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Microalgae as an Oil Producer for Biofuel Applications

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Abstract

Microalgae are more promising feed stocks to their widespread availability and higher oil yields. As with any biological lipid, this is a potential feedstock for making the renewable fuel biodiesel. However, extracting and purifying oil from algae continues to prove a significant challenge in producing both microalgae byproducts and biofuel, as microbial oil extraction is relatively energy-intensive and costly. The aim of the research work is to produce biodiesel from micro algal species. Chaetoceros sp were identified for the research work and studied for their lipid, carbohydrate and protein content. The main aim of the project is to make use of the algae present in the water bodies and to extract the useful algal oil meant for biodiesel production to meet the challenges of fuel requirement in the present scenario. Microalgal oil was extracted from Chaetoceros sp. and the physico-chemical properties were determined. The density, viscosity, acid value, saponification value and free fatty acids were recorded as 1.305gm/ml, 6.2mm^2/s, 2.5339mg/gm of oil, 173.56mg/gm of oil, and 0.71gm/100 gm of algae (Oleic acid). The fatty acid profile showed pentadecanoic acid (17.56%), 1.Nonadecenoic acid (20.1%), methyl palmitate (2.91%), methyl linoleate (12.07%), palmitic acid (1.97%) as major fatty acids.

Key Words: *Chaetoceros* sp., single cell oil, solvent extraction, and GC-MS analysis.

Introduction

In recent years, global warming, world oil supply, energy demand have all played a part in the push for alternatives to petroleum-based fuels. The Intergovernmental Panel on Climate Change (IPCC) affirms that during the 20th century, the Earth's average temperature increased by 0.6°C and will continue to increase anywhere from 1.5°C to 4.5°C by the year 2100[1]. This increase in global temperature is enough to cause flooding in coastal regions and make storms like Hurricane Katrina a more common occurrence[2]. The major force in rising global temperatures is anthropogenic carbon dioxide emissions, which accounts for 80% of all greenhouse gases produced[1].

In contrast to the hydrogen alternative, biodiesel is a more immediate option as a renewable fuel source. Biodiesel contains no sulfur or aromatics, and use of biodiesel in a conventional diesel engine results in substantial reduction of unburned hydrocarbons, carbon monoxide and particulate matter (Biodiesel Emissions). Biodiesel consists of alkyl esters of long chain fatty acids that are derived from oils and fats produced by organisms[3]. There are a number of animal fats and plants that can be used to produce biodiesel. Animal fats that are usually used include lard, yellow grease, and tallow[4]. Plants that are typically grown for biodiesel include corn, cottonseed, peanut, rapeseed, and soybean[5]. Currently, the supply of biodiesel from animal fats and plant oils is not enough to completely support the world's energy needs. It is also not economically feasible to rely completely on plants and animal fats for biodiesel. A large amount of land conversion from forestry and food agriculture would be required; furthermore, not all land may be suitable for crops[1]. In addition, an increase in crops would be conducive to increased amounts of pesticide and fertilizer, which would damage the environment[6].

Microalgae have been investigated as a potential source for biodiesel. Microalgae can build a global oil supply chain that is sustainable and delivers fuel. It will be the supplier to the biodiesel business. Marine algae or sea weeds are the oldest members of plant kingdom. The existing large scale natural sources of algae are: Bogs, marshes and swamps (salt marshes and salt lakes). Marine algae contain lipids and fatty acids as membrane components, storage products, metabolites and source of energy. The US Department of Energy reports that biodiesel produced from algae could see yields greater than oilseed crops[7]. Micro-algae are the fastest growing photosynthesizing organisms. They can complete an entire growing cycle every few days. Algae produce 100 times more oil per acre than traditional food oilseed crops such as soy, etc. Marine algae contain lipids and fatty acids as membrane components, storage products, metabolites and source of energy. Algae contain about 2% lipid and 40% fatty oils by weight[8]. Today Algae are used by humans in many ways; for example, as fertilizers, soil conditioners and livestock feed. They can double their mass several times a day and produce at least 15 times more oil per acre than alternatives such as rapeseed, palms, soybeans, or jatropha. Moreover, algae-growing facilities can be built on coastal land unsuitable for conventional agriculture.

Chaetoceros algae are marine algae. They are pelagic diatom cultures to be one of the best algal groups to culture and as a food source, unparallel. It is a medium size diatom (microscopic unicellular marine alga having cell walls impregnated with silica) that is extensively used in the aqua culture industry, primarily for bivalve shell fish and shrimp. It has high reproductive potential characteristic.

