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Selection of microalgae for accumulation of lipid production under different growth conditions

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ABSTRACT

To achieve sustainable production of biofuel from microalgae, a well-optimized and sustained biomass production is prerequisite. One of the most important factors in obtaining oil from microalgae is the choice of the right algal species to be used. Seven isolates (*Chlorella*, *Haematococcus*, *Ulothrix*, *Chlorococcum*, *Scenedesmus*, *Rivularia*, *Scytonema*) of the 20 microalgal cultures isolated were selected based on their purity and growth rates. Under similar environmental conditions, *Scenedesmus* sp. and *Chlorococcum* sp. produced maximum calorific value (21.46 MJ/kg and 16.13MJ/kg) and lipid content (34.5±0.3% and 32.3±0.28%). Growth analysis in *Scenedesmus* sp. and *Chlorococcum* sp. was studied under various culture conditions such as nitrate concentrations, temperature and CO_2 concentration. The most significant growth was recorded in *Scenedesmus* sp. and *Chlorococcum* sp., with N+/28ºC/CO_2. Best biomass productivity was obtained at 28ºC under conditions of nitrogen sufficiently and CO_2 supplementation (N+/28ºC/CO_2). The adequate fatty acid profile was analysed by gas chromatography. The presence of oleic and linoleic fatty acid as major constituents makes *Scenedesmus* sp. and *Chlorococcum* sp. as suitable feedstock for biodiesel production.

Introduction

Algae are photosynthetic microorganisms that are able to use the solar energy to combine water with carbon dioxide to create biomass. Microalgae grow much faster than higher plants and require much less land areas. However, the utilization of microalgae to overcome global warming is not enough without utilizing an algal biomass before degradation (Widjaja, 2009). The study of algae-for-fuel has become a hot topic in recent years with energy prices fluctuating widely and green house gas emissions becoming a cause for concern (Gouveia and Oliveira, 2009). Some microalgae appear to be suitable group of oleaginous microorganism for lipid production (Chisti, 2007). Microalgae have been suggested as potential candidates for fuel production because of a number of advantages including higher photosynthetic efficiency, higher biomass production and higher growth rate compared to other energy crops (Milne *et al*., 1990; Minowa *et al*., 1995; Dote *et al.*, 1994). Moreover according to biodiesel standard published by the American Society for Testing Materials (ASTM), biodiesel from microlage oil is similar in properties to the standard biodiesel, and is also more stable according to their flash point values.

In this study, we have instigated the selection of freshwater microalgae with high lipid content in our microbiology laboratory, Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Tamil Nadu, India. *Scenedesmus* sp. was identified and selected from 20 different strains. *Scenedesmus* sp. was characterized by growth analysis with CO_2 and lipid composition analysis. Furthermore, the lyophilized cells of *Scenedesmus* sp. were subjected to calorimetric measurements to compare the calorific value of dried biomass with fossil fuels.

Materials and methods

Isolation, Purification and Identification of microalgae

Water samples for microalgae isolation were collected from different locations (in and around Dindigul district, Gandhigram, Tamilnadu, India.) that appeared to contain algal growth in a fresh water bodies. All samples were collected at about the same time between 0800 to 1100 hrs. Surface water and water at a depth of 0.50 meter were collected at each location. Water samples were taken from the sites to laboratories in bottles cooled in ice. 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. BBM was composed of (mg/L) $NaNO_3$, 250; K_2HPO_4, 75; KH_2PO_4, 175; $CaCl_2$, 25; NaCl, 25; $MgSO_4$, 75; $FeCl_3$, 0.3; $MnSO_4$. $7H_2O$, 0.3; $ZnSO_4$. $7H_2O$, 0.2; H_3BO_3, 0.2; $CuSO_4$. $5H_2O$, 0.06. Every two days, the flasks were examined for algal growth using optical microscope, with serial dilutions being made in BBM from flasks showing growth, isolation were made by inoculation of 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 freshwater microalgae were identified and authenticated based on the guidelines of the standard manual (Prescott, 1959).

