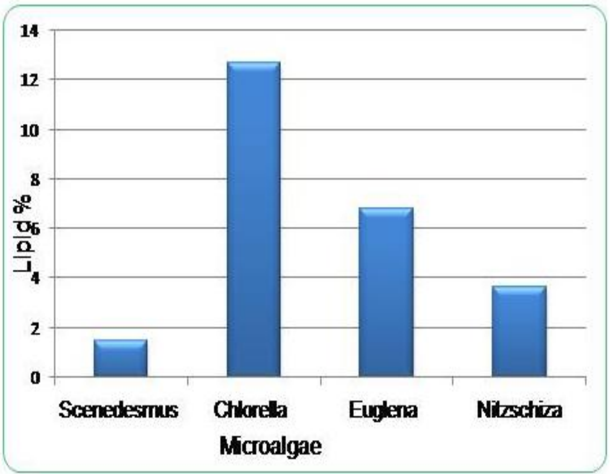


Figure 1: Percentage of lipid productivity in four selected microalgae

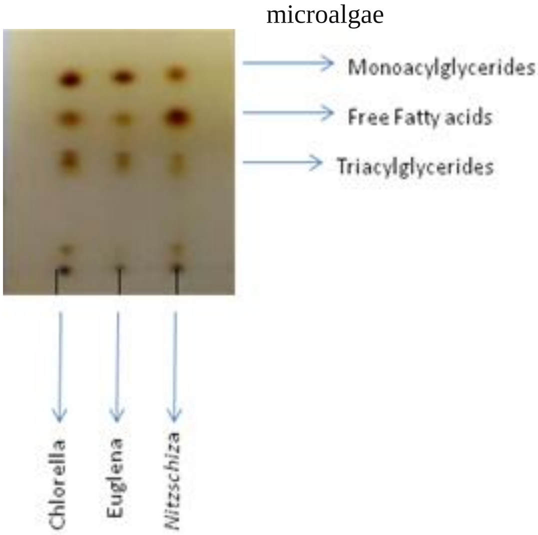


Fig. 2: Thin layer chromatogram showing present in *Nitszshia, Chlorella and Euglena species*

