STUDY POTENTIAL OF MICROALGAE FOR BIODIESEL PRODUCTION

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Abstract : Use of petroleum sourced fuels is extensively considered as unsustainable due to their finite resources and enhanced pollution load on the ambient environment. On the other hand, need of energy is increasing continuously with rapid increase in urbanization and industrialization. Microalgae are abundantly available and being renewable offer potential opportunities for biodiesel production as it is carbon neutral fuel. Oil content in microalgae can exceed 80% by weight of dry biomass, but such algae have low productivities. Microalgae with high productivity and oil levels between 20 and 50% include *Chlorella, Dunaliella, Isochrysis, Nannochloropsis, Neochloris, Porphyridium, Schizochytrium, and Tetraselmis.* In the present study, pure cultures of *Chlorella* and *Spirulina* algae were investigated for their characteristic properties with an aim to examine their potential for biodiesel production. The algae species were grown in sterile BG media, non-sterile tap water and pond water. Characterization of algae biomass and oil extracted from algae was done using proximate analysis, ultimate analysis and FTIR spectroscopy. Keeping in mind viability of large scale application, a comparative study was also carried out using mixed culture algae procured from natural pond water. The findings of the above study strongly indicate the suitability of algae oil for biodiesel production.

***IndexTerms* - Microalgae, Biodiesel, Characterization, Renewable**.

I. Introduction

Due to the significant contribution to GHG emissions, and the unsustainable reserves of crude oil, the clean and renewable energy fuels (biofuels) are gaining importance as alternative forms of energy. According to International Panel on Climate Change (IPCC), the annual emissions of CO_2 (the principal anthropogenic GHG) increased by about 80 per cent between 1970 and 2004 [1]. About 80% of energy which are used today is derived from petroleum, coal, and natural gas which result in about 98% carbon emissions [2].

Microalgae are considered as very good alternative for biofuel production because of their advantages of high photosynthetic efficiency, higher biomass production, and faster growth and also because high quality agricultural land is not required to grow the biomass compared to other energy crops [3], [4],[5]. The quite common oil content by weight of dry biomass present in microalgae is 30% which can be upto 70% in some species like *Botryococcus braunii* (25-75%) and *Schizochytrium* spp (50-77%) [6]. The oil in microalgae is mainly composed of four unsaturated fatty acids, namely palmitolleic (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3) (Ratio indicates presence of number of carbon atom: number of double bonds in fatty acid). Biodiesel is highly biodegradable as 90–98% of biodiesel is mineralized within 21–28 days [7]. According to life cycle analysis (LCA) on the process of biodiesel production from microalgae, about 90% of the process energy is consumed by oil extraction. This suggests that there is a need to improve lipid production which will decide the overall economy of biofuel [8]. Also, there is a need to develop the microalgae molecular biology to genetically modify the microalgae in order to get the best strain suitable for oil extraction by improving the photosynthetic efficiency of algae, enhancing the growth rate and increasing the oil content. In comparison to petroleum diesel, the use of biodiesel in engine results in reduction of emissions such as particulate matter from 53% to 69%, dry soot from 79% to 83%, hydrocarbons from 45% to 67%, and carbon monoxide from 4% to 16% with the minor increase in NOx emissions from 10% to 23% resulting in the improvement of thermal efficiency of the engine [8], [9].

Several recent works have been done in the area of biodiesel production from algae feedstocks. Z. Du et al.(2011) performed the microwave assisted pyrolysis under different microwave power levels and found that at 750W there was the maximum yield of bio oil (28.6%) for the *Chlorella sp* [10]. Aresta et al (2005) compared the liquefaction technology with supercritical-CO_2 technology for oil extraction and came to the conclusion that liquefaction is better than supercritical-CO_2 extraction from the quantitative point of view. However, in liquefaction fatty acids may degrade due to working experimental conditions [11]. Direct transesterification approach (Wahlen et al., 2011) was studied for biodiesel production in single step using algae [12]. P.D. Patil et al. (2011) explored supercritical methanol conditions to extract the oil using wet algae paste of *Nannochloropsis sp* [13]. The potential worth of any feedstock depends on the chemical and physical characterization of that biomass. The present study is focused on the examination of biomass properties of three varieties of algae (i.e. *Chlorella, Spirulina* and pond water algae) which affect the performance of biodiesel derived from them. The findings of such studies will determine the potential of the algae feedstock for biodiesel production. The study also investigates the proper growth conditions required for the algae cultivation.