Determination of calorific value

Calorific value was determined using an automatic adiabatic bomb calorimeter (Changsha Kaiyuan Instruments Co., 5E-1AC/ML). The calorific value was calculated by the measurement of elevated water temperature due to combustion of the microalgae powder. Appropriate dry weights of micro algal powder were wrapped with paper and combusted in the bomb calorimeter. The calorific value of wrapping paper without biomass was also measured to subtract from the raw calorific values of biomass samples to remove the background value (Matsunaga *et al*., 2009)

Lipid extraction

The total lipids were extracted from microalgal biomass using a modified method of Bligh and Dyer (1959). The lipids were extracted with chloroform-methanol (2:1, v/v), and then separated into chloroform and aqueous methanol layers by the addition of methanol and water to give a final solvent ratio of chloroform:methanol:water of 1:1:0.9. The chloroform layer was washed with 20 ml of a 5% NaCl solution, and evaporated by rotary vacuum evaporator (Rotavapor R-210, Buchi). The weight of the crude lipid obtained from each sample was measured using an electronic scale.

Growth evaluation

The microalgae were grown in 2L glass bubble column bioreactor with continuous stirring by bubbling filtered air in BBM (Yoo *et al*., 2010). The microalgae cultures were tested for growth parameters as influenced by two temperatures (28 and 30°C) with 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 cultures were continuously illuminated with six fluorescent lamps at 3500 lux (maximum) for 21 days. Growth was evaluated over time in terms of dry weight. All the samplings and testing were performed in triplicate.

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). Fifty milligram samples were placed into capped test tubes, saponified with 1 ml of saturated KOH-CH3OH solution at 75^0C for 10 min, and then subjected to methanolysis with 5% HCl in methanol at 75^0C for another 10 min (Schreiner, 2006). 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 (Xu *et al*., 2001). Six fatty acids (C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3) were used as the standard materials.

Results and discussion

The successful implementation of algal biomass as a potential bioenergy feedstock is largely governed by the quantum of producible biomass. Therefore enhancement of the growth rate of algae in terms of biomass productivity is one of the most important parameters (Phukan *et al*., 2011). In this study, from the six different water bodies 20 microalgal were collected and the microalgal cultures were purified and maintain in pure culture. Only seven isolates (*Chlorella, Haematococcus, Ulothrix, Chlorococcum, Scenedesmus, Rivularia, Scytonema*) of the 20 microalgal cultures were selected based on their purity and growth rates (Table 1). Many algal species exhibit rapid growth and high productivity, and certain microalgal species can be induced to accumulate substantial quantities of lipids, often greater than 60% of their dry biomass (Richmond, 2004)

The lyophilized cells of seven microalgae cultured in BBM for 21 days were estimated for their calorific value. The calorific values (Table 2) of microalgae were as follows; 21.46 MJ/kg for *Scenedesmus* sp., 16.13MJ/kg for *Chlorococcum* sp., and 15.88 MJ/kg for *Chlorella* sp. The highest calorific value was from dried cells of *Scenedesmus* sp. These results are agreed in with the results obtained by Phukan *et al*. (2011).

After mass multiplication, the microalgae was harvested and used for oil extraction. The total lipid contents for the microalgae cultured in this study ranged from 6.5% to 34.5% of the dry weight. Among the isolated microalgae, *Scenedesmus* sp., *Chlorococcum* sp. and *Chlorella* sp. produced high lipid content of 34.5±0.3%, 32.3±0.28% and 29.7±0.25% of dry weight (Fig. 1) respectively, *Ulothrix* recorded lowest lipid content of 6.5±0.42%. Liu *et al*. (2008) reported that total lipid contents representing 20-50% of the dry biomass weight were found to be quite common, and some microalgae even exceeded 80%. The oil content of *Chlorella* typically ranges between 28 and 32% dry weight but can reach 46% dry weight under stress (Rodolfi *et al*., 2009). Chisti (2007) reported that oil content of microalgae is usually between 20-80%.

