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A Comparative Study on Three Algal Strains as Feedstocks for Biodiesel Production.

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ABSTRACT

Microalgae have been proved as the potential source for biodiesel production due to their high lipid production. Three microalgae Scenedesmussp., Chlorella sp. And Chlamy domonas sp. were studied as feed stocks for biodiesel production. Uni-algal culture were established and characterized for growth and lipid production potential. Scenedesmussp. showed highest lipid content of about 23% compared with Chlorella sp. And Chlamydomonas sp where as the lipid contents were about 14% for both strains. From GC analysis of biodiesel, ten group of saturated and unsaturated fatty acid were identified (C16–C24), and the most predominant fatty acids were C16 (palmitic acid) and C18 (Stearic, linoleic and oleic acid). These results confirm that an efficient production of biodiesel from the three microalgae could be possible. Scenedesmus was found to be the best algal strains for biodiesel production due to high lipid content followed by Chlorella and Chlamydomonas.

Keywords: Biodiesellipid content, Scenedesmus, Chlamydomonas, Chlorella

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INTRODUCTION

Carbon dioxide emissionsas a result of continued use of fossil fuel contribute one of the great problems to the earth which is global warming. Thus there is significant need for an alternative fuel that is renewable and carbon neutral.

Biodiesel is biodegradable, nontoxic and a low emission profiles, environmentally friendly biofuel. Also it has emerged as the most suitable alternative to petroleumdiesel fuel owing to its ecofriendly characteristics andrenewability. Comparedwith traditional fuels, biodiesel is carbon neutral, contributes lessemission of gaseous pollutants and hence is environmentally beneficial. Biodiesel is a mix of monoalkyl esters of long-chain fatty acids, obtained by chemical reaction (transesterification), coming from renewable feedstock suchas vegetable oil or animal fats, and alcohol with a catalyst [1].

Actually, one of the most promising feedstock for biodiesel production is unicellularalgae [2, 3].Microalgae are believed to be excellent organisms for fuel production because they exhibit a high photosynthetic efficiency and a strong capacity to adapt to the environment, high oil content and growth rate, and higharea-specific yield [4]. Moreover, microalgae can be cultivatedin saline/brackish water and on non-arable land includingmarginal areas that are unsuitable for agricultural purposes(e.g., desert and seashore lands); therefore,this precludes competition for the conventional crop land[5]. Also theyare potential organisms for utilizing excessive amounts ofCO_2, so they are capableof fixing CO_2 to produce energy and chemical compoundsupon exposure to sunlight [6, 7].These organisms use solar energy to create biomassand accumulate triacylglycerides (TAGs), which can be converted into biodiesel via transesterificationreaction [8, 9].

The average yield of microalgal biodiesel production is 10 to 20 times higher and requires less land area than the other oleaginous seeds[10]. Therefore,microalgae have been predicted to be a new biofuel source that is renewable and is environmentally and economicallysustainable [11-13] and thebiodiesel derived from microalgae have emerged as one of the most promising alternative sources of lipid for use in biodiesel production[14].

The success of biodiesel production from microalgae depends on the content of triglyceride (TAG) which is more than 70% of the lipid content [15-17].Transesterification of triglycerides by an alcohol(generally methanol) inthe presence of a catalyst will produce fatty acid methyl esters (FAMEs) [18-20].This process is the one thatis most commonly used to produce industrial biodiesel.

However, due to the high cost and low lipid yield, microalgae-based biodiesel production still lacks economic viability at a large-scale. Therefore, optimization of lipid production is important for biodiesel production from microalgae. Extensive research revealed that environmental conditions can modify the lipid metabolism of microalgae efficiently [21, 22].

Many parameters including lipid content, growth rate, fatty acid composition and cultivation conditions is considered an important factors to identify the most promising microalgae species and to maximize oil yield for biodiesel production [23].Also, Selection of species/strains that are robust and displayhigh growth and lipid accumulation rates is an important prerequisite for the success of micro algalbiofuel in future[24]. Most common algae like Chlorella, Dunaliella, Nanno chloropsis and Scenedesmushave oil levels between 20 and 50% [25-29].

