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Research Article

A study of increasing biomass and lipid content from *Chlorella* sp. for biodiesel conversion

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Abstract

Biodiesel has received significant attention since it was made from nontoxic and biodegradable materials, and its use leads to a huge decrease in the emissions of greenhouse gases and air pollutants. Therefore, improving lipid content of microalgal strains could be a cost effective second generation feedstock for biodiesel production. Growth and lipid accumulation in *Chlorella* sp. was studied under various culture conditions such as nitrate concentrations, temperature and CO_2 concentration. The most significant increase in lipid reached 37.5±0.21% of dry cell weight, which was recorded in N-/30ºC/CO_2 culture in BG 11 medium. Best biomass productivity was obtained at 30ºC under conditions of nitrogen sufficiently and CO_2 supplementation (N+/30ºC/CO_2). The adequate fatty acid profile was analysed by Gas chromatography. The presence of oleic and linoleic fatty acid were the major constituents makes *Chlorella* biomass a suitable feedstock for biodiesel production.

Keywords: Biodiesel; *Chlorella* sp.; Fatty acid profile; Lipid content; Biomass.

Introduction

As a result of continued fossil fuel depletion, increasing air pollution and global warming, the world has put increasingly more attention into the development and utilization of alternative energy resources [1]. The biofuel production from photosynthetic microorganisms is considered as an effective strategy to produce renewable energy [2,3]. One promising candidate of biomass for alternative fuel production is micro-algae, which have high growth rates [4]. Recent studies have found that the lipid content of algae could be increased through changing cultivation conditions, such as CO_2 aeration fixation, temperature, salinity, and nutrient concentration [5,6].

Most research work have focused on the growth and lipid accumulation through cultivation in photo-bioreactors and the lipid content of algae could be increased dramatically under certain stress factors. However, relatively few reports focused on fatty acid methyl esters (FAME) and fatty acids composition analysis [7], especially the content and accumulation of C16 and C18 series (as % of total FAME), which are the principle sources for algal biodiesel [6].

In the present work, *Chlorella* sp. was evaluated as a potential source for biodiesel production. This optimization of the growth environment, especially in terms of nitrate and CO_2 as well as temperature, was undertaken, and the lipid content was determined. Furthermore fatty acid profile was analysed by Gas chromatography.

Materials and methods

Isolation, purification and identification of microalgae

Algae samples were collected from six different water bodies in and around Dindigul district, Tamil Nadu and purified by standard plating methods, identified and authenticated based on a standard manual [8].

Lipid extraction

The total lipids were extracted [9] from seven microalgal biomass basis on purity. In brief, 50 mL of microalgae culture was harvested by centrifugation at 4000 rpm, re-suspended in 1 mL distilled water, the sample was then mixed with 1.25 mL chloroform and 2.5 mL methanol (1:2 v/v) and subjected sonication at 50 Hz for 30 min. After sonication, the tubes were

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incubated overnight at 27ºC at 100 rpm. The next day, an additional portion of chloroform (1.25 mL) was added, and the extraction mixture was sonicated again for 30 min. To separate the chloroform and aqueous methanol layers, 1.25 mL water was added and then centrifuged at 4000 rpm for 10 min. The chloroform layer was gently removed from the bottom, and a second extraction was performed by adding 2.5 mL chloroform and vortexing. The chloroform portions were collected and washed with 5 mL 5% NaCl solution and evaporated in an oven at 80ºC to dryness. Thereafter, the weight of the crude lipid obtained from each sample was measured gravimetrically. Experiments were performed in triplicate and data are expressed as mean ± SD.

Effect of Nitrate, CO_2 and Temperature

Chlorella sp. was selected for this study based on the potential of lipid content. *Chlorella* sp. was grown in 2L glass bubble column bioreactors with continuous stirring by bubbling filtered air in BG 11 medium [10]. For the treatments viz., temperature (26 and 30ºC), CO_2 (without or with supplementation: 0.04 and 5% [v/v], respectively), and nitrate (with or without), and all combinations of these variables. The culture was continuously illuminated with six fluorescent lamps at an irradiance level of 2500 lux for 21 days. Growth was evaluated over time in terms of dry weight (DW). Lipid content was analyzed by modified method of Bligh and Dyer.

Fatty acid composition analysis

A fatty acid composition analysis was performed using a Shimadzu 2010 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector and a DEGS capillary column (30mx0.25x0.25µm). *Chlorella* sp. oil (100 µL) was placed into capped test tubes, saponified with 1 ml of saturated KOH-CH_3OH solution at 75^0C for 10 min, and then subjected to methanolysis with 5% HCl in methanol at 75°C for another 10 min [11]. Thereafter, the phase containing the fatty acids was separated by adding 2 ml of distilled water and then recovered. The components were identified by comparing their retention times and fragmentation patterns with those for standards [12]. Six fatty acids (C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3) were used as the standard materials.

