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Lipid extraction and CO_2 mitigation by microalgae

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Abstract

Due to increasing oil prices and climate change concerns, biodiesel has gained attention as an alternative energy source. Biodiesel derived from microalgae is a potentially renewable and carbon–neutral alternative to petroleum fuels. Microalgae were isolated in different water bodies and five species were selected based on purity. *Chlorella* sp. reached high growth rate (0.21 g l^{-1} dw) when compared to the growth rate of *Chematococcus* sp. (0.1 g l^{-1} dw) on 24^{th} day. Rate of CO_2 fixation and carbon, in terms of quantity was estimated. Results showed that CO_2 fixation was maximum at 0.486 g/dw and carbon biomass was 0.68 g/ml/day in *Chlorella* sp. *Chlorella* sp. reached a high lipid content 24.3±0.81% on 24 days of incubation. The fatty acid compositions were analysed by Gas chromatography, *Chlorella* sp. showed highest amount of oleic acid (16.43 mg g^{-1} dw). The results suggest that *Chlorella* sp. can be useful for producing biodiesel.

Key words: Microalgae, CO_2 mitigation, *Chlorella* sp., Biodiesel, Gas chromatography

Introduction

The growing economy together with rising population and industrialization has led to a steady increase in the global energy demand. If the government around the world sticks to the current policies, the world will need almost 60% more energy in 2030. Of this 45% will be accounted by China and India together (International energy agency 2007). Among the many problems concerning the global environment, the greenhouse effect due to an increase in atmospheric CO_2 levels has attracted worldwide attention. The growing demand for fuels can be met by bioenergy. Large scale introduction of biomass energy could contribute a sustainable development of several fronts viz., economical, social and environmental problems (Turkenburg 2000).

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Biodiesel is one of the alternative sustainable energy source used instead of petroleum in internal combustion engine. The use of biodiesel fuel is increasing as oil price continues to rise in the global scenario. Microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production (Prabakaran and Ravindran 2011). In recent years, use of microalgae as an alternative biodiesel feedstock has gained renewed interest to researchers, entrepreneurs and the general public. Recent soaring oil price, diminishing world oil reserves and environmental deterioration generates renewed interest in using algae as an alternative and renewable feed stock for fuel production (Hu et al. 2008). Based on these facts the present work is an attempt to generate biodiesel from microalgae and also to apply algae for carbon dioxide sequestration.

Materials and methods

Isolation and identification of microalgae

Water samples for microalgae isolation were collected aseptically from different sites (around Dindigul district, Tamilnadu, India.) that appeared to contain algal growth in a fresh water bodies. Ten ml of water samples were transferred to a 500 ml conical flask containing 200 ml of sterilized Bold's Basal Medium (BBM) (Kanz and Bold 1969) and then incubated on a rotary shaker at 27°C and 100 rpm under continuous illumination using white fluorescent light (maximum 2500 lux) for three weeks. Every two days, the flasks were examined for algal growth using optical microscope, with serial dilutions being made in BBM from flasks showing growth. Subcultures were made by inoculation 50 µl culture solution onto petri plates containing BBM solidified with 1.5% (w/v) of bacteriological agar. These procedures were repeated for each of the original flasks. Petri plates were incubated at 27°C under continuous illumination for two weeks. The purity of the cultures was confirmed by repeated plating and by regular observation under a microscope. The microscopic identification was done using botanical approaches (John et al. 2003). The microalgae identified and authenticated based on a standard manual (Prescott 1959).

Growth analysis

At first, cells of identified microalgae were cultivated in 2 L flask using BBM and incubated batch wisely at 24°C. for 27 days. The cultures were bubbled with a sterile air and illuminated (2500 lux maximum). Every three days the microalgal cells were harvested by centrifugation and washed twice with deionized water. Microalgal pellets were dried at 80°C for dry weight measurements (Ehimen et al. 2010).

Estimation of carbon content and carbon dioxide fixation rate

Dried algal (0.2 mg) samples were placed in 500 ml conical flask and 10 ml of 1N potassium dichromate and 20 ml of conc. H_2SO_4 mixture was diluted with 200 ml of distilled water and 10 ml of H_3PO_4 and 1 ml of diphenyl amine was added. Finally, titrated against 4 N ferrous ammonium Sulphate (FAS). The end point was brilliant green colour appeared. The carbon content was estimated using the following formula

$$a = \frac{3.951}{g}\left(1 - \frac{T}{S}\right)$$

where a is carbon content, g is weight of the sample, T is FAS with blank (ml) and S is FAS with sample (ml). And the amount of carbon dioxide fixation rate was estimated using the formula, (Yun et al. 1997)

$$R_{CO_2} = C_C * \mu_L \left(M_{CO_2} / M_C\right)$$

Where R_{CO2} and μ_L are the CO_2 fixation rate (g CO_2 m^{-3} h^{-1}) and the volumetric growth rate (g dry weight m^{-3} h^{-1}), respectively, in the linear growth phase. M_{CO2} and M_C represented the molecular weights of CO_2 and elemental carbon, respectively, C_C is average carbon content (algal dry weight [g]).

