Study of Lipid Accumulation from *Chlorococcum* Microalga for Biodiesel Conversion

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Abstract

Microalgae-derived biodiesel seems to be a viable substitute for petroleum-based transportation fuels as a renewable biofuel. Raising the lipid content of microalgal strains may therefore provide a low-cost second-generation feedstock for the synthesis of biodiesel. Chlorococcum growth and lipid accumulation were investigated under a range of culture conditions, including CO2 concentration, temperature, and nitrate concentrations. The lipid content increased significantly by 33.1 ± 0.28% of dry cell weight in N-/30 ºC/CO2 cultivation in Bristol medium. The highest biomass productivity was achieved at 30ºC when there was adequate nitrogen and CO2 supplementation (N+/30 oC/CO2). Gas chromatography was employed to determine the proper ratio of fatty acids. The biomass of chlorococcum shows great potential as a feedstock for the manufacture of biodiesel since it is mostly composed of oleic and linoleic fatty acids.

Keywords: Biodiesel; Chlorococcum; Fattyacid Profile; Lipid Content

Introduction

Global warming, increasing air pollution, and the ongoing depletion of fossil fuels have caused people to pay increased attention to the creation and utilization of alternative energy sources (Farrell et al., 2006). It is thought that producing biofuel from photosynthetic microorganisms is a successful way to provide renewable energy (Ma and Hanna, 1999; Vicente et al. 2004). Microalgae offer a promising biomass choice for alternative fuel production due to their rapid growth rates (Rittmann, 2008). Recent research has shown that

adjusting cultivation parameters such as CO2 aeration fixation, temperature, salinity, and nutrient concentration can improve the lipid content of algae (Chiu et al., 2009; Converti et al., 2009). Most studies have focused on the growth and accumulation of lipids in algae grown in photo-bioreactors; under specific stress conditions, algae can have a significantly higher lipid content. However, few studies have focused on fatty acid methyl esters (FAME) and fatty acid composition analysis (Damiani et al., 2010), particularly the content and accumulation of the C16 and C18 series (as% of total FAME), which are the primary sources of algal biodiesel (Converti et al., 2009).

This study evaluated chlorococcum as a possible biodiesel source. The growth environment was optimized, specifically with regard to temperature, nitrate and CO2 levels, and lipid content measurements. Furthermore, gas chromatography was used to investigate the fatty acid profile.

Materials and Methods

Isolation, Purification and Identification of Microalgae

Algae samples were obtained from six different water bodies in and around Dindigul district, Tamil Nadu. They were purified using standard plating method, identified and validated using a standard manual (Prescott 1959).

Lipid Exraction

Total lipids were extracted (Bligh and Dyer, 1959) from seven microalgal biomass samples based on purity. In brief, 50 mL of microalgae culture was harvested by centrifugation at 4000 rpm, resuspended in 1 mL of distilled water, mixed with 1.25 mL chloroform and 2.5 mL of methanol (1:2 v/v), and sonicated at 50 Hz for 30 minutes. After sonication, tubes were incubated overnight at 27ºC and 100 rpm. The extraction mixture was sonicated for an additional half-hour the following day after 1.25 mL of chloroform was added. The layers of aqueous methanol and chloroform were separated by centrifuging 1.25 mL of water for 10 minutes at 4000 rpm. The chloroform layer was gently scraped from the bottom, and a second extraction was carried out by adding 2.5 mL of chloroform and vortexing. The chloroform components were collected, rinsed with 5 mL of 5 % NaCl solution, and evaporated in an oven at 80°C. Next, each sample's crude lipid weight was determined by gravimetric analysis. Data from trials conducted in triplicate are shown as mean ± SD.

Effect of Nitrate, CO_2 and Temperature

Chlorococcum sp. was chosen for this investigation due to its potential lipid content. Chlorococcum sp. was cultivated in 2L glass bubble column bioreactors with continuous stirring using filtered air and Bristol medium (James, 1978). Temperature (26 and 30ºC), CO2 (0.04 and 5 % [v/v] without or with supplementation), nitrate (with or without), and combinations of these factors were the treatments. For 21 days, the culture was illuminated constantly with six fluorescent lamps at a 2500 lux irradiance level. Growth was measured throughout time in terms of dry weight (DW). The lipid content was measured using a modified Bligh and Dyer method.