Result and discussions

Microalgae are present in all existing earth ecosystems, not just aquatic, but also terrestrial representing a large variety of species living in a wide range of environmental conditions [13]. In this study, a total of 16 microalgal cultures (Table 1) were isolated from six different water bodies. Only seven microalgae (*Chlorella Haematococcus, Spirulina, Chlorococcum, Desmococcus, Sytonema* and *Tolypothrix*) were selected for extraction of lipids based on their purity. The total lipid contents for the microalgae cultured in this study ranged from 5±0.81% to 29.4±0.72% of the dry weight. The lipid content from *Chlorella* sp. was 29.4±0.72% of the dry weight, which was about 5.8 times higher than that from *Spirulina* sp. (Fig. 1). Many microalgae species can be induced to accumulate substantial quantities of lipids, contributing to high oil yield [14]. In previous studies [15, 16] observed that the total lipid contents ranged from 20-50% of dry bio mass weight were quite common, and some microalgae even exceeded 90% as a response to different culture conditions.

Table 1. Isolation of microalgae from different water bodies in and around Dindigul district

S. No	Location	Latitude	Longitude	Name of the microalgae
1	Kamarajar dam	10°17’43.44” N	77°48’44.06” E	*Chlorella* sp.
2	Palar dam	10°24’30.61” N	77°29’38.39” E	*Haematococcus* sp.
3	Palani pond	10°26’12.59” N	77°30’52.27” E	*Spirulina* sp., *Chlorococcum* sp.
4	Manjalar dam	10°11’37.15” N	77°37’55.86” E	*Desmococcus* sp.
5	Nerhu pond	10°16’39.12” N	77°56’04.75” E	*Sytonema* sp.
6	Anaippatti dam	10°05’20.15” N	77°51’10.28” E	*Tolypothrix* sp.

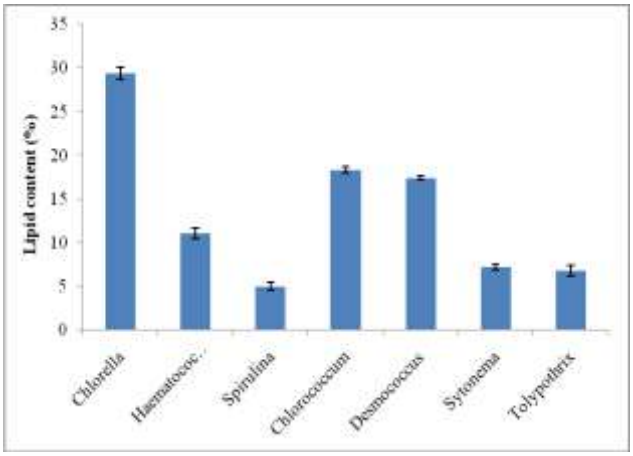

Fig 1. Lipid content in isolated microalgae

Microalgal biomass growth data of *Chlorella* sp. under the six experimental conditions are depicted in Fig. 2. Culture curves showed the fastest growth for N+/30ºC/CO_2 (nitrate sufficient medium with CO_2 enrichment at 30ºC). Comparing this culture with N-/30ºC/CO_2 culture (nitrogen free medium with CO_2 enrichment at 30ºC), it can be observed that the former attained a 2-fold higher growth after 15 days. The CO_2 enrichment in the inlet air flow enhanced algal growth as expected, which can be seen by comparing the culture curves for N+/30ºC/CO_2 with N+/30ºC and N-/30ºC/CO_2 with N-/30ºC, showing that cultures under air bubbling were C-limited. In terms of temperature influence on *Chlorella* sp. biomass productivity, Fig. 2 curves showed that *Chlorella* sp. grew faster at 30 ºC than 26ºC, irrespective of supplemented CO_2.

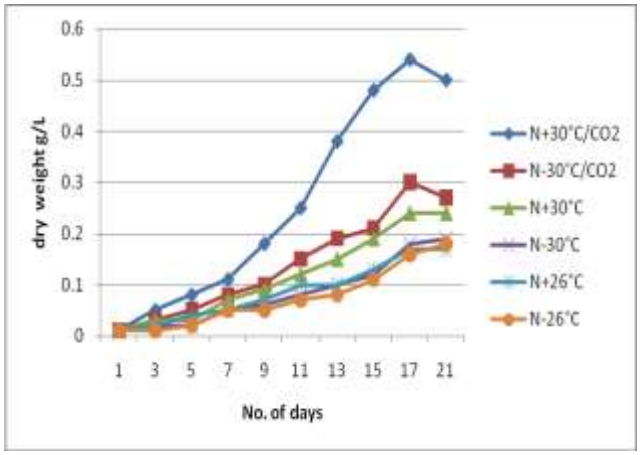

Fig. 2. Average dry weights of *Chlorella* sp. under different growth conditions over time

