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Microspora Floccosa; A Potential Biofuel Producer

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Abstract

The current study is focused on biofuel production from local specie of algae. Initially samples were observed to identify the algal specie. Afterward oil was extracted from algae by Soxhlet extraction method, retention time was optimized to improve the yield of oil at different intervals. The recovered oil from algae was subjected to qualitative analysis by Gas Chromatography. Four major peaks were appeared on GC chromatogram which correspond to methyl esters of Dodecanoic acid, Tetradecanoic acid, 8,11,14-Eicosadienoic acid and 9,10-Dihydroxy octadecanoic. The results reflect that Microspora floccosa algae considered to be favorable for biofuel production.

Keywords: Microspora floccose, Algae, Biofuel, Renewable Energy, Extraction, Characterization

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Introduction

The constant increase in scarcity of power generation fuel supplies and global warming is a direct result of indiscriminate fossil fuel exploitation. The global energy demand has already pushed for energy carrier alternative that is clean, renewable and that reduce the load on fossil fuels. In present situation, 80% of the total energy utilized derives from fossil fuels. The combustion of fossil fuel yield about 98% of carbon dioxide while cultivation of algae consume carbon dioxide, therefore algae can be used for CO_2 capture from industrial plants and thermal power stations and help them reduce their overall impact on atmosphere. Reduction in the use of fossil fuel will considerably reduce the emission and other pollutants. Also the fossil fuels are unevenly distributed. Biofuels have already gained World's interest due to their relatively low emission profile, non-toxicity, biodegradability, renewability and relatively evenly distribution. Biofuels are distilled from organic matter i.e. algae, and many edible crops that photo synthetically transform solar energy into chemical energy in form of carbohydrates, oils and proteins and it is one of the best ways of producing 'green' energy.

Temperature near or at tropics encourages the production of algae because of higher and quicker biomass yield and lower production costs. Therefore, renewable fuels can potentially provide developing countries the opportunities to improve economics and have better access of energy. Although, the increasing global demand for biomass derived fuels is of great concern, and this includes change in land use directly and indirectly, competition with edible crops, and land tenure conflicts.

Among the existing options for biofuel production, algae are most promising. Algae are one of the most photo synthetically efficient plants on earth. If it gets suitable conditions, it may grow

double in quantum within 24 hours. Algae are reported to be among the best feedstock option for biodiesel production by Shay [4]. The oil produced from algae is 250 times the oil produced from soybeans per acre per year. Only algae have the tendency to produce sufficient automobile fuel so as to substitute the present gasoline demand. The oil yield from algae is 7 to 31 times greater than from palm. Algae produce 7 to 31 time greater oil than on oil [2]. Microalgae are the best algae for biodiesel production [3]. Growth and harvest of microalgae is far easy and faster than that of macro algae, also microalgae yields more oil than macro algae [4].They can grow even on the arid lands as well as saline soil. They can grow faster than food crops and can be harvested in days rather months. [5, 7]. Algae are the most efficient biological oil producer crop and it can produce different types of biofuels. Such as anaerobic digestion of algal biomass produces methane gas [8], transesterification of microalgal oil yield biodiesel [9, 10], and photo-biologically process of algae results in bio-hydrogen [11, 12] etc.

Algae can be grown naturally and also in semi artificial environment i.e. open pond and photo-bioreactor (PBR). Open pond are shallow to the algae exposed to sunlight. Photo bioreactor is a transparent controlled ideal conditions system. PBR ensures prevention of contamination and maintenance of integrity of specie. Light reception by algae can be managed by wall thickness of PBR, light intensity, position of source and photo period. With PBR the whole system can be monitored efficiently.

Production of the renewable energy is highly demanded since a decade. It has been globally realized that the capability of first generation biofuels especially the oil from seeds is not sufficient to meet the demand of biofuel, mitigate global warming and improve the economy. To overcome this issue research has been conducted for producing second generation biofuels, from non-edible feed stocks like microalgae. As reported by Laungsuwon et.al, [13] some microalgal species were found to be efficient antidote for some diseases. The extracts of *C. glomerata* and *M. floccosa* algal extracts showed the antimicrobial activity against *Bacillus cereus* and *Vibrio parahaemolyticus*, so that it could be a source of valuable bioactive materials for health products.

Chaetoceros sp algae was identified by Ananadhi Padmanabhan et.al, [14] and further determined its carbohydrate and protein content. Ananadhi Padmanabhan et.al aimed to utilize the algae developed in water structures and recover useable oil from them and convert in biodiesel to satisfy the demand of fuel for present energy need.