The objectives of the proposed research work are identifying a species which is acclimatized to Indian climatic conditions. The selection of species was completely identified based on the composition of lipid content and the previous literature. The present research work was designed to extract and study different physico-chemical parameters of microalgal oil so as to explore a source to produce biodiesel in India. The algal species *Chaetoceros* alga *was* chosen for this study for several reasons. This strain is native to Chennai; Tamil nadu and was found in high concentrations in Chennai coastal area. Algae are periodically separated from water by harvesting algae through dissolved air floatation. Oil will then be extracted from the algal biomass using solvent extractions and an autoclave operated at elevated temperatures. This algal oil will then be converted into biodiesel using a transesterification process. Transesterification after called alcoholysis is the reaction of a fat or oil with an alcohol to form esters and glycerol. In transesterification, triglycerides in microalgal oil react with alcohol to form a mixture glycerol and fatty acid alkyl esters called biodiesel.

Material and Methods

Culture Collection: *Chaetoceros* species was obtained from Centre for Advanced studies, Madras University, Chennai, Tamil Nadu, India.

Culture Cultivation: The Marine microalgae were grown in F/2 Medium. F/2 medium is an enriched seawater medium designed for growing marine algae. The concentration of the original formulation termed “F medium” has been reduced by half. F/2 medium was prepared using 950 ml of filtered natural. *Chaetoceros* sp., is cultivated under batch culture consists of a single inoculation of cells into a container of sterilized seawater followed by a growing period of 22 days and finally harvested when the algal population reaches its maximum or near-maximum density nutrient level, contamination with predators and other competing algae. pH was maintained as 7.9 throughout the growth study. The algae were grown in 1 L volumes in 2 L Erlenmeyer flasks. The inoculums for the strain were 5ml, containing about $5 x 10^4$ cells /ml. The strains were cultivated at 24^0C with a light: dark photoperiod of 14:10 h, with a light intensity of 115 $\mu molm^{-2}s^{-1}$ and were continuously stirred for aeration.

Algal oil Extraction: Solvent extraction of bio-oils historically has the highest yield of any extraction process, thus it sets the standard for comparison[9]. With ethanol solvent extraction, the water from the concentrated algae slurry is fully evaporated off leaving only the algae. After this, the dried algae are transported into a vessel where it is agitated in the presence of ethanol. The oils are extracted into the ethanol solvent and a filtration step is introduced to remove the solid biomass from the liquid oil phase; the ethanol is then boiled off and recycled. The remaining algal oil then continues to the biodiesel conversion step. In our research the ground algae were dried for 20 min at 80°C in an incubator for removal of moisture. The oil was extracted through solvent extraction process. To 0.5gm of dry algae, ethanol and con HCL added and it is heated well in water bath at 90˚C for 45 min and stirred well often. Then the mixture was extracted with petroleum ether and anhydrous sodium sulphate. At last the oil and petroleum ether mixture is heated in water bath at 50˚C and placed in a desicator for removal of moisture. The species of algae used will determine the net energy production. The algae with the higher oil content will inevitably produce more energy per process. The oil percentage was calculated on the basis of following formula:-

$$\text{Percent oil in Microalgae (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

Conversion to Biodiesel: Once the oil is extracted, it must be processed further to convert it into biodiesel. Raw fats and oils (glycerides) cannot be used directly in diesel engines without modifications to the fuel systems. The glycerides can contaminate the lubricating oil, induce the formation of carbon deposits in the engine, and affect engine durability, among other issues. Although other methods exist (pyrolysis, microemulsion, and blending of oils) current emphasis is on the process known as transesterification (alcoholysis). In transesterification, fats or oils are reacted with an alcohol to produce esters and glycerol in a one step reaction. The catalyst and alcohol are mixed together prior to the reaction with glycerides[10]. First the catalyst and alcohol are mixed together. This mixture is then fed to a reactor, where it is then combined with raw oils and continuously stirred. Next, the mixture of glycerin, biodiesel, and unreacted methanol is fed to a separator. Biodiesel and methanol are separated from the glycerin byproduct by use of either density loops or gravity settling. Finally, biodiesel and methanol are purified through evaporation to allow for collection of pure biodiesel[11]. The ester products can be used directly in diesel engines without blending it with petroleum diesel fuel[5].

