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Characterization and fatty acid profiling in two fresh water microalgae for biodiesel production: Lipid enhancement methods and media optimization using response surface methodology

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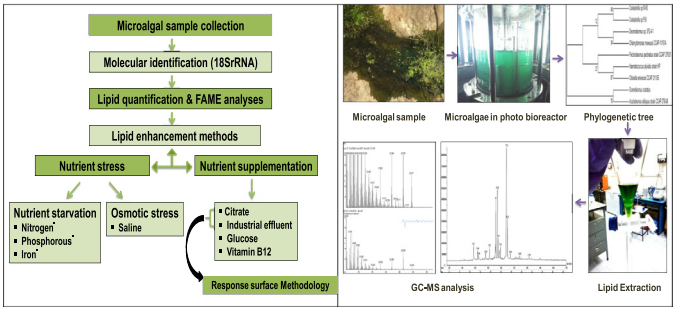
HIGHLIGHTS

- Fresh water indigenous microalgal isolates were characterized as *Coelastrella* sp. M-60 and *Micractinium* sp. M-13 as biodiesel feedstock.
- The media supplemented with vitamin B_{12}, glucose, citric acid, effluent and salinity stress greatly influenced the biomass, lipid productivity and lipid content by these microalgae.
- Media optimization with the variables citric acid and effluent using RSM increased the potential of lipid productivity by microalgae.
- FAME characterization and its fuel properties of microalgae are in accordance with the international standards.

GRAPHICAL ABSTRACT



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ABSTRACT

Two fresh water microalgae, *Coelastrella* sp. M-60 and *Micractinium* sp. M-13 were investigated in this study for their potential of biodiesel production. For increasing biomass and lipid production, these microalgae were subjected to nutrient starvation (nitrogen, phosphorous, iron), salinity stress and nutrient supplementation with sugarcane industry effluent, citric acid, glucose and vitamin B_{12}. The lipid productivity obtained from the isolates *Coelastrella* sp. M-60 (13.9 ± 0.4 mg/L/day) and *Micractinium* sp. M-13 (11.1 ± 0.2 mg/L/day) was maximum in salinity stress. The media supplemented with all the four nutrients yielded higher lipid productivity than the control. The response surface methodology (RSM) was employed to evaluate the effect of sugarcane industry effluent and citric acid on growth and lipid yield. Fatty acid profile of *Coelastrella* sp. M-60 and *Micractinium* sp. M-13 were composed of C-14, C-16:0, C-18:0, C-18:1 and C-18:2 and their fuel properties were also in accordance with international standards.

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1. Introduction

Microalgae have currently gained much attention as a new resource for the production of biodiesel and other types of fuel from their biomass. Production strategies and fuel properties of algal biodiesel have potential advantages than petro diesel (Mata et al., 2010) that has attracted researchers and industrialists worldwide towards the direction of biodiesel production for the escalating energy demand due to growing population, increasing urbanization and transportation across the world. Extensive use of petro fuel emitting carbon dioxide, sulfur dioxide, nitrous oxide, carbon monoxide and particulate matter etc., thus significantly increasing the level of green house gases in the atmosphere emphasizes the research of alternate fuel in order to reduce the global warming (Chisti, 2007; Tale et al., 2014; Yamik et al., 2014).

Reports states that due to continuous increase of energy demand make an indication that conventional oil reserves, may be commercially utilized after 2050 (Maity et al., 2014). The oil production per acre from microalgae is greater when compared to vegetable oils and thus microalgae fuel may be the one of the alternate to produce adequate automotive fuel to substitute the current petro diesel demand (Chisti, 2007). It is noteworthy to mention that microalgae accumulates remarkably high lipid bodies as triacylglycerols (TAG) and value added products by their higher photosynthetic efficiency, growth in limited nutrient inputs, higher lipid production per unit area, utilization of waste water and industrial effluents (Hammouda et al., 1995; Mallick, 2002; Devi et al., 2013). Most of the biodiesel produced from microalgal lipid and fatty acid methyl ester (FAME) were found to be in accordance with the biodiesel standards (Nascimento et al., 2013). Higher lipid storage was also found in mixotrophic microalgae in nutrient stress along with glucose supplementation with the variation in FAME constituents (Chandra et al., 2014). Enhancement of lipid production in microalgae by scheming the cultivation conditions is equally essential as the microalgal selection for high lipid-producing algae (Duong et al., 2012; Mansour et al., 2005). Major advantages of biodiesel from microalgae include higher stability than oil crop feedstocks (Rajvanshi and Sharma, 2012) and 1 kg of algal biodiesel can fix 1.83 kg of CO_2 with higher CO_2 mitigation rate (Saifullah et al., 2014).