So this study aimed to compare between three microalgal strains (Scenedesmussp., Chlorella sp. and Chlamydomonas sp.) as feedstocks for biodiesel production

MATERIALS AND METHODS

Isolation and Purification of microalgae:

Different fresh water samples were collected in sterilized bottles for isolation of microalgae and then samples were inoculated in Bold’s Basal medium and incubated at 25^0C under light intensity with 16:8 h for 10 days. After incubation, individual colonieswere picked and transferred to the same media for purificationin 250 ml conical flask. The culture broth was shakenmanually for five to six times a day. The pre-cultured sampleswere streaked on BBM medium-enriched agar plates andcultured for another 15 days with cool white fluorescent lightusing the same light intensity. The single colonies on agar were picked up and cultured inliquid BBM medium, and the streaking and inoculationprocedure was repeated until pure cultures were obtained.

Identification of the pure cultures was done by observing under themicroscope. Isolated and purified microalgae were inoculated in 250-mlErlenmeyer flasks containing 125 ml culture medium (BBM). Flasks were grownat room temperature with Light was provided by coolwhite fluorescent lamps at an intensity of 3000 Lux irradiance with 16:8 hours light and dark cycle for 30 days.The inoculums were then transferred to 1000-ml Erlenmeyer Flasks.

Biomass concentrations:

The growth of algae and biomass concentration was monitored by measuring optical density at a wavelength of 440 nm[30].

Lipid Extraction:

Lipid extraction was done by using chloroform/methanol (2:1) and estimated gravimetrically [31].

Determination ofFatty acid Methyl Ester (FAME) Content and Transesterification:

The fatty acids were converted to methyl esters [32]. The samples were esterified in 1% sulphuric acid in absolute methanol and extracted with hexane to separate the layers. The mixtureformed two phases, and the upper hexane layer contained thefatty acid methyl esters (FAMEs). Analysis of Fatty acids was carried out usinggas chromatography.

RESULTS AND DISCUSSION

Regarding to the growth of algal strains and biomass concentrations, Fig. 1 shows the progress of growth of three micro algae species measured as optical density (OD) of cultures at 440 nm.The optical density of the culture of all three species reached a maximum after 20 day of cultivation. Highest optical density (OD) was observed for Scenedesmus followed by Chlorella and least for Chlamydomonassp. The algal biomass were harvested at the end of an experiment and used to determine dry biomass yield, lipid content and lipid productivity (Table 1). Highest biomass yield was observed for Scenedesmus followed by Chlorella and sp. For lipid production Scenedesmussp. showed highest lipid content of about 23% followed by Chlamydomonas of 14.3% and Chlorella of 14%. The biomass and lipid productivity of the species under investigation is in agreement with that of observed by earlier reports [33-35].Jena et al.[36] screened three brackish water microalgal strains (Chlorococcum sp., Chlorella sp. and Scenedesmus sp.) of Odisha coast for the suitability for biodiesel production. They found Scenedesmus sp. to be the best one for high lipid productivity biomass yield. Similarly, Prabakaran and Ravindran[37] suggested that Scenedesmus sp. is useful for producing biodiesel, among different microalgal cultures isolated from six different water bodies from Gandhigram, Tamil Nadu based on its high lipid and oleic acid contents.