Testing of Algal Oil and Biodiesel: Algal oil and the biodiesel produced from the transesterification process will be tested in order to determine if the conversion was successful or not. The extracted oil was analyzed for density, viscosity, acid value, saponification value and free fatty acids. In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a micro syringe (or, solid phase micro extraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column. The fatty acid composition of algal oil was determined through Gas Chromatograph. The fatty acids were identified by chromatographic retention time by comparison with standards.

Results and Discussion

Growth Study: The culture growth was determined for every 24 hours. Cell numbers were counted under a microscope with a haemocytometer. Growth rate of *Chaetoceros* species reached to a maximum of upto 750 cells per ml at 20th day of growth period and shown in figure1. The growth rate of *Chaetoceros* species expressed in cell counts in the F/2 media is illustrated in figure 2. The first-day counts showed that the culture was in lag phase, but from the second day onwards, there was a gradual increase in the multiplication of cells in all the flasks. In this study, though there was initially a slow growth, about 2.2 x 10' cellslml were noted. In the F/2 medium, although fluctuations of growth were noted in the middle, the rate of growth reached the peak on the 8th day, with 3.5 x 10* cellslml. All the cultures entered the declining phase on the 24th day.

Figure-1
***Chaetoceros* species grown in F/2 medium**

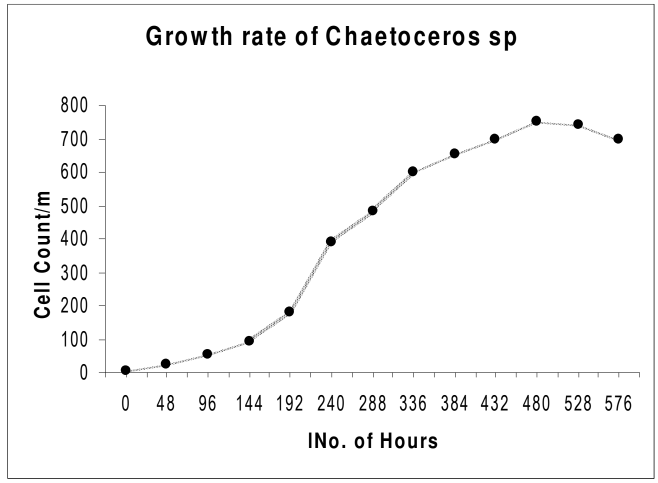


Figure-2
Graph representing growth rate of *Chaetoceros* species

Algal Oil Extraction: Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils[12]. Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops. On average basis 70 percent oil was recovered from marine microalgae. Author[13] also reported similar results of oil recovery from rice bran for different rice varieties. Microbial algal oils, also called single cell oils. It has been demonstrated that such microbial oils can be used as feed stocks for biodiesel production, and compared to other vegetable oils and animal fats; the production of microbial oil has many advantages such as short life cycle, less labor required, less affection by venue, season and climate, and easier to scale up[14]. Therefore, microbial oils might become one of potential oil feed stocks for biodiesel production in the future. If we let the algae grow naturally then the oil yield will be low, around one percent by the weight of the algae. If more carbon dioxide and organic material would be available to the algae, oil yield can be increased to as much as 40 percent by weight is shown figure 3.

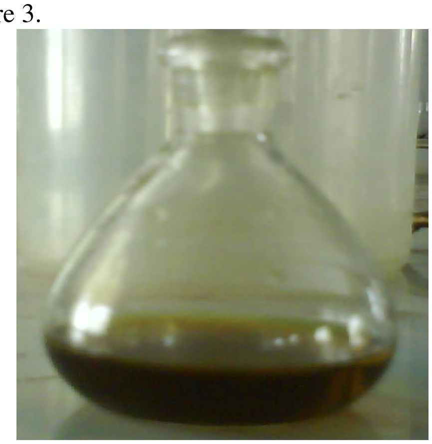


Figure-3
Picture representing microalgal oil

Biodiesel production: The process of converting vegetable and plant oils into biodiesel fuel is called transesterification. Chemically, transesterification means taking a triglyceride molecule or a complex fatty acid, neutralizing the free fatty acids, removing the glycerin and creating an alcohol ester. In making biodiesel, triglycerides are reacted with methanol in a reaction known as transesterification or alcoholysis. Transestrification produces methyl esters of fatty acids that are biodiesel, and glycerol. The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol. Transesterification is catalyzed by acids, alkalis[14] and lipase enzymes[15]. Alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction[14]. In order to shift the reaction to the right, it is necessary to either use excess alcohol or remove one of the products from the reaction mixture. The second option is usually preferred for the reaction to proceed to completion. The reaction rate was found to be highest when 100% excess methanol was used. Sodium hydroxide (1%) was used as catalyst for biodiesel production. Biodiesel production reached higher than 90% (v/v) at 50 – 60^{o}C within one hour is shown in figure 4.