II. Methods

II.1. Microorganisms, growth medium and growth conditions

Spirulina strain and the green alga strain *Chlorella* pyrenoidosa were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune-Maharashtra, India. The algae from a pond nearby India Gate, New Delhi, India was also collected. All the three samples were maintained in conical flasks containing 50 mL sterile BG11

[14] media [Table1] and kept under shaking conditions at 120 rpm and 24°C for half an hour and then in the direct sunlight. Regular sub culturing was also performed after every 15-18 days.

Growth was also observed by placing the cultures in the growth chamber. In growth chamber cultures were incubated under illumination (2000 Lux) at 25±1°C with light: dark cycles of 12:12 h for 15 to 18 days [Table2] with bubbling air at normal pressure. Growth was also attempted in tap water as well as in pond water without BG 11 medium.

Table 1: Media (BG media) composition used for culturing algae species

Ingredients	g/L
Sodium Nitrate	1.50
Dipotassium hydrogen tartarate	0.0314
Magnesium sulphate	0.036
Calcium chloride dihydrate	0.0367
Sodium carbonate	0.02
Disodium magnesium EDTA	0.001
Citric acid	0.0056
Ferric ammonium citrate	0.006

Table 2: Growth Conditions for algae

Media: BG 11(Final pH after sterilization 7.1)
Illumination in growth chamber : 2000 Lux
Temperature Conditions: 25±1°C
Light: dark cycle of 12:12 h (15 to 18 days)

II.2. Growth Evaluation

The growth of the species was monitored at regular interval for total period of 20 days using UV visible spectrophotometer (Systronics117) by reading the culture absorbance at 480nm and 360nm for *Chlorella pyrenoidosa* and pond water algae respectively (Table 3).

II.3 Biomass Harvesting

The cultures were harvested by centrifugation at 7000 rpm for 15 min. The biomass collected was washed twice with distilled water after centrifugation and then sun- dried for 2-3 days. Pond water algae was sun dried for two weeks.

II.4. Oil Extraction

After sun drying the biomass was further dried in an oven at 80°C for 24hrs. It was then pulverized in a mortar and lipids were extracted using hexane solvent with the Soxhlet apparatus (500 mL). About 300 mL of solvent was used for 30 grams of dried biomass and was heated upto the boiling point of the solvent (56^0C). The procedure was repeated until the colour of the solvent used for the extraction was clear. The solvent phase was separated from oil phase using distillation set up.

II.5. CHNS analysis

C, H, N, S analysis of dried biomass and algae oil was carried out using CHNS analyzer and the oxygen content was calculated by difference (Table 4).

II.6 FTIR analysis

The FTIR spectra of dried algal biomass and deoiled mass were obtained by using FTIR spectrometer (Nicolet 6700) at room temperature. The dried algal powder was mixed with potassium bromide (KBr) powder and pressed into pellets before analysis. Similarly, pellets were made for algae oil.

3. Results and discussions

Table 3 shows the growth pattern studied for 20 days using UV visible spectrophotometer (Systronics117). In the table, it is observed that there is gradual increase in absorbance which indicates the growth of algae in all the three cases. However, comparing the *Chlorella* growth in BG 11 medium and tap water alone, growth is slower in tap water which signifies that BG 11 is required for the rapid growth of algae. With the concern for large scale cultivation of algae, it can be even grown in tap water. Algal growth is also observed with pond water but the growth is slower if compared to *Chlorella* growing in BG 11 medium. The growth study was conducted for the first 20 days and then the algae were allowed to grow for several months with an aim to obtain sufficient algae biomass needed for oil extraction. Growth of the cultures could also be observed visually.

The proximate analysis gives the information about percentage of moisture, ash, fixed carbon and volatile matter present in the biomass. Moisture and ash content are important determinant of heating value. The moisture content also determines the transport and storage potential of biomass. Ash content leads to high disposal costs which results in an unconstructive economic impact on overall biomass conversion to biodiesel. Ash content affects the energy content as chemically it is non-combustible inorganic.

Table 3: Growth evaluation shown by absorbance measured after every four days using UV visible spectrophotometer

Algae	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20
Chlorella (BG 11) at 480 nm	0.002	0.017	0.037	0.042	0.062	0.064
Chlorella (Tap water) at 480nm	0.001	0.001	0.002	0.004	.006	0.014
Pond water algae in pond water at 360 nm	0.002	0.003	0.005	0.008	0.009	0.018

Higher the ash value lower the energy content. Comparing the results obtained from the proximate analysis for the three different varieties of algae, it is clear that ash content in pond water algae is very high (65%) due to silt deposition in the pond as compared to Spirulina and *Chlorella.* Thus it indicates that very less percentage of pond water algae biomass (35%) will be available for lipid extraction, which will lower the overall yield of the oil extracted. Looking at the values of volatile matter of the three species, *Spirulina* has the highest percentage of volatile matter (74.41%) which indicates high percentage of biomass available for lipid extraction. The volatile matter of *Chlorella* (70.14%) is comparable to that of *Spirulina.* On the other hand, pond water algae have the low percentage of volatile matter. Moisture content in pond water is low as compared to *Chlorella* and *Spirulina.* Low moisture content helps in better oil extraction resulting in higher yields.

