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Growth Kinetics and Fatty Acid Composition of *Chlorella vulgaris* (Trebouxiophyceae), *Scenedesmus quadricauda*(Chlorophyceae) & *Isochrysis galbana* (Prymnesiophyceae)

Biotechnology

KEYWORDS : Biodiesel, Biomass, Fatty acid, Microalgae

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ABSTRACT

Microalgae are the promising renewable source for future biodiesel production. The screening of potent microalgae species are more important to produce sustainable biofuel. In this present investigation, 2 fresh water microalgae Chlorella vulgaris Beyerinck [Beijerinck], Scenedesmus quadricauda (Turpin) Brebisson and marine microalga, Isochrysis galabana Parke were taken and the cultures were maintained at photo flux of 150µE m-2 s-1 (12 light/ dark), 25˚C in an orbital shaker at 150 rpm up to 30 days. The main parameters used in this study were spectroscopy for growth rate determination, FT-IR and GC-MS to detect the fatty acid esters. The maximum growth rate and increased biomass were obtained at 12 – 16 days culture. In the three tested microalgae, C12:0, C13:0, C14:0, C15:0, C16:0, C14:1, C16:1, C17:1, C18:1, 16:2, 18:2, 16:3, 18:3, 24:2. 24:3 and C26:3 were commonly present. C. vulgaris showed 0.210 day-1 growth rate and 34.75% Poly Unsaturated Fatty Acids (PUFA) whereas growth rate (0.202 and 0.181 day-1) and PUFA content (39.59 and 61.12) of S. quadricauda and I. galbana were comparatively higher. This study proved that the marine microalga, I. galbana are valuable candidate for PUFA to produce biodiesel.

Introduction

Globally, 80% of the energy supply based on fossil fuel, coal, gas and petro based oils. The renewable energy represents only 13% of total energy supply currently with biomass. Increased demand of fuel is the biggest motivation behind the increasing number of researches.

Microalgae are primary photosynthetic unicellular microorganisms, have several advantages, including higher photosynthetic efficiency, biomass production and growth rates as compared to other energy crops (Chisti, 2007). Many microalgae can produce substantial amounts (e.g. 20–50% dry cell weight) of triacylglycerols (TAG) as a storage lipid under different adverse environmental conditions. The lipid classes may differ depending on the species (Metzger & Largeau, 2005) and lipid productivity depends on medium composition, irradiance and temperature (Huerlimann *et al*., 2010). Microalgae biodiesel are biodegradable, renewable, economically cheap, eco-friendly and non-toxic fuel (Gouveia & Oliveira, 2009).

In this study, growth rate, biomass production, lipid content, and fatty acid composition of fresh water microalgae *Chlorella vulgaris, Scenedesmus quadricauda* and marine microalgae *Isochrysis galbana* were analyzed.

Materials and Methods

Microalgae Strain and Biomass Production

Two fresh water Microalgae *Chlorella vulgaris, Scenedesmus quadricauda* and marine microalgae *Isochrysis galbana* were obtained from the Central Institute for Brackish Water Aquaculture (CIBA), Chennai. Each microalgal strains were cultured in 1 L of BBM medium (Guillard & Ryther. 1962) for fresh water microalgae and f/2 medium (Bold 1949) for marine microalgae with 150 µmol m^{-2} s^{-1}, at 25˚C in an orbital shaker at 150 rpm in 12:12 hours light and dark up to 30 days.

Growth rates were determined by spectrophotometer (Hitachi, U-2900) at 680nm wavelength (Lee *et al.,* 1998). Growth rate (K) and generation time (G) was calculated by the equation 1 and 2 (Qin, 2005).

Growth (K) rate for Microalgae using optical densities can be calculated by,

$$K = \frac{(\log ODf - \log ODi)}{T} X\ 3.322 \text{ ---- (1)}$$

ODf: final Optical Density
ODi : Initial Optical Density
T : Time in days

Generation time (G) in days can be obtained by using the following formula,

$$G = \frac{0.301}{K} \text{ -------------- (2)}$$

Extraction

The biomass was harvested and disrupted using ultra sonic bath (Equitron) at frequency of 153 KHz for 1 minute. Each Microalgal samples were extracted with 3 ml of chloroform/methanol (1/2, v/v) by vortexing (1 min) and centrifugation at 8000rpm for 15 min at room temperature (RT). The supernatants were collected and residues reextracted thrice with 2 ml of chloroform/methanol (1/1, v/v) by centrifugation as stated above. All the supernatants were pooled together, filtered with Whatman No.1 paper and washed with 2 ml of milli-Q water, followed by centrifugation at 8000rpm for 5 min. The lower organic phases were collected and evaporated to dryness (Bligh & Dyer, 1959).

