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Screening of Microalgae Based on Biomass and Lipid Production at Indoor and Outdoor Cultivation Condition

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ABSTRACT

The seven microalgae cultures such as Chlorella vulgaris, Nitzschia palea, Scenedesmus quadricaudus, Chroococcus turgidus, Desmococcus olivaceus, Synechococcus lividus and Phormidium autumnale were screened based on their growth, biomass and lipid production in their respective medium at both indoor and outdoor cultivation condition. C. vulgaris exhibited maximum growth followed by S. quadricaudus and D. olivaceus. Lower percentage of total lipid content was recorded in C. turgidus, D. olivaceus and S. lividus. Though S. quadricaudus and D. olivaceus produced moderate biomass, it didn't achieved optimum lipid content for biodiesel production. C. vulgaris showed good growth with maximum production of biomass and optimum lipid content whereas N. palea attained highest lipid content in both the cultivation conditions. Hence C. vulgaris and N. palea were selected as promising candidates for biofuel feedstock.

Key words: *Microalgae, Indoor, Outdoor, Biomass, Lipid content*

INTRODUCTION

Algae, especially microalgae, were found to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels[4,5]. The three most important classes of microalgae in terms of abundance are the diatoms (*Bacillariophceae*), the green algae (*Chlorophyceae*) and cyanobacteria (*Cyanophyceae*).

Economic feasibility of microalgae based bio fuel production depends basically on two major factors viz. the cost of commercial scale cultivation (appropriate technology), and the selection of