The CHNS analysis gave the percentage of carbon, hydrogen, oxygen and nitrogen present in the algal biomass and the oil extracted as seen in Table-4. Empirical formulae and molar ratios of H/C and O/C are also calculated Table 6. Comparing the elemental percentage of biomass and oil, an increase in carbon and hydrogen percentage is observed in algae oil, which depicts the high heating value of algae oil. The decrease in the percentage of sulphur in oil describes the lower SO_2 emissions for the algae biodiesel. Pond water algae biomass has the highest percentage of oxygen which will lead to increase in the viscosity of the oil extracted. On the other hand, pond water algae has the lowest percentage of nitrogen and sulphur in comparison to *Spirulina* and *Chlorella,* which will lower the NO_x and SO_2 emissions. Chlorella has the highest percentage of carbon thus increasing heating value of the oil extracted. Looking at the results obtained for algae oils in Table 5; Spirulina oil has the highest percentage of carbon and very low percentage of nitrogen, sulphur and oxygen.

Table 4: CHNS analysis (wt. %)

	C	**H**	**N**	**S**	**O (by difference)**
Spirulina (biomass)	48.10	6.97	10.14	0.76	34.02
Chlorella (biomass)	51.33	7.90	9.8	0.49	31.47
Pond water Algae (biomass)	44.09	1.22	0.70	0.34	53.64
Spirulina (oil)	76.73	12.40	0.10	0.001	10.76
Chlorella (oil)	56.75	8.77	4.94	0.19	29.34
Pond water Algae (oil)	59.94	11.57	0.11	0.51	27.86

Table 5

	H/C	O/C	Empirical formula
Spirulina (biomass)	0.14	0.7	$CH_{1.74}O_{0.53}N_{0.18}S_{.006}$
Chlorella (biomass)	0.15	0.61	$CH_{1.85}O_{0.46}N_{0.16}S_{5.9}$
Pond water Algae (biomass)	0.03	1.2	$CH_{0.33}O_{0.91}N_{0.016}S_{.003}$
Spirulina (oil)	0.16	0.14	$CH_{1.9}O_{0.105}N_{.001}$
Chlorella (oil)	0.15	0.52	$CH_{1.85}\ _{0.39}N_{0.074}\ S_{.001}$
Pond water Algae (oil)	0.19	0.46	$CH_{2.3}O\ _{0.35}N\ _{.001}S_{.003}$

The FTIR spectra of pond water algae biomass, de-oiled biomass and the oil extracted from the biomass are shown in Fig. 1. The region 3300–3000 cm $^{-1}$ is characteristic for C-H stretching vibrations of alkenes, alkynes and arenes, whereas the region from 3000 to 2850 cm $^{-1}$ is recognized by C-H stretching vibrations of $–CH_3$, $>CH_2$ and CH .Two bands (2690-2840) are observed for –C-H stretching of aldehyde functional group. The absorption between 1650-1740 cm $^{-1}$ implies the presence of C=O functional group. The region between 1800 and 1500 cm $^{-1}$ shows characteristic bands for proteins. The region from 1200 to 900 cm $^{-1}$ indicates a sequence of bands due to C-O, C-C,

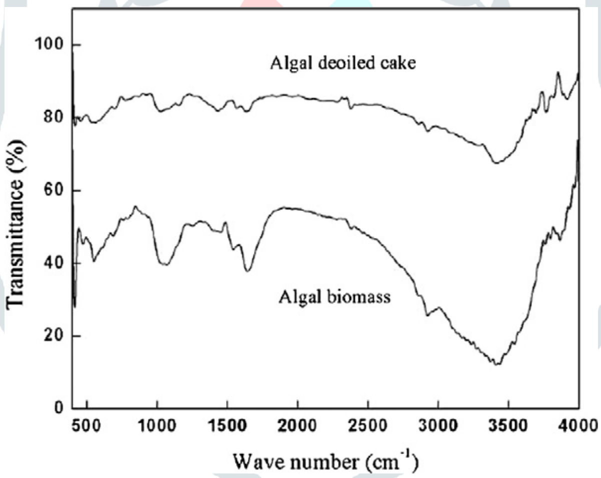

Figure 1: FTIR spectra of Chlorella sp. MP-1 biomass and deoiled cake [12].