Lipid extraction

The total lipids were extracted from the fresh microalgal biomass using a slightly modified method of Bligh and Dyer. 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 to sonication for 30 min at maximum power. After sonication, the tubes were incubated overnight at 27°C 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 50°C 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.

Analysis of fatty acid profile

A fatty acid profile analysis was performed using a Shimadzu 2010 gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector and a Diethylene glycol succinate (DEGS) capillary column (30mx0.25x0.25µm). Initial column temperature and injection port temperature both were 180°C. Detector temperature was 230°C, and the temperature was increased to 300°C at temperature gradient of 15°C/min. 100µl lipid samples were placed into capped test tubes, saponified with 1 ml of saturated KOH-CH_3OH solution at 75°C for 10 min, and then subjected to methanolysis with 5% HCl in methanol at 75°C for another 10 min (Schreiner 2006). Thereafter, the phase containing the fatty acid methyl esters was separated by adding 2 ml of distilled water and then methanol was recovered. The components were identified by comparing their retention times and fragmentation patterns with those for standards (Xu et al. 2001). Six fatty acid methyl esters (C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3) were used as the standard materials.

Result and Discussion

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 (Sydeny et al. 2010). In this study, from the three different water bodies were collected and 16 microalgal cultures were isolated (Table 1). Only five isolates (*Chlorella, Haematococcus, Ulothrix, Chlorococcum, Rivularia,*) of the 16 microalgal cultures were selected based on their purity and growth rates (Fig. 1).

Table 1: Isolation of microalgae from different location in and around Dindigul District.

Location	Latitude	Longitude	Name of the microalgae
Kamarajar dam	10°17'43.44" N	77°48'44.06" E	*Chlorella* sp., *Rivularia* sp.
Palar dam	10°24'30.61" N	77°29'38.39" E	*Haematococcus* sp., *Scenedesmus* sp.
Manjalar dam	10°11'37.15" N	77°37'55.86" E	*Ulothrix* sp., *Chlorococcus* sp.,

Under suitable conditions and sufficient nutrients, microalgae can grow profusely. Commonly, they double their biomass within 3.5 h or 24 h during the exponential growth phase (Chisti 2007). Fig.1 growth of the test organisms increased steadily with a lag of 2 days followed by the logarithmic phase and attained the stationary phase at about 23 days. The figure shows increasing incubation times enhanced the growth tremendously. The biomass productivity was expressed as dry cell weight per liter indicated that *Chlorella* sp. exhibited two times higher growth rate (0.21 g l^{-1} dw) when compared to the growth rate of *Haematococcus* sp. (0.1 g l^{-1} dw) on 24th day.

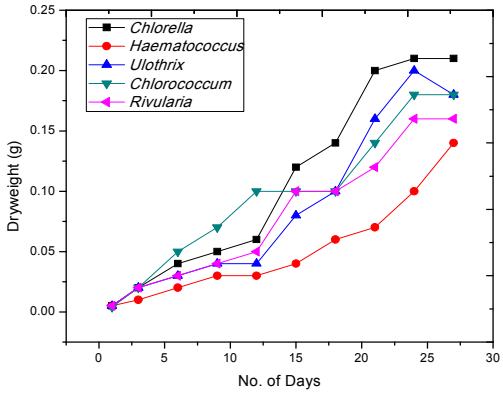

Figure 1: Growth analysis of selected microalgae

The carbon content of the dried algal biomass was estimated by Walky and Black method**.** Three (*Chlorella* sp., *Ulothrix* sp., and *Chlorococcum* sp.) isolated microalgae were selected based on the growth. Table 2 shows increasing carbon content enhanced the

fixation of CO_2. Carbon content of Chlorella sp. was maximum at 0.486 g/dw and CO_2 fixation was 0.68 g/ml/day. In *Ulothrix* sp. was minimum carbon and CO_2 fixation were 0.323 g/dw and 0.46 g/ml/day respectively. This value coincides approximately with carbon content of *Chlorella vulgaris* (0.5906 g/dw) and fix 26.0 g $CO_2/m^3/h$, when cultivated at 15% v/v of CO_2 concentration in waste water (Yun et al. 1997).