Transesterification

Fatty acid methyl esters (FAMEs) were prepared by transmethylation of fatty acids from oil extracts of each strain. The samples were reacted with 1ml of 1% NaOH in MeOH followed by heating at 55˚C for 15 minutes, after adding 2ml of 5% methanolic HCl, again heated for 15 minutes at 55˚C and then adding 1 ml milli-Q water. FAMEs were extracted by hexane (3:1) and evaporated to dryness under nitrogen. They were redissolved in 200 µl of hexane and stored in -40˚C in glass vials. (Carreau & Dubacq, 1978).

The FAME Samples were identified by GC and MS (JEOL GC mate) and FTIR (Perkin Elmer model spectrum-I PC).

Results

Growth Rates of Microalgae

Out of these 3 strains, *C. vulgaris* and *S. quadricauda* had the fastest growth rate during 12 - 16 days; this is a period suitable for large-scale production. Their biomass usually doubles within 34.4h and 35.63h during the late log growth phase. The net growth rates differed among the examined species under similar environmental conditions. The average growth rates of *C. vulgaris*, *S. quadricauda* and *I. galbana* were 0.210, 0.202 and 0.181 $days^{-1}$, respectively. The growth rate of *Chlorella vulgaris* after 16 days of incubation was 6.572 compared with an initial reading of 3.944 at OD 680 nm. This result proved that *Chlorella vulgaris* is suitable for mass culturing. Algal growth rate is directionally proportion to availability of nutrients, light, the stability of pH, temperature and the initial inoculums density. An increase in the initial inoculums density leads to better algal growth and increases the nutrient removal efficiency.

Maximum dry biomass was observed at the 12th and 16th days. Among three strains including *C. vulgaris* and *I. galbana* had the highest optical density of the cells, which ranged between 4.88 and 6.57. The growth rate and generation time of each selected microalgal species is shown in Table 1. Based on the daily readings, the highest productivity was for *Chlorella vulgaris*.

Fatty Acid Analysis

Fig.1 represented the GC spectra of the 3 microalgae. In a previous report (Knothe, 2008), palmitic, stearic, Palmitoleic acid, oleic, and linolenic acid were recognized as major fatty acids in biodiesel. In the three tested microalgae, C12:0, C13:0, C14:0, C15:0, C16:0, C14:1, C16:1, C17:1, C18:1, 16:2, 18:2, 16:3, 18:3, 24:2. 24:3 and C26:3 were commonly present. C14:1 and C16:3 were higher in *Chlorella vulgaris* at 28.81 % and 25.20 % respectively, whereas C16:2 (26.63 %), C16:1 (24.20 %) were highest in *S. quadricauda* and C16:2 (39.99 %), C16:0 (19.27 %) were present in *I. galbana*. The lipid productivity of each microalgal species is represented in Table 1. In particular, oils with high Linoleic acid content have been reported to have a reasonable balance of fuel properties (Rashid *et al.,* 2008).

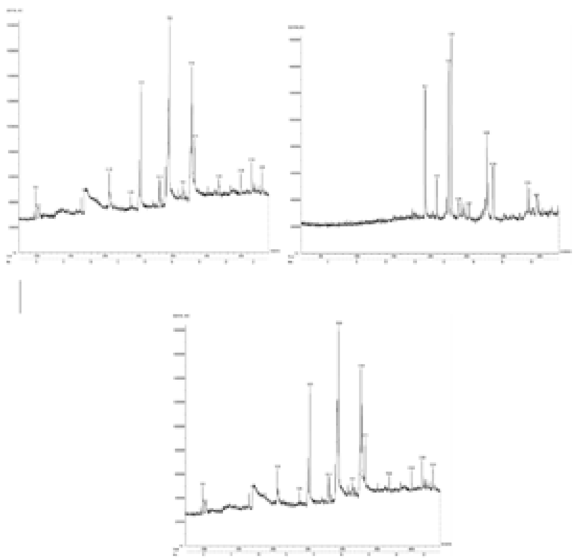

Fig. 1 GC Spectra of *Chlorella vulgaris*, *Scenedesmus quadricauda* & *Isochrysis galbana*

PUFAs were 34.75%, 39.59%, and 61.12% for the lipids extracted from *Chlorella vulgaris*, *S. quadricauda* and *I. galabana* respectively. The current state shows, biodiesel that is produced from these crude lipids will have a high cetane number and a high oxidative stability. In addition, high methyl ester yields with 16 and 18 carbon atoms were achieved by transesterification of the cellular crude lipids.