Fatty Acid Composition Analysis

The fatty acid composition was analysed on a Shimadzu 2010 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) with a flame ionisation detector and a DEGS capillary column (30mx0.25x0.25µm). Chlorococcum sp. oil (100 µL) was placed in closed test tubes, saponified with 1 ml of saturated KOH-CH3OH solution at 750C for 10 min, and then treated to methanolysis with 5% HCl in methanol at 750C for another 10 min (Schreiner, 2006). The fatty acid-containing phase was then separated with 2 ml of distilled water and recovered. The

components were identified by comparing their retention and fragmentation patterns to those of standards (Xu et al., 2001). Six fatty acids (C16:1, C17:0, C18:0, C18:1, C18:2, and C18:3) were utilised as the standard materials.

Result and Discussions

Microalgae comprise a vast range of organisms that exist in a variety of environmental circumstances and are present in all contemporary earth ecosystems, both terrestrial and aquatic (Sydney et al. 2010). Six distinct water basins provided the 16 microalgal cultures (Table 1) used in this investigation. A selection of seven microalgae, selected for lipid extraction, were found to be pure: Chlorella haematococcus, Spirulina, Chlorococcum, Desmococcus, Sytonema, and Tolypothrix. The microalgae cultivated in this study had lipid levels ranging from 5±0.81% to 29.4±0.72 % in dry weight. Chlorococcum sp. has a lipid content of 29.4±0.72% of dry weight, almost 5.8 times more than Spirulina sp. (Fig. 1). Many microalgae species can be encouraged to accumulate large amounts of lipids, resulting in a significant oil output (Sheehan et al., 1998). Previous research (Spolaore et al., 2006; Li et al., 2008) found that total lipid concentrations ranging from 20-50 % of dry biomass weight were relatively common, with some microalgae exceeding 90 % in response to different culture conditions.

Figure 2 depicts the microalgal biomass growth data of Chlorococcum sp. under six experimental conditions. N+/30ºC/CO_2 (a nitrate-sufficient medium with CO_2 enrichment at 30ºC) had the fastest growth curves. When compared to the N-/30ºC/CO_2 culture (nitrogen-free medium with CO_2 enrichment at 30ºC), the former showed double the growth after 15 days. CO2 enrichment in the inlet air flow increased algal growth, as expected. C-limited cultures were found in air-bubbled cultures when comparing N+/30ºC/CO_2 with N+/30ºC and N-/30ºC/CO_2 with N-/30ºC, according to culture curves. Chlorococcum sp. biomass productivity increased at 30 ºC compared to 26 ºC, regardless of CO_2 supplementation (Fig. 2 curves).

Cultures under nitrogen deprivation had a higher lipid content. After 21 days of nitrogen scarcity, the N-/30 ºC/CO_2 culture had the highest lipid content (33.1±0.28%) (Fig. 3), which is comparable with previous studies (Gouveia et al., 2009). The lipid content of the N-30 ºC and N-/26 ºC cultures was 31.5% apiece. Since protein production is restricted, nitrogen-limited development might cause cells to generate more lipids per cell (Suen et al., 1987).

Fatty acids in the Chlorococcum sp. microalga were primarily esterified, and their contents were measured by gas chromatography (Table 2). Oleic acid (C18:1) and linoleic acid (C18:2) were prevalent, accounting for 52.8% and 43.2%, respectively. Chlorococcum sp. had the highest concentrations of oleic acid (11.77 mg g-1 dw) and linoleic acid (9.63 mg g-1 dw). The structure of the fatty esters that make up an oil's constituents determines its ignition quality, combustion heat, cold filter plugging point (CFPP), oxidative stability, viscosity, and lubricity. It has been demonstrated that oils with a high oleic acid content have an appropriate fuel balance (Knothe, 2008). As a result, Chlorococcum sp. can be used to produce good quality biodiesel.

Conclusions

To obtain microalgae with high biomass and lipid production, seven microalgae were chosen based on purity and cultivation. The highest lipid content (29.4%) was found in Chlorococcum sp. The Chlorococcum sp. growth analysis revealed that the N+/30ºC/CO_2 (nitrate sufficient medium with CO_2 enrichment at 30ºC) cultures grew the fastest, whereas the N-/30ºC/CO_2 cultures had the highest lipid content. The composition of fatty acids in Chlorococcum sp. comprised primarily C18:1 and C18:2. The study results indicate that increased lipid production can be obtained by altering not only nutrient starvation but also standard nutrition. Chlorococcum sp. is an excellent and suitable microalga for biodiesel production.

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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.