4. Systematic account of microalgae

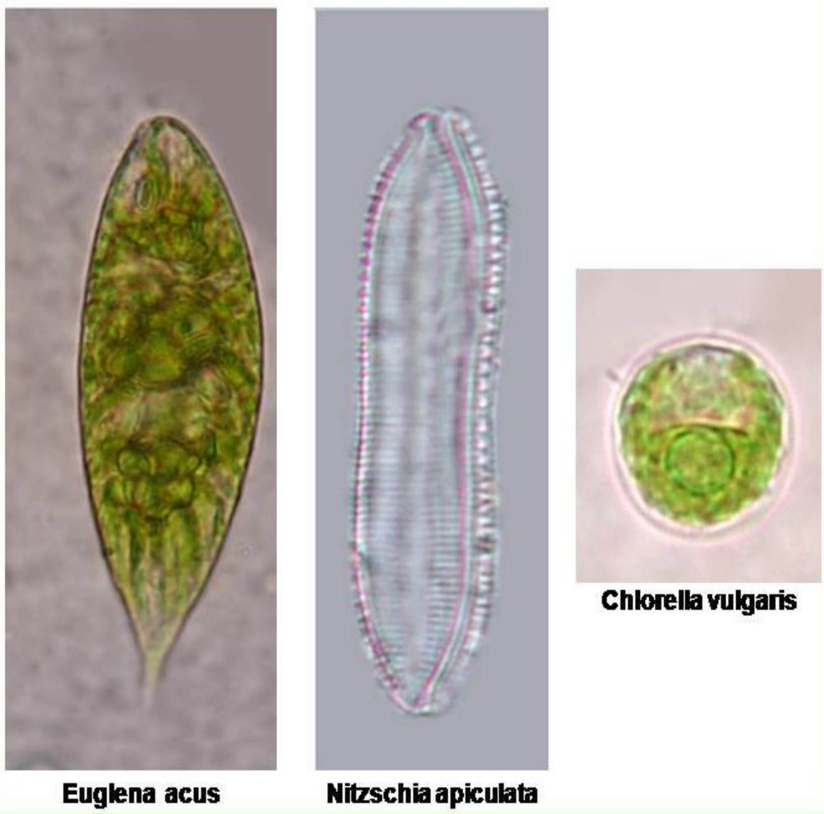
Chlorella vulgaris Beijerinck

Alga free living. Cells usually solitary or in small colonies, spherical and with a thin cell membrane. Chloroplast parietal, cup-shaped and with a pyrenoid which is sometimes indistinct. Cells usually 5-10 µ in diameter.

Euglena acus Ehr.
Cell s cylindrical; anterior end narrowed and truncated; posterior end with a long sharp tail. Pellicle striated, striae nearly parallel to the body. Chromatophores numerous, discoidal; pyrenoids absent. Paramylum consisting of rods of variable in shape, size and numbers. Flagellum up to ¼ of the body length. Eyespot reddish, small, oval. Nucleus oval, central. Metabolic. 100.6-163-(202) × 9-14-(19) µ.

Nitzschia apiculata (Greg.) Grun.
Valves 42-70 µ long, 7-8.5 µ broad, linear with slightly concave margins and cuneate, slightly constricted apiculate ends; keel narrow and excentric; keel punctae 14-16 in 10 µ, not distinguishable from the striae; striae 14-16 in 10 µ, distinct, interrupted by a wide longitudinal hyalin e space.

Fig. 3: Photographs of *Euglena acus, Nitzschia apiculata* and *Chlorella vulgaris*



5. Conclusion

Microalgae is great potential source of sustainable feedstock for the production of third generation biofuels, such as biodiesel. This research has shows that microalgae, present in the Aundha region environment can be a major source of biofuels. Lipid content ranged from 3.62- 12.7%. percentage of lipid is not much higher as compared to other feedstock, so there is need further investigation in this field. The oil can successfully be converted to biodiesel using a single step, acid catalyzed transesterification method. In addition, the biofuels produced from *Euglena* oil was found to have more favorable fuel properties. Overall, regional cultivation of the microalgae and processing into bio-fuels can provide economic benefits to the Aundha rural communities and save a large proportion of foreign exchange used on importing fossil fuels.

References

[1] Abubakar, L.U., Mutie, A.M., Kenya, E.U. and Muhoho A., 2012. Journal of Drawing plied hytotechnology in environment sanitation, Vol. 4: 147-153

[2] A.P.H.A. 1985. Standard methods for the examination of water and waste water. 16th Edition. American Publ. Hlth. Asso. New York. Abraham, B.T., Bissibore, K.S., Heno, T. N. 2002. Study of some physicochemical parameters and treatment of industrial effluent. J. of Environ. Poll. 1(2): 213-216.

[3] Brennan L, Owende P., 2010. Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*. 2010; 14:557-577.

[4] Bridgwater, A. V.; Toft, A. J.; Brammer, J. G., 2002. A techno-economic comparison of power production by biomass fast pyrolysis with gasification and combustion. *Renewable Sustainable Energy Re* V.*: 6* (3), 181-248.

[5] Casadevall, E., D. Dif, Gudin, D. Chaument, and O. Desantit. 1985. Studies on batch and continuous cultures of *Botryococcus braunii*: Hydrocarbon production in relation to physiological state, cell ultra structure, and phosphate nutrition. *Biotech. and Bioeng.* 27(3): 286-295.