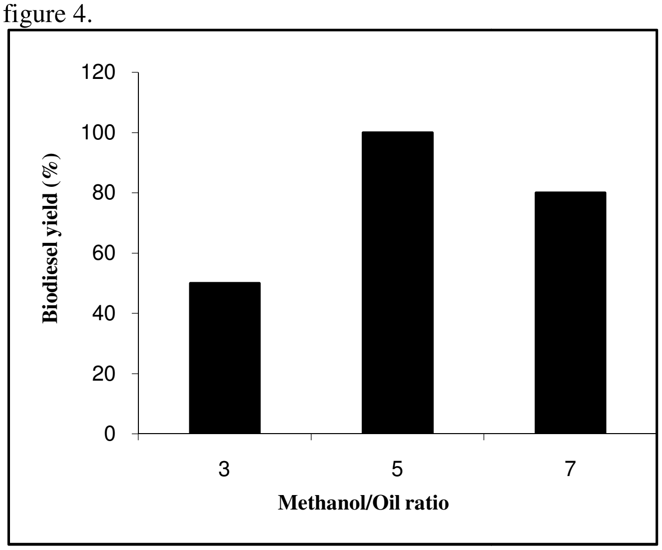


Figure-4
Biodiesel production from microalgal oil

Biochemical Analysis: Lipids are fatty acids and their derivatives, as well as substances related biosynthetically or functionally to these compounds. The most typical lipid classes of plant and animal origin consist of fatty acids linked by an ester bond to the trihydric alcohol, glycerol, or to other alcohols such as cholesterol, or by amide bonds to long-chain bases, or on occasion to other amines. Lipid type is important to biodiesel production because the quality and variety of lipids will determine the need for pretreatment before it is converted to biodiesel, as well as the final fuel properties. Microalgal oil contained about Lipid value of 400mg/l, Protein value 350mg/l and Carbohydrate about 380mg/l and it was graphically represented in figure 5

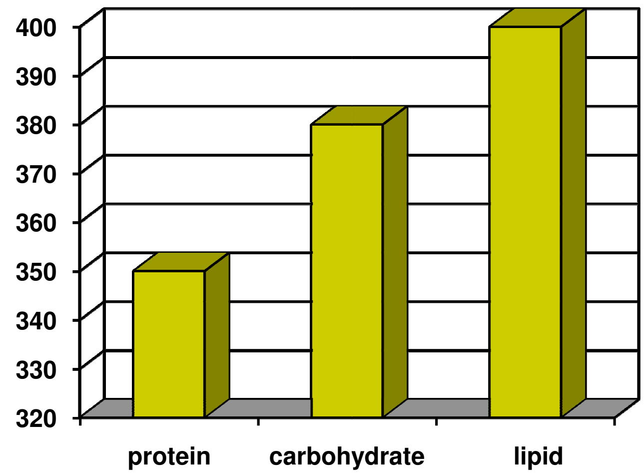
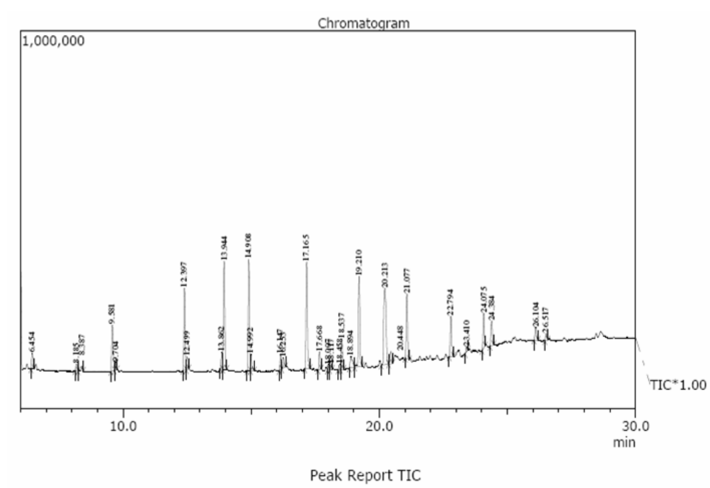


Figure-5
Biochemical representation of microalgal oil

The results on physico-chemical characteristics of marine microalgae indicated that the Density, Viscosity, Acid value, Saponification value and free fatty acids were recorded as 1.305gm/ml, 6.2mm^2/s, 2.5339mg/gm of oil, 173.56mg/gm of oil, and 0.71gm/100 gm of algae (Oleic acid) is shown in Table 1.