Table 2:Estimation of carbon and CO_2 fixation rate of microalgae.

S. No	Microalgae	Carbon content (g/dw)	CO_2 fixation rate (g/ml/day)
1	*Chlorella* sp.	0.486	0.68
2	*Ulothrix* sp.	0.323	0.46
3	*Chlorococcum* sp.	0.387	0.53

The total lipid contents for the microalgae cultured in this study ranged from 6.1 ± 0.7% to 24.3 ± 0.81% of the dry weight. The lipid content from *Chlorella* sp. was 24.3 ± 0.81% of the dry weight, which was about nearly four times higher than that from *Ulothrix* sp. (6.1 ± 0.7%). The lipid content in *Chlorella* species can, however, be increased to 53-66% by nitrogen deprivation (Hsieh and Wu 2009; Xiong et al. 2008). Lipid content data for different algal species are readily available and have been consistently reported in the literature (Griffiths and Harrison 2009). Many microalgae species can be induced to accumulate substantial quantities of lipids (Sheehan 1998) contributing to a high oil yield. In previous studies (Spolaore et al. 2006 and Li et al. 2008), total lipid contents representing 20–50% of the dry biomass weight were found to be quite common, and some microalgae even exceeded 90% as a response to different culture conditions (Mata et al. 2010).

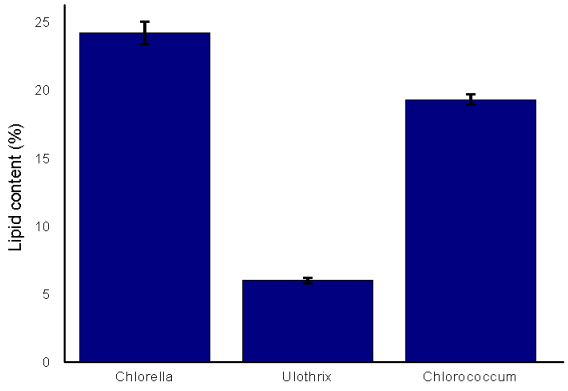

Figure 2. Lipid content of selected microalgae

Fatty acids in the three strains of microalgae were primarily esterified, and the major fatty acid compositions were determined using GC analysis (Fig. 3). In the three tested microalgae, linoleic acid (C18:2) and oleic acid (C18:1) were commonly dominant. The most commonly synthesized fatty acids have chain lengths that range from C16 to C18, similar to those of higher plants (Ohlrogge and Browse 1995). Specifically, the major fatty acids were C16:0 and C16:1 in the Bacillariophyceae, and C16:0 and C18:1 in the Chlorophyceae (Cobelas and Lechado 1989). 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 the structure of its component fatty esters (Stournas et al. 1995 and Knothe 2008). Therefore, among the tested microalgal species, *Chlorella* sp. showed the highest oleic acid content (16.43 mg g^{-1} dw), making it the most suitable for the production of good quality biodiesel.

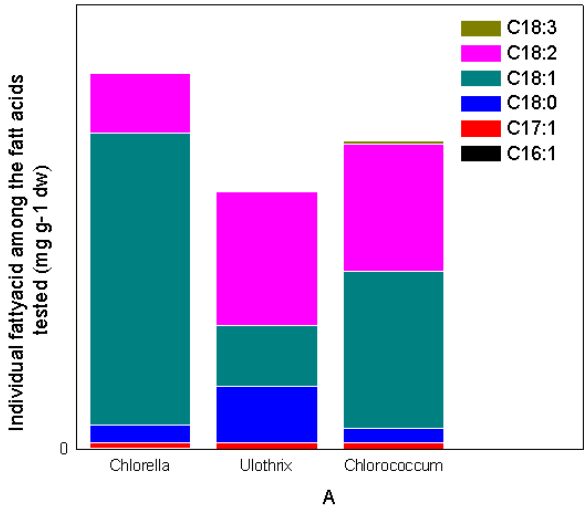

Figure 3. Fatty acid composition of the different microalgal species

Conclusions

This study revealed certain freshwater microalgae having high growth, fixation of CO_2 and biodiesel production; five microalgal cultures were shortlisted based on purity and growth rate. *Chlorella* sp. showed the best growth rate on 27 day of incubation. The dry biomass of *Chlorella* used to estimate carbon content at 0.486 g/dw and carbon dioxide fixation rate was 0.68 g/ml/day. The highest total lipid contents (24.3 ± 0.81%) and highest oleic acid content (16.43 mg g^{-1} dw) were found in *Chlorella* sp. Finally, the results of the study indicate that the naturally isolated *Chlorella* sp is a suitable microalga for use in CO_2 mitigation and biodiesel production.

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