C-O-C and C-O-P stretching vibrations of polysaccharides. CH_2 stretching vibrations in the range of 3100–2800 cm^{-1} indicate the presence of lipid. The absorption at 2928 and 2860 cm^{-1} means CH_2 asymmetric and symmetric stretching in lipid. In Fig:1 , by comparing the bands characteristic for lipids in the spectra of algae biomass and deoiled cake, it is clearly visible that there is decrease in the intensity of absorption band in the deoiled cake spectra which states that lipids are extracted after the application of solvent. Similar pattern was observed with *Spirulina and Chlorella as* well [Fig: 2(a), (b), (c) and Fig: 3 (a), (b)]. The above results are in close agreement to the results obtained by Phukan et al (2011) with *Chlorella* sp. MP-1 [15].

Conclusion

In the present study, it is observed that algae can be grown in tap water as well as in natural pond water. However, growth is rapid in BG 11 medium. Algae can be grown both in the direct sunlight as well as in the artificial growth chamber. Proximate studies showed that *Chlorella* and *Spirulina* have comparable high volatile matter and low ash content and therefore, high oil extraction can be expected. Spirulina oil has the highest percentage of C which increases its heating value and lower N and S leading to reduced SO2 and NO_x emissions. FTIR spectra also indicate the extraction of lipids from the biomass. Thus, the above study experimentally recognized the feasibility of algae for the biodiesel production. The above feedstocks display quite a few important characteristics for futuristic research biodiesel production. The characterization of algae biomass makes certain that it has the potential to offer feedstock for biodiesel production. Future studies should concentrate on the development of the algal species with fast growth rate and high lipid content through genetic engineering.

References

[1] H. C. Greenwell, L. M. L. Laurens, R. J. Shields, R. W. Lovitt and K. J. Flynn, Placing microalgae on the biofuels priority list: a review of the technological challenges, J. R. Soc. Interface 7(2010)703-726

[2] Kirtay E, The role of renewable energy sources in meeting Turkey's electrical energy demand, Energy Educ Sci Technol Part A 23(2009)15–30

[3] TA Milne, RJ Evans, N Nagle, Catalytic conversion of microalgae and vegetable oils to premium gasoline, with shape selective zeolites, Biomass 21(1990) 219–232.

[4] Ginzburg, B.Z., Liquid fuel (oil) from halophilic algae: a renewable source of non-polluting energy, Renew. Energy 3(1993)249–252

[5] T Minowa, S Yokoyama, M Kishimoto, T Okakura ,Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction, Fuel 74(1995)1735–1738

[6] Chisti, Y., Biodiesel from microalgae, Biotechnol. Adv. 25(2007) 294–306.

[7] Pasqualino JC, Montane D, Salvado J., Synergic effects of biodiesel in the biodegradability of fossil-derived fuels, Biomass Bioenergy 30 (2006),874–9.

[8] Chincholkar SP, Srivastava S, Rehman A, Dixit S, Lanjewar A, Biodiesel as an alternative fuel for pollution control in diesel engine, Asian J Exp Sci 19(2) (2005), 13–22.

[9] Lapuerta M, Armas O, Rodrı´guez-Ferna´ndez J, Effect of biodiesel fuels on diesel engine emissions, Prog Energy Combust Sci 34(2) (2008), 198–223.

[10] Zhenyi Du, Yecong Li , Xiaoquan Wanga, Yiqin Wana, Qin Chen, Chenguang Wang , Xiangyang Lin, Yuhuan Liu, Paul Chen, Roger Ruan, Microwave-assisted pyrolysis of microalgae for biofuel production. Bioresource Technology 102(2011), 4890–4896.

[11] Michele Aresta ,Angela Dibenedetto , Maria Carone, Teresa Colonna ,Carlo Fragale, Production of biodiesel from macroalgae by supercritical CO2 extraction and thermochemical liquefaction.Environ Chem Lett 3(2005), 136–139.

[12] Bradley D. Wahlen, Robert M. Willis, Lance C. Seefeldt, Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresource Technology 102(2011), 2724–2730.

[13] Prafulla D. Patil , Veera Gnaneswar Gude , Aravind Mannarswamy , Shuguang Deng , Peter Cooke , Stuart Munson-McGee , Isaac Rhodes , Pete Lammers , Nagamany Nirmalakhandan. Optimization of direct conversion of wet algae to biodiesel under, supercritical methanol conditions. Bioresource Technology 102(2011),118–122.

[14] Rippka R; Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J Gen Microbiol 111(1979)1-61

[15] Mayur M. Phukan, Rahul S. Chutia, B.K. Konwar, R. Kataki, Microalgae Chlorella as a potential bio-energy feedstock.Applied Energy (2011).