Infra-red (IR) spectrometry

Fig 2 showed the FTIR spectra wave number of *C. vulgaris*, *S. quadricauda* and *I. galbana* showed 3400–736 cm^{-1}, 3900 – 129 cm^{-1} and 3395 – 713 cm^{-1}. Two bands of *Chlorella* sp had of particular interest, the band at 1644, 1452 and 1317 cm^{-1} which was associated with (C=O) of ester groups, primarily from lipids and fatty acids. The bands range at 3402 and 2136 cm^{-1} which indicates C-H correspond to the asymmetric and symmetric vibrational modes of methyl groups and CO_2. Whereas 1085 and 736 cm^{-1} peaks formed by H_2O and CO_2.

S. quadricauda showed 8 absorption peaks between 3395 – 713 cm^{-1} of wavelength, with these 3395, 2953 and 713 cm^{-1} denoted as H_2O, 2922 and 2851 cm^{-1} were CO_2, C=O stretching absorption band in the region1642 and 1456 cm^{-1}.

In the case of *I. galbana* we observed 12 bands from 3900 – 729 cm^{-1}, the bands near 4000 – 3000 cm^{-1} showed formation of water and CO_2. It produced 4 peaks (2955, 2923, 2852 and 2314 cm $^{-1}$) between 3000 – 4000 cm^{-1} because the formation of C-H represented the methyl groups. The band 1641 cm^{-1} denoted the C=O groups. C-O-C corresponding to ethers (1213, and 1080 cm^{-1}) stretching vibrations produces a strong band in the 1200–900 cm^{-1} regions. The least wave numbered band 729 cm^{-1} formed by CO_2 in spectra (Antonio Marcilla *et al.,* 2009).

Discussion

Microalgae can produce largest biomass and having attracting attention as a source biofuel production. Highest growth rate, the maximum biomass production and the increased lipid productivity were obtained after 16th day of culture. *I. galbana* proved to be suitable as raw materials for commercial production, due to their high PUFA content (61.2%) whereas *C. vulgaris* and *S. quadricauda* had 34.75% and 39.53%. But *Chlorella vulgaris* had high growth rate of about 0.210 which showed high biomass productivity. The main fatty acid composition that found in biofuel and which obtain through this studied species was mainly C12:0, C14:0, C16:0, C18:0, C18:1, C18:3, C16:1, and C14:0. The result of this study proves that the marine microalgal strain *I. galbana* have high PUFA, a valuable candidate for biodiesel production.

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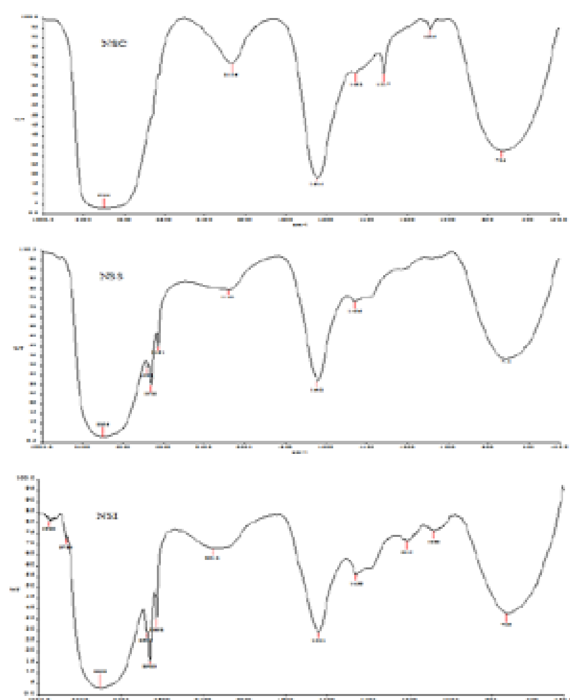

Fig. 2 FT-IR Spectra of *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Isochrysis galbana*

Table.2 The lipid productivity of *Chlorella vulgaris, Scenedesmus quadricauda* and *Isochrysis galbana*

Fatty Acid	Chlorella vulgaris	***Scenedesmus quadricauda***	Isochrysis galbana
Saturates			
12:0	nd	nd	7.39
13:0	15.58	nd	nd
14:0	nd	21.98	nd
15:0	17.61	5.17	nd
16:0	nd	6.38	19.27

MUFA			
14:1Δ^{9}	28.81	nd	nd
16:1Δ^{11}	3.19	24.20	nd
17:1Δ^{9}	nd	2.71	nd
18:1Δ^{9}	nd	Nd	12.19
PUFA			
16:2Δ$^{7, 10}$	nd	26.63	39.99
18:2Δ$^{6, 11}$	2.18	12.96	nd
16:3Δ$^{6, 9, 12}$	25.20	nd	nd
16:3Δ$^{9, 12, 15}$	1.89	nd	nd
18:3Δ$^{3, 9, 12}$	nd	nd	6.80
24:2Δ^{15,18}	3.45	nd	nd
24:3Δ$^{5, 9, 17}$	2.03	nd	nd
26:3Δ$^{5, 9, 19}$	nd	nd	14.33

*nd: not detected

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