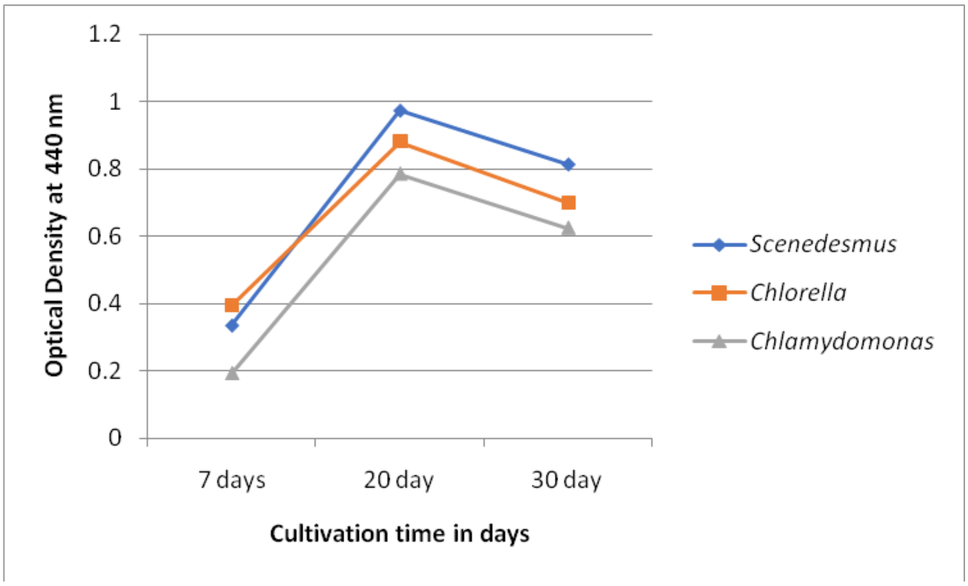

Fig 1: Growth monitoring of microalgae isolates on BBM liquid media at 440 nm.

Table 1: Specific growth rate and lipid productivity of microalgal strains

Microalgal strain	Biomass(dry weight)	Total lipid content	% of lipid to dry weight
Chlorellasp.	0.0529	0.0076	14%
Chlamydomonassp.	0.0526	0.0081	14.3%
Scenedesmussp.	0.083	0.0191	23%

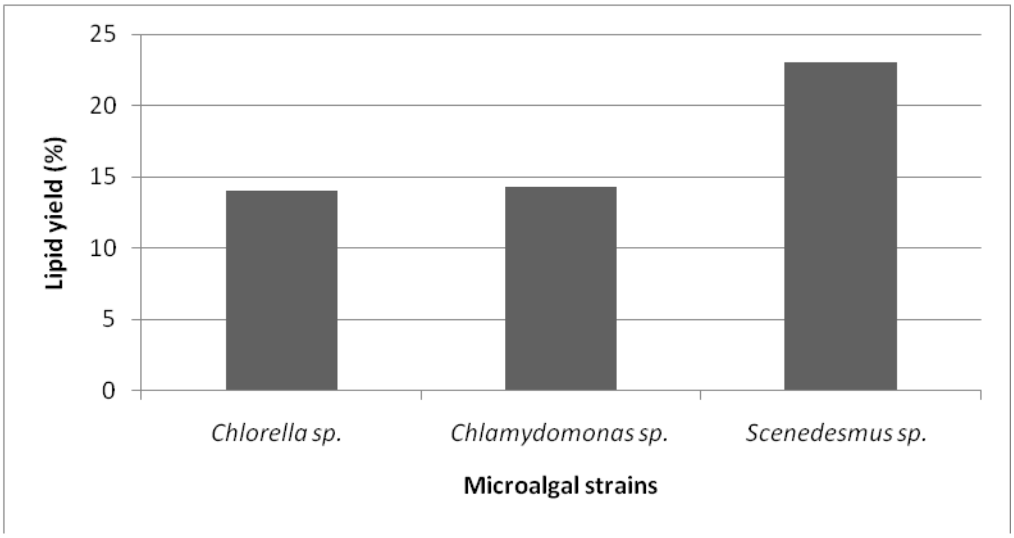

Fig 2: Total lipid content of microalgal strains

The lipids from the three microalgal strains were primarily transesterified and the major FAME compositionwere determined by GC analysis. The FAME composition was calculated as percentage of the total esters present in the sample, as shown in Table 2. The results obtained show that FAME obtained from the lipids of Chlorella, Chlamydomonas and Scenedesmusare mainly composed of saturated esters of 29.4 %, 29.3% and 28.9% respectively, among which palmitic (C16:0) is the most significant with a relative percentage of 16.7 % (wt) for Scenedesmus while Stearic (C18:0) was in relative percentage of about 13 % (wt) for Chlorella and Chlamydomonas.

With regard to the unsaturated esters, the percentages were ranged between 71 % for Scenedesmusand70.7%for Chlorella and Chlamydomonassp. From the unsaturated fatty acids, special attention should be given to linoleic (C18:2) and oleic (C18:1) where they recorded the highest percentages in the algal strains of 44% and 26.4% for Chlorella and Chlamydomonassp., where they were 47.8 and 22.7% for Scenedesmus.