Kumar and co-authors [15] reported physio-chemical characterstics of algal oil. The species of algae from India were identified including Tolypotryx (a blue-green algae type) and five were green algae Hydrodictyon, Spirogyra, Cladophora, Rhizoclonium and Pithophora. Gas Chromatography analysis showed high percentages of Methyl Stearate, Methyl linoleate, Methyl Palmitate and Methyloleate. The physical and chemical properties of oil obtained from algae satisfy the standards given by American Society for Testing and Materials (ASTM) D6751, ISO 15607 and EN14214-Europe; it shows potential to be used as a biofuel.

As delineated by Liu et.al, [16] about possibilities of heterogeneous algae as a high quality biodiesel producer. Phototrophic algal cells accumulated predominantly the membrane lipids phospholipids (PL) and glycolipids (GL). Along with the greater quantity of Oleic Acid (C18:1) which was 35.2% of the total fatty acids, the recovered oil from the heterotrophic Chlorella Zofingiensis was found to be more feasible for production of biodiesel. Gouveia et. al, [17] found six different species of microalgae, namely Nannochloropsis sp., Spirulina maxima, Chlorella vulgaris, Dunaliella tertiolecta, Scenedesmus obliquus and Neochloris oleabundans. Nannochloropsis sp. (a marine microalga) with 28.7% oil and Neochloris Oleabundans (a freshwater microalgae) with 29% oil yield were found suitable as feedstock for production of biofuel.

In other report, algae suspended in water were subjected to heat at 80^{o}C to 95^{o}C and extraction was carried out for 30 minutes.

Maximum oil was recovered when the algae-water suspension was kept in Microwave system at 95°C for 30 minutes. Around 76-77% of recoverable oil was extracted at 95°C in Microwave system for 20-30 minutes. Whereas; the water bath control recovered only 43-47%. This research work indicated the efficiency of continuous microwave system for recovering oil content from algae. [18].
Hydrothermal liquefaction method was also investigated, the liquefaction of microalgal species Dunaliella Tertiolecta hydrothermally with various temperatures, catalyst dosages and holding periods. The maximum yield of bio-oil obtained was 25.8%, under the reaction condition of 360°C temperature, using 5% Na_2CO_3 as catalyst and 50 minutes holding time. [19]

Single step processes which carry out extraction and transesterification processes simultaneously were presented by patil et.al [20]. This process enables liquefaction and turns wet biomass of algae (containing 90% water) into biodiesel using supercritical methanol method. This research work emphasizes on producing Fatty acid methyl esters (FAMEs) from free fatty acids, triglycerides and polar phospholipids. The effect of variables like reaction time, reaction temperature and wet algae to methanol ratio (wt/vol.). The proposed process in this study was considered to be an economical to obtain algae biodiesel.

Microalgae subjected to fast pyrolysis tests in the fluid bed reactors were reported by Mioo et.al [21]. The experiments undertook at 500°C temperature; having heating value as 600°C/s. N_2 swept out with a flow rate of 0.4m^3/h while the vapor resisted for 2 to 3s. In comparison to slow pyrolysis, this work on fast pyrolysis recovered higher quantity of high quality oil produced directly from microalgae by continuously processing at the feed rate of 4g/min. Chllorella protothecoides yielded 18% liquid from its biomass on fast pyrolysis, whereas Microcytic aeruginosa gave up 24% of its biomass as oil. The polar and saturated fractions were recorded as 31.17 and 1.14 respectively as average of the oil obtained from microalgae, which were greater than that of the oil extracted from wood. The chemical composition analysis by Gas Chromatography indicated that the straight chain alkanes of saturated compounds were distributed in microalgal oil quite similar to that in diesel fuel. The oil produced from the fast pyrolysis of microalgae had low Oxygen content and greater heating value of 29 MJ/kg, it was also found to have viscosity of 0.10 Pa.s, and density equals to 1.16kg/L. Such characteristics of algal oil prove it to be more suitable for use as substitute for fossil fuels as compared to the yields of past pyrolysis of lignocellulosic biomass. Aim of this research was to find new algal specie and study about its potential for oil production. Also the oil yield was observed under various conditions.

Material and Method

Algal sample

The algae sample was handpicked in bulk from the water treatment plant in Kotri (25°26'6.84" N68°20'9.53" E), a satellite city of Hyderabad. The sample was collected in the morning of March, 2014. Immediately after collection it was brought to laboratory. A part of sample was observed with microscope for identification. The algal specie was morphol-ogically same as Microspora floccose. The rest of the sample was washed and then dried in oven for 2-3 hours to get 100% dry algae.