Table1-1
Physico–chemical characteristics of microalgal oil

S. No	Parameters	VALUES
1	Density	1.305gm/ml
2	Viscosity	6.2mm^2/s
3	Acid Value	2.5339mg/gm of oil.
4	Saponification value	173.56mg/gm of oil
5	Free fatty acid value	0.71gm/100 gm of algae (Oleic acid)



PEAK#	R.TIME	AREA	AREA%	NAME
1	6.454	162931	1.39	1-Decene
2	8.185	76984	0.66	Undecane
3	8.387	65229	0.56	Dimethoxymethylbenzene
4	9.581	511307	4.35	1-Dodecene
5	9.704	87068	0.74	Tetradecane
6	12.397	888039	7.56	1-Pentadecene
7	12.499	128692	1.10	pentadecane
8	13.862	186411	1.59	N,N-DIMETHYL-HEPTYLAMINE
9	13.944	1193140	10.16	2,4-Di-tert-butylphenol
10	14.908	1207835	10.28	1-Pentadecene
11	14.992	172113	1.47	1-Bromo-2-methyldecane
12	16.147	154971	1.32	Nonadecane
13	16.253	125003	1.06	DI-METHYLDODECYLAMINE
14	17.165	1187709	10.11	1-Nonadecene
15	17.668	181214	1.54	NEOPHYTADIENE
16	18.007	53194	0.45	Isobutyl phthalate
17	18.117	53446	0.46	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol
18	18.458	64183	0.55	(2r,4r)-3-benzoyl-4-benzyl-2-(tert-butyl)-4-isopropyl-1
19	18.537	341957	2.91	Methyl palmitate
20	18.894	231863	1.97	Palmitic acid
21	19.210	1030913	8.78	1-Nonadecene
22	20.213	1418071	12.07	Methyl linoleate
23	20.448	161043	1.37	Methyl behenate
24	21.077	700517	5.96	1-Nonadecene
25	22.794	446299	3.80	9-Hexacosene
26	23.410	52885	0.45	rac-1,3,4,6-tetraphenylhexane
27	24.075	357880	3.05	Monoethylhexyl phthalate
28	24.384	276854	2.36	9-Hexacosene
29	26.104	153689	1.31	1-Eicosanol
30	26.517	74850	0.64	Squalene
		11746290	100.00	

Figure- 6
GC chromatogram and fatty acid composition of C_ALGAL OIL

The fatty acid profile of marine microalgal oil indicated that Pentadecanoic acid (17.56%), 1.Nonadecenoic acid (20.1%), methyl palmitate (2.91%), methyl linoleate (12.07%), palmitic acid (1.97%) as major fatty acids and were represented in figure 6. The extracted microalgal oil consists of major fatty acids which is similar to rice bran oil in fatty acid composition.

Conclusion

Algae are more promising feed stocks to their wide spread availability and higher oil yields. Depending on species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils[12]. Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops. The experimental oil yield obtained from *Chaetoceros* species was about 70%. Algal oil is highly used for Biodiesel production which is eco-friendly. Once the oil is extracted from the algae the residue left is used for feed animals. The Density, Viscosity, Acid value, Saponification value and free fatty acids were recorded as 1.305gm/ml, 6.2mm^2/s, 2.5339mg/gm of oil, 173.56mg/gm of oil, and 0.71gm/100 gm of algae (Oleic acid). The obtained algal oil was analyzed by using Gas Chromatography. The fatty acid profile showed Pentadecanoic acid (17.56%), 1.Nonadecenoic acid (20.1%), methyl palmitate (2.91%), methyl linoleate (12.07%), palmitic acid (1.97%) as major fatty acids. In view of the physico-chemical parameters of microalgal oil extracted from *Chaetoceros* species can be efficiently used for biodiesel production.

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