In these circumstances, response surface methodology (RSM) is one of the suitable and efficient approaches for optimizing the media to obtain maximum lipid production in microalgae (Yang et al., 2014). Among the biochemical engineering approaches of lipid enhancement in microalgae, nutrient-starvation is universally employed for scheming metabolic fluxes to lipid biosynthesis.

Presently the cost of producing algal biodiesel is higher than petro fuels, thus cost effective considerations need to be explored for cheap and easily cultivable alternate feedstock for biodiesel (Cheng et al., 2009). Therefore, fuels derived from microalgae have been proposed as a promising alternative renewable energy source for current and future energy requirement. Hence this present study was focused to enhance the yield of biomass and lipid productivity through media optimization of citric acid and sugarcane industry effluent to decrease the production cost. Thus, the objectives of this study were to enhance the lipid production of two microalgal isolates through nutrient starvation and supplementation. Osmotic stress was also induced by exhibiting various salinity conditions in the growth media, further the growth media was supplemented with vitamin B_{12}, organic carbon such as glucose, citric acid, sugarcane industry effluent. Since the limited number of algal species are exploited as suitable biodiesel feedstock for ever increasing energy demand, this study would explore these microalgae for their potential, which may accomplish in the search of efficient economically competent and sound alternate for biodiesel production.

2. Methods

2.1. Molecular identification and growth study of microalgae

Fresh water samples were collected for the isolation of microalgae from a pond near Checkanurani, Madurai, Tamil Nadu, India (9° 56′ 33.0900″ N, 77° 59′ 41.3916″ E) and identified by partial 18S rRNA sequencing (Hu et al., 2013). The microalgae *Coelastrella* sp. M-60 and *Micractinium* sp. M-13 were cultured and maintained as pure cultures in 100 ml BG-11 medium (Rippka et al., 1979) in 250 ml Erlenmeyer's flask under constant illumination of 1500 lux of alternate photoperiod (light:dark – 12:12 h cycle) at 25 °C. The growth phase was measured as optical density at 670 nm using colorimeter (Elico, CL 63, India) at a constant interval of 4 days.

2.2. Cultivation of microalgae in nutrient stress and supplementation

Nutrient stress conditions like nitrogen, phosphorous and iron starvations were imposed by excluding the nitrogen ($NaNO_3$), phosphorous (KH_2PO_4) and iron (Fe $(NH_4)_3(C_6H_5O_7)_2$) sources in normal BG-11 media respectively. Osmotic stress was also created in the BG-11 media by the addition of 1%, 2% and 3% NaCl. Other than starvation studies, nutrient supplementation studies were also carried out with the cultures of microalgae by the addition of different concentrations of sugar industry effluent (0.625, 1.25 and 2.5 ml/L), citric acid (10, 20, 30 mg/L), glucose (0.05%, 0.1%, 0.15% and 0.2%) and vitamin B_{12} (0.001%, 0.002% and 0.003%) to the BG-11 media. *Coelastrella* sp. M-60 (13 ± 0.04 mg/100 ml) and *Micractinium* sp. M-13 (23.5 ± 0.08 mg/100 ml) were used as initial inoculum in 250 ml Erlenmeyer's flask for the aforementioned studies and normal BG-11 media was used as a control. All the experiments were performed with triplicates and the results were expressed as mean ± standard deviation.

2.3. Biomass and lipid productivity of microalgae

Microalgae biomass was harvested in stationary phase at 45th day by centrifugation at 8000 rpm for 10 min. Cells were dried at 60 °C in hot air oven and dried biomass was calculated gravimetrically (the growth was expressed as dry cell weight DCW), further the total lipid was extracted using chloroform:methanol (2:1) based on modified method of Folch et al. (1957) and then quantified gravimetrically. Biomass and lipid productivities were calculated from the initial cell dry weight and final cell dry weight from the following equations (Griffiths and Harrison, 2009) and expressed as DCW per mg/L/day. All the experiments were performed with triplicates and the results were expressed as mean ± standard deviation. One-way ANOVA followed by Tukey's honestly significant difference (HSD) test was used for the data analyses (http://vassarstats.net).

$$BP = (B_2 - B_1)/(t_2 - t_1) \quad (1)$$

$$LP = BP \times \text{Lipid content in } \% \quad (2)$$

where, BP is the biomass productivity expressed in unit mg/L/day and LP is the lipid productivity expressed in unit mg/L/day and B_1 and B_2 are biomass concentration in mg/L harvested from the two sampling points t_1 and t_2 respectively.

2.4. Separation of neutral lipids by thin layer chromatography (TLC)

Microalgae total lipids extracted from different nutrient conditions and control were separated by TLC using the developing solvent (hexane; diethyl ether; acetic acid in the ratio of 70:30:1 (Hu