Oil extraction

The algae was oven dried at 70°C, after drying it was ground and sieved. Then 2g of dried algae was tied in filter bag and that filter bag was loaded in extraction chamber of Soxhlet apparatus. The extraction chamber was kept over a boiling flask containing 50ml of extraction solvent. Hexane was used as solvent. The solvent was allowed to reflux at 60°C. After a certain retention period, and many cycles the algal oil was concentrated in the boiling flask with hexane. After the algal oil collection, hexane was removed from the oil by rotary evaporator. The flask with the oil and without oil was weighed to determine the amount of oil obtained.

Testing of algal oil

Algal oil was tested to identify the fatty acids methyl esters. Fatty acid methyl esters were

prepared by saponification and esterification of lipid by standard IUPAC method. In typical procedure, Fatty acids were methylated in round bottom flask (100 mL), with methanolic potassium hydroxide solution (0.5N, 5 mL) and refluxed for 10 min at 99 °C. Further reflux was carried out after addition of BF_3/Methanol solution 5 mL. Thereafter, solution was boiled and taken to separatory funnel. 10mL of n-Haxan was added and FAME was extracted and separated. Hexane layer, containing the FAME, was placed into a glass vial and sodium sufate anhydrous was used to dehydrate the sample. The vial was capped and placed at -20°C until GC analysis.

Gas chromatography (GC) analysis was carried out by Shimadzu gas chromatograph Plus of model 2010 with a DB-5column (5% phenyl methyl polysiloxane, 15 m x 0.25 μm, film thickness 0.25 μm) and FID detector. The GC was used under following operating condition: oven temperature program was 40°C (hold 2 min) to 150°C at 5°C min-1 and then to 270°C at 15°C min^{-1}. Injector and detector temperature were kept at 280°C. Nitrogen was used as a carrier gas and column flow was adjusted to 1.5 mL/min. Split ratio was 20:1 and the 1 μL of extracted oil sample was injected into the system using micro syring. Identification of the components was achieved based on retention time compared with FAME standards [13].

Results and Discussion

Microspora floccosa algae characterization and Bio-oil yield

Before oil extraction the characterization studies from morphology of *Microspora floccose* were conducted. Fig.1a and b shows the microscopic image of said specie, Fig. 1b represents the morphology of *Microspora floccose* applied in current study, while Fig. 1b is image of already reported specie as comparative study.

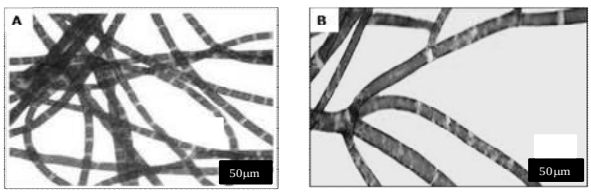


Figure 1. **Comparative microscopic image of *Microspora floccose* *(A) current study (B) reported***

After characterization the algae were subjected to Soxhlet extraction for different period of time. Initially 1.25 g/g oil was achieved at 2 hours soxhlet extraction which was increased with increase in extraction time and maximum 3.75g/g oil was obtained at 5 hours. It is about 10-15 times higher than reported algae [17-19, 21]. The yield was decreased at further extraction process which proves that no more oil is retained in algae. The algal oil yields are elaborated in graph presented in (Fig. 2).

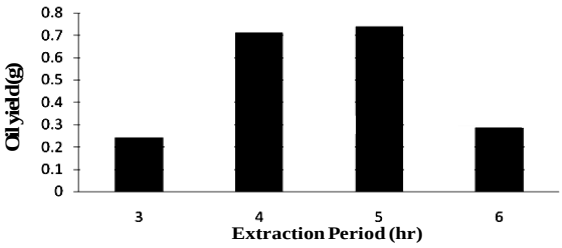


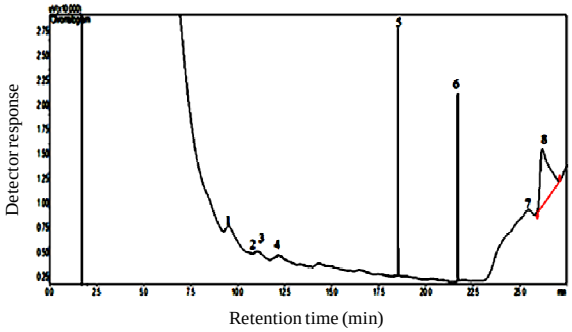
Figure 2. **Oil yield from algae at different extraction period**

Gas chromatography of algal oil

Fig. 3 represents the chromatograph of recovered algal oil, showing the retention time of different eluting chemical substances. Standards of fatty acid methyl esters (FAME) were dissolved in 5 mL hexane and the resulting solution injected into gas chromatograph. The FAME obtained in algal oil was identified by comparing with FAME standards peaks in Gas Chromatography result reported as in (Table 1).