Demirbas and Demirbas[38] reported that C16:0 and C18:1were the most important fatty acids, which were considered as the indicators for the quality of biodiesel. Yang et al. [39] observed that in Scenedesmussp. C16:0 and C18:1 presented in major quantities (about 60% of the total fatty acids). Similar results were reported by Chen et al. [40].

Table 2: Fatty acid composition of different algal isolates determined as FAME

Algal strains / FAME composition	Chlorella sp. (wt %)	Chlamydomonas sp. (wt %)	Scenedesmus sp. (wt %)
14:0 Myristic	0.033	0.033	0.040
15:1 Pentadecenoic	0.004	0.005	0.003
15:0 Pentadecanoic	0.006	0.005	0.006
16:1 Palmitoleic	0.071	0.071	0.117
16:0 Palmitic	15.239	15.039	16.683
17:0 Margaric	0.60	0.80	0.106
18:2 Linoleic	44.009	44.009	47.847
18:1 Oleic (9)	26.075	26.175	22.187
18:1 Oleic (10)	0.430	0.330	0.620
18:0 Stearic	13.030	13.030	11.670
19:1 Nonadecenoic	0.014	0.014	0.175
19:0 Nonadecenoic	0.020	0.020	0.032
20:1 Gadoleic	0.061	0.051	0.061
20:0 Arachidic	0.314	0.314	0.340
22:0 Behenic	0.051	0.061	0.059
23:0 Tricosanoic	0.031	0.011	0.012
24:0 Tetracosanoic	0.022	0.042	0.042

The relative degree of unsaturation and saturation of fatty acids in biodiesel feedstock influence the biodiesel properties [41]. Therefore, the ratio of saturated and unsaturated fatty acid is very important to microalgae as a biodiesel feedstock [36].

The high concentration of unsaturated fatty acids in the extracted lipids is determinant for the fuel quality. Unsaturated FAMEs comprised over 82% of the total biodiesel content [42, 43]. The FAMEs content is mainly composed of oleic, linoleic, linolenic, palmitic and stearic acids [44-46]. The higher saturated fatty acid content would cause higher oxidative and thermal stability, leading to a slower deterioration rate of the lipid characteristics [47].

Gour et al. [24]reported significant higher percentage of oleic acid was observed in Scenedesmussp.(15-16 %) compared to Chlorellasp. (4.58%).Rodolfi et al. [26] also reported highest oleic acid content in Scenedesmussp. among microalgal species they investigated. It has been reported by many workers that oleic acid methyl esters in biodiesel improve fuel properties of biodiesel[48, 49].

It was found that most predominant FAMEs were C16 and C18 methyl esters such as methyl palmitate (C16:0), methyl palmitolate (C16:1), methyl stearate (C18:0), methyl oleate (C18:1) and methyl linolate (C18:2). The order of the most abundant fatty acids contents were C18:2> C16:0> C18:1> C18:0. These results showed good agreement with the lipid compositions from different microalgal strains (Chlorella,Chlamydomonas and Scenedesmussp.) that contained higher amounts of unsaturated fatty acids than other microalgae in general even though their fatty acid compositions (Figs. 3-5). These results revealed that FAME from this microalgae had better quality of biodiesel since higher saturated fatty acids such as palmitic acid or stearic acid in biodiesel enhances the oxidative stability while higher unsaturated fatty acid such as linoleic acid or olenic acid reveals the opposite properties of saturated fatty acid [37; 50-52].