Table 2 Fatty Acid Composition of Chlorococcum 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

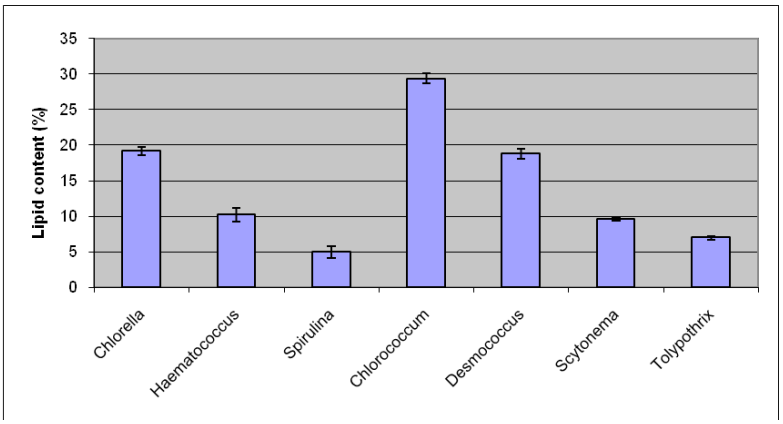

Figure 1 Lipid Content in Isolated Microalgae

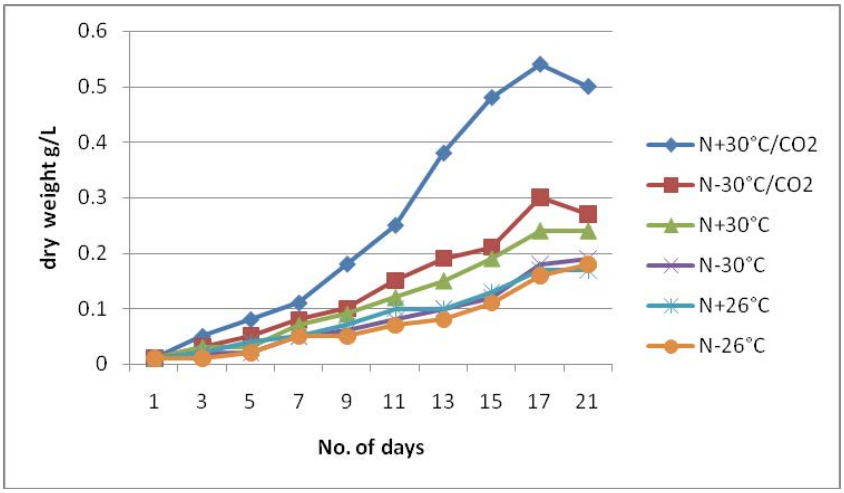

Figure 2 Average Dry Weights of Chlorococcum sp. Under Different Growth Conditions Over Time

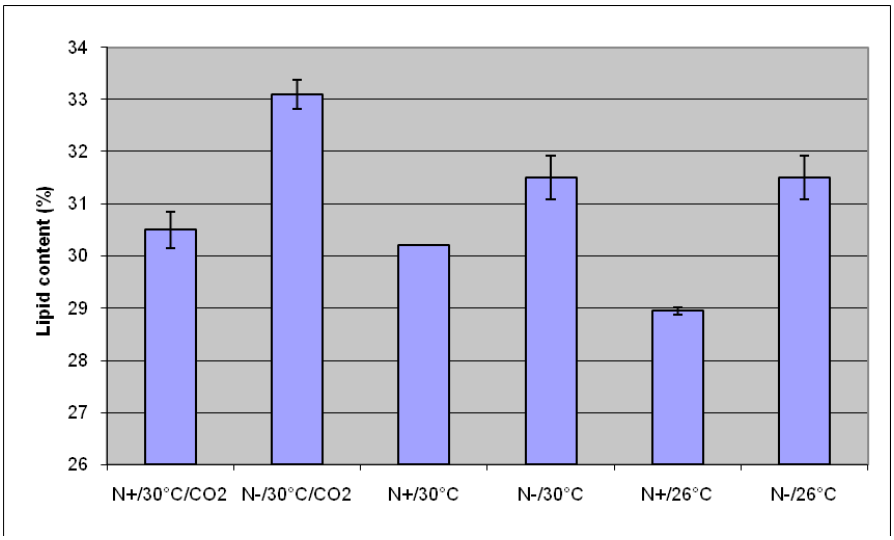

Figure 3 Lipid Content of Chlorococcum sp. Under Different Growth Condition After 21 Days of Incubation