Based on the lipid content *Scenedesmus* sp. and *Chlorococcum* sp. were selected for the growth evaluation. Microalgal biomass growth data of under the eight experimental conditions are depicted in Fig. 2 and 3. *Scenedesmus* and *Chlorococcum* culture curves showed the fastest growth for N+/28ºC/CO_2 (nitrate sufficient medium with CO_2 enrichment at 28ºC). Comparing with N-28°C culture (nitrate free medium at 30°C), it can be observed that the former attained seven fold in *Chlorococcum* sp. and eight fold in *Scenedesmus* sp. higher growth after 18 days. The CO_2 enrichment by sparging in culture media enhanced algal growth as expected, which can be seen by comparing the culture curves for N+28°C/CO_2 with N+28°C and N-28°C/CO_2 with N-28°C, showing that cultures under air bubbling were C-limited. In terms of temperature influences on *Chlorococcum* sp. and *Scenedesmus* sp. biomass productivity were higher in 28°C than 30°C, irrespective of supplemented CO_2.

Fatty acids in the two strains of microalgae were primarily esterified based on the potential of lipid content and the six fatty acid methyl ester profiles were determined using GC analysis (Table 3). In previous reports Lee *et al*. (2011), the most commonly synthesized fatty acids have chain lengths that range from C16 to C18, similar to those of higher plants and palmitic, stearic, oleic and linolenic acid were recognized as the most common fatty acids contained in biodiesel. In the two tested microalgae, oleic acid (C18:1) and linoleic acid (C18:2) were dominant, which ranged from 19.9-52.8% and 43.2-74.4% respectively. The total amount of fatty acid methyl ester of the two microalgae ranged from 19.62 to 22.29 mg g^{-1} dw. The highest amount of oleic acid (11.77 mg g^{-1} dw) was detected in *Scenedesmus* sp., while linoleic acid (14.61 mg g^{-1} dw) was higher 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 the structure of its component fatty esters (Knothe, 2008). Thus, it was concluded, among the tested microalgal species, *Scenedesmus* sp. showed the highest oleic acid content (52.8%), making it the most suitable for the production of good quality biodiesel.

Conclusions

This study revealed that certain freshwater microalgae having high calorific value, lipid productivity and growth rate when grown within CO_2. Seven microalgal cultures were shortlisted based on purity and growth rate. The maximum calorific value (21.46 MJ/kg) and high lipid content (34.5±0.3%) was found for *Scenedesmus* sp. The highest total fatty acid and oleic acid of 22.29 mg g^{-1} dw and 11.77 mg g^{-1} dw respectively were mainly found in *Scenedesmus* sp. The results of this study indicate that the isolated microalga *Scenedesmus* sp. is a valuable microlage for use in biodiesel production.

Table 1. Isolation of microalgae from different locations 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. *Ulothrix* sp.
3	Palani pond	10°26'12.59" N	77°30'52.27" E	*Chlorococcum* sp.
4	Manjalar dam	10°11'37.15" N	77°37'55.86" E	*Scenedesmus* sp.
5	Nanganji dam	10°35'35.34" N	77°29'38.39" E	*Rivularia* sp.
6	Anaippatti dam	10°05'20.15" N	77°51'10.28" E	*Scytonema* sp.

Table 2. Calorific value of microalgae.

S. No	Microalgae	Calorific value (MJ/kg)
1	*Chlorella* sp.	15.88
2	*Haematococcus* sp.	10.54
3	*Ulothrix* sp.	3.23
4	*Chlorococcum* sp.	16.13
5	*Scenedesmus* sp.	21.46
6	*Rivularia* sp.	8.78
7	*Scytonema* sp.	6.54

Table 3. Fatty acid composition of *Scenedesmus* sp. and *Chlorococcum* sp.