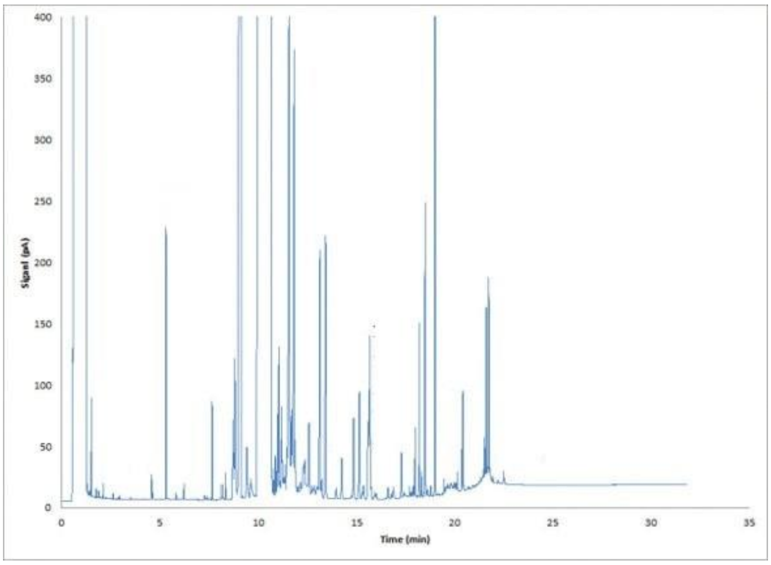

Fig 3: GC chromatogram of FAMEs from Chlorellasp

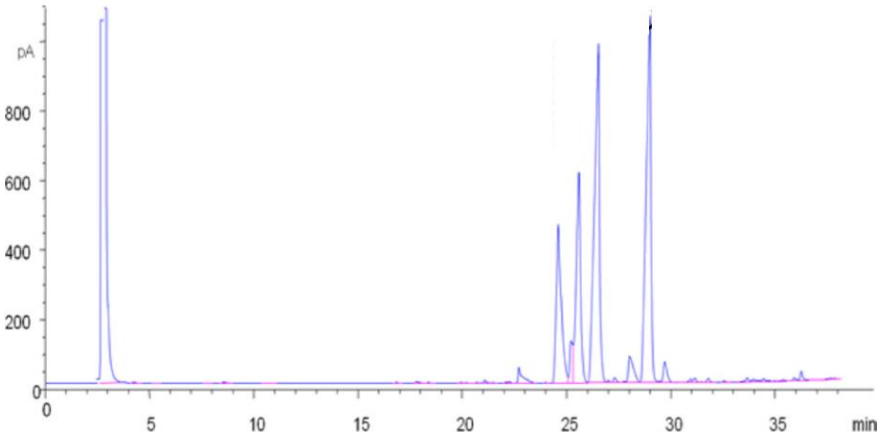

Fig 4: GC chromatogram of FAMEs from Chlamydomonassp

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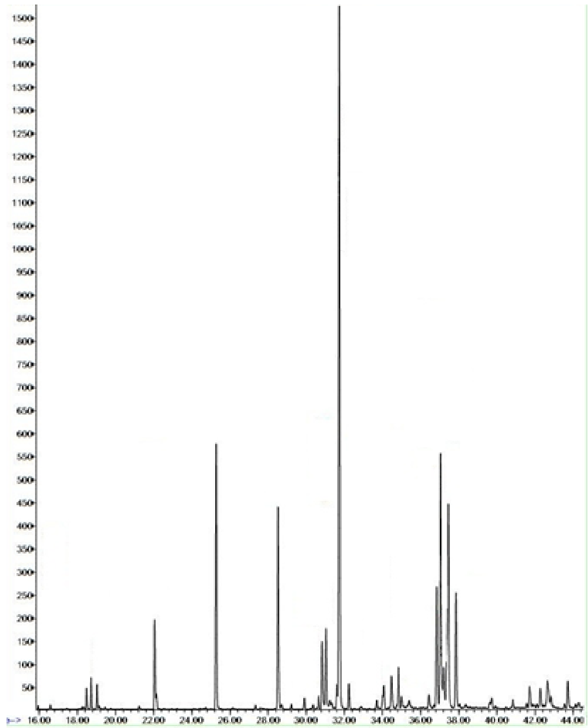

Fig 5: GC chromatogram of FAMEs from Scenedesmussp

CONCLUSIONS

The present study deals with three microalgal strains for biodiesel production. The experimental result suggests thatamong the tested strains, Scenedesmussp. was found to be the best algal strains for biodiesel production due to high lipid content followed by Chlorella and Chlamydomonas. Also the qualitative analysis of fatty acid show high value of palmitic acid along with maximum amount of unsaturated fatty acids (linoleic and oleic acid).

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