[6] Chisti Y., 2007. Biodiesel from microalgae. *Biotechnology Advances*. 25:294-306.
[7] Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnol. Adv.*, *25*, 294–306.
[8] Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. *Trends Biotech. 26*, 126–131.
[9] Desikachary, T. V. 1959. Cyanophyta. I.C.A.R.New Delhi. pp. 1-686.
[10] Desikachary, T. V., 1959. Cyanophyceae. ICAR Monographs on Algae. New Delhi.
[11] Dote, Y., S. Sawayama, and S. Yokoyama. 1992. Liquefaction of hydrocarbon-rich microalga. In *Preprints of Papers Presented at the 204th American Chemical Society National Meeting*, 1836-1839. R. G. Jenkins, ed. Washington, D.C.: American Chemical Society.
[12] Fritsch, F. E. 1913. Observations on phytoplankton of the river Tames Ann. Bot.17: 631-647.
[13] Ganapati, S. V. 1960. Ecology of tropical waters. Proc. Symp. Algol. ICAR, New Delhi. 1: 214-218.
[14] Inoue, H., T. Korenaga, H. Sagami, T. Koyama, H. Sugiyama, and K. Ogura. 1994. Formation of farnesyl oleate and three other farnesyl fatty acid esters by cell-free extracts from *Botryococcus braunii* B race. *Phytochem.* 36(5): 1203-1207.
[15] Iyengar, M. O. P. 1938. The vegetation of Madras and its environs In. Scientific survey of Madras and its environs. Univ. Madras. Pp: 52-59.
[16] Iyengar, M. O. P. and Desikachary. 1981. Volvocales ICAR Monograph, New Delhi. 1-532.
[17] Iyengar, M. O. P. and G. Venkataraman. 1951. The ecology and Seasonal sccession of the river Cooum at Madras with specialreference to Diatomataceae. J. Madras Univ. 21: 140-192.
[18] Koh L. P, Ghazoul J. Biofuels, biodiversity, and people: understanding the conflicts and finding opportunities. *Biological Conservation*. 2008; 141: 2450-2460.
[19] Lewis, N. S.; Nocera, D. G., 2006., Powering the planet: Chemical challenges in solar energy utilization. *Proc. Natl. Acad. Sci. U.S.A.* 2006, *103* (43), 15729-15735
[20] **Mulbry W., Konrad S. and Buyer J., 2008.** Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates" Journal of Applied Phycology, 9314-9318
[21] **Philipose, M. T. 1960.** Freshwater phytoplankton of Inland fisheries, Proc. Symp. Algol., ICAR., New Delhi. 272-291.
[22] **Philipose, M. T. 1967.** Chlorococcales. I.C.A.R. New Delhi. pp.1-365.
[23] **Philipose, M. T. 1983.** Contribution to our knowledge of Indian algae III. Euglenineae - Part-I. The genus Euglena Ehrenberg. Proc. Indian Acad. Sci. (Plant Sci.). **91 (6):** 551-599.
[24] **Richmond, A.**, 2000. Microalgal biotechnology at the turn of the millennium: A personal view. *J. Appl. Phycol. 12* (3), 441-451.
[25] **Rodolfi L., Zitelli G.C. Bassi N., Padovani G., Bonini G., Biondi N. And Tredici M.R., 2009.** Lipid production from microalgae: Strain selection, induction of lipid synthesis and outdoor cultivation in pilot photobioreactors. Biotechnology and Bioengineering 102 (1): 100-112
[26] **Thompson GA., 1996.** Lipids and membrane function in green algae. *Biochemica et Biophysica* 1306:17-45
[27] **Xiufeng L., Han Xu and Qingyu W., 2007**. Large-scale production from microalgae *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotechnology and Bioengeneering 98 (4): 764-771.
[28] **Abubakar, L.U., Mutie, A.M., Kenya, E.U. and Muhoho A., 2012.** Journal of Drawing plied hytotechnology in environment sanitation. http://www.trisanita.org/japes
[29] **Y.P.Nagarajal, Chandrashekhar Biradar1, K.S.Manasa and H.S.Venkatesh,** International journal of current Microbiology and applied sciences. http://www.ijcmas.com