Higher lipid content for the cultures under nitrogen starvation. N-/30 ºC/CO_2 culture reached the maximum lipid content (33.1±0.28%) after 21 days of nitrogen shortage (Fig. 3), which is comparable with results from other authors [17], followed by the N-30 ºC and N-/26 ºC cultures reached 31.5% lipid content. Nitrogen limited growth can stimulate the cells to produce more lipids per cell since protein biosynthesis is limited [18].

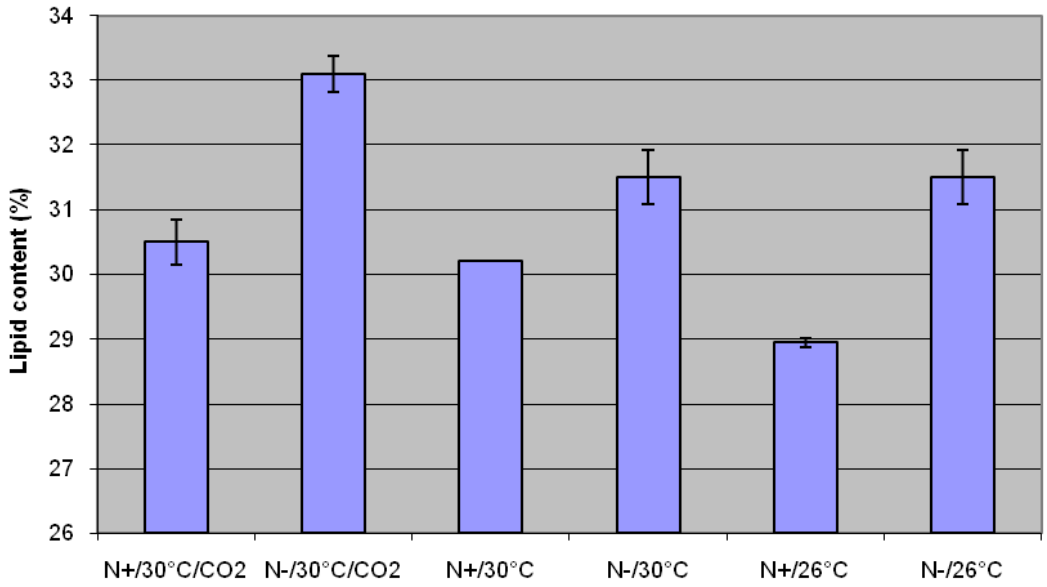

Fig. 3. Lipid content of *Chlorella* sp. under different growth condition after 21 days of incubation

Fatty acids in the *Chlorella sp*. microalga were primarily esterified, and the fatty acid compositions were determined using GC analysis (Table 2). Oleic acid (C18:1) and linoleic acid (C18:2) were dominant, which ranged 52.8% and 43.2% respectively. The highest amount of oleic acid (11.77 mg g^{-1} dw) and linoleic acid (9.63 mg g^{-1} dw) was detected

in *Chlorella* sp. Oils with high oleic acid content have been reported to have a reasonable balance of fuel, including its ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity and lubricity, which are determined by structure of its component fatty esters [19]. Therefore, *Chlorella* sp. is suitable for the production of good quality biodiesel.

Table 2. Fatty acid composition of *Chlorella* sp.

Fatty acid methyl ester	Amounts of fatty acids (mg g^{-1} dw)	Fatty acid methyl ester composition (wt %)
C16:1	ND	
C17:0	0.21	(0.94)
C18:0	0.59	(2.64)
C18:1	11.77	(52.8)
C18:2	9.63	(43.2)
C18:3	0.09	(0.4)
Total	22.29	(100)

ND: not detected

Conclusions

To get microalgae with high biomass and lipid productivity, seven microalgae were selected based on purity and easy cultivation. The highest lipid content (29.4%) was found from *Chlorella* sp. In the growth analysis of *Chlorella* sp., the fastest growth was observed in N+/30ºC/CO_2 (nitrate sufficient medium with CO_2 enrichment at 30ºC) but maximum lipid content were in N-/30ºC/CO_2 cultures. The composition of fatty acids in *Chlorococcum* sp. was mainly C18:1 and C18:2. The results of the study indicate that the higher lipid productivity can be obtained by varying not only the nutrient starvation but also normal nutrition and *Chlorococcum* sp. is a suitable microalga for biodiesel production

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Conflict of interest

Authors declare there are no conflicts of interest.

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