Fatty acid methyl ester	Amounts of fatty acids (mg g^{-1} dw)			
	Scenedesmus sp.		*Chlorococcum* sp.	
C16:1	ND		ND	
C17:0	0.21	(0.94)	0.27	(1.37)
C18:0	0.59	(2.64)	0.76	(3.87)
C18:1	11.77	(52.8)	3.92	(19.9)
C18:2	9.63	(43.2)	14.61	(74.4)
C18:3	0.09	(0.4)	0.06	(0.30)
Total	22.29	(100)	19.62	(100)

ND: not detected

( ): Fatty acid methyl ester composition (wt %)

Fig. 1. Lipid content of microalgae

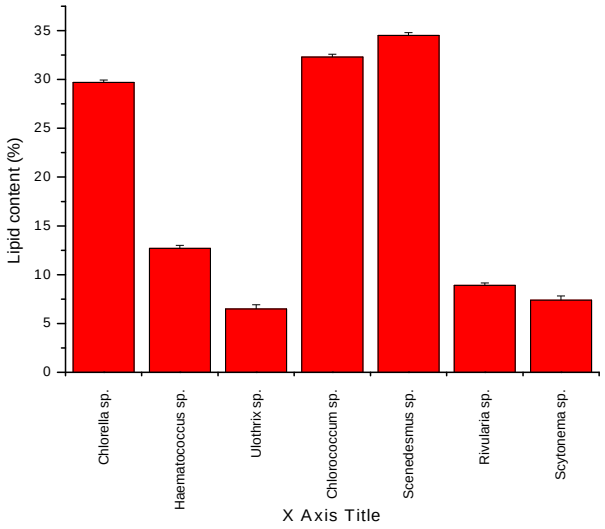


Fig. 2. Average dry weights of *Scenedesmus* sp. under different growth conditions over time

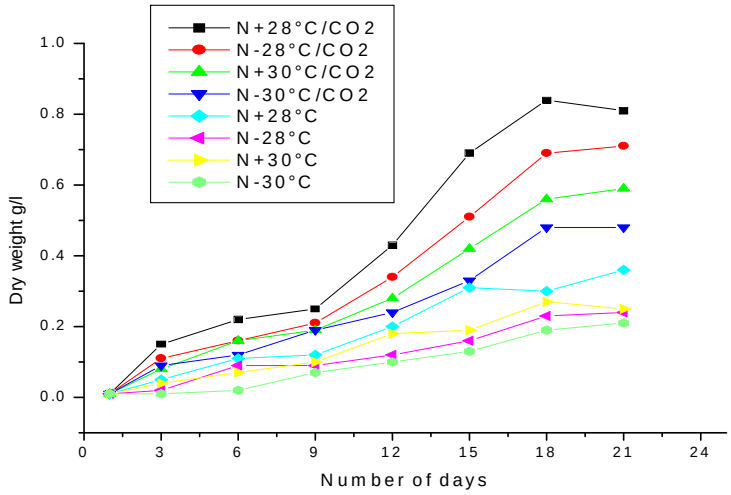
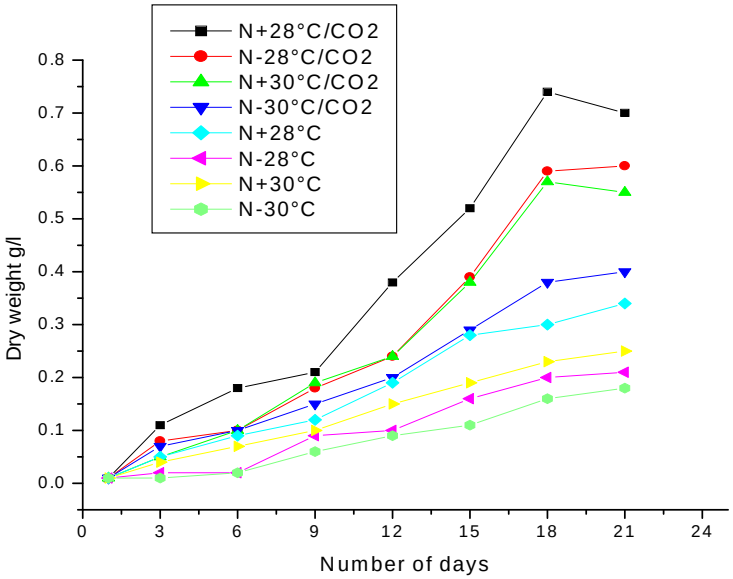


Fig. 3. Average dry weights of *Chlorococcum* sp. under different growth conditions over time



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