Table 1. **FAMEs profile of bio-oil obtained from Microspora Floccosa by GC.**

Retention Time (min)	Compound	Molecular Formula	Molecular weight (g)	Relative %
18.50	Dodecanoic acid methyl ester	$C_{13}H_{26}O_2$	214.34	52
21.70	Tetradecanoic acid methyl ester	$C_{15}H_{30}O_2$	242.39	20
25.47	8,11,14-Eicosadienoic acid methyl ester	$C_{21}H_{36}O_2$	320.51	4
26.185	9,10-Dihydroxy octadecanoic acid methyl Ester	$C_{19}H_{38}O_4$	330.50	16
	Unidentified			-
Total				92



Conclusion

Soxhlet extraction was applied on 2 g oven dried algae using hexane (50mL) as solvent, with different retention times. The maximum yield was obtained when hexane refluxed over algae for 5 hours continuously at 60ºC. The last four peaks of GC represented the following compounds: Dodecanoic acid methyl ester, Tetradecanoic acid methyl ester, 8,11,14-Eicosadienoic acid methyl ester and 9,10-Dihydroxy octadecanoic acid methyl ester. The presence of these compounds shows the feasibility of Microspora Floccosa as a feedstock for biodiesel. The earlier few peaks can be further studied to know the corresponding compounds.

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References

1. E. Kirtay. The role of renewable energy sources in meeting Turkey's electrical energy demand. *Energy Educ. Sci. Technol. Part A.*, 23 (2009) 15.
2. A. B. Hossain, A. N. Salleh, P. Boyce, Chowdhury and M. Naqiuddin. *American J. Biochemistry and Biotechnology.*, 4 (2008) 250.
3. A. K. Bajhaiya, S. K. Mandotra, M. R. Suseela, Kiran Toppo and S. Ranade, *Asian J. Exp. Boil.*, 1 (2010) 728.
4. E. G. Shay, *Biomass Bioenergy*., 4 (1993) 227.
5. Y. Chisti, *Biotechnol Adv*., 25 (2007) 294.
6. Y. Chisti, *Trends in biotechnology*., 26 (2008) 126.
7. J. Tickell, From the fryer to the fuel tank. The complete guide to using vegetable oil as an alternative fuel. Tallahasseee, (2000) USA.
8. P. Spolaore, C. Joannis-Cassan, E. Duran and A. Isambert, *J. Biosci. Bioeng*, 101 (2006) 87.
9. F. R. Thomas, Oakhaven Permaculture Center. *Permaculture Activist*, 59 (2006) 1.
10. A. Banerjee, R. Sharma, Y. Chisti and U. C. Banerjee, *Crit. Rev. Biotechnol*, 22 (2002) 245.
11. M. Gavrilescu and Y. Chisti, *Biotechnol, Adv*. 23 (2005) 471.
12. A. S. Fedorov, S. Kosourov, M. L. Ghirardi and M. Seibert, *Appl. Biochem. Biotechnol.*, 124 (2005) 403.
13. R. Laungsuwon, W. Chulalaksananukul, *J. Bio. Sci. Biotech.* 3 (2014) 211.
14. M. R. Ananadhi Padmanabhan and A. Shaleesha Stanley, *Res. J. Recent Sci.*, 1 (2012) 57.
15. P. Kumar, M. R. Suseela and K. Toppo, *Asian J. Exp. Biol. Sci.*, 2 (2011) 493.
16. J. Liu, J. Huang, Z. Sun, Y. Zhong, Y. Jiang, and F. Chen, *Bioresource Technology*, 102 (2011) 106.
17. L. Gouveia, and A. C. Oliveira, *J. Industrial Microbiology & Biotechnology*, 36 (2009) 269.
18. S. Balasubramanian, J. D. Allen, A. Kanitkar and D. Boldor, *Bioresource Technology* 102 (2011) 3396.
19. Z. Shuping, W. Yulong, Y. Mingde, I. Kaleem, L. Chun and J. Tong, *Energy*, 35 (2010) 5406.
20. P. D. Patil, V. G. Gude, A. Mannarswamy, S. Deng, P. Cooke, S. Munson-McGee, I. Rhodes, P. Lammers and N. Nirmalakhandan, *Bioresource Technology*, 102 (2011) 118.
21. X. Miao, Q. Wu and C. Yang, *J. Analy.and Applied Pyrolysis*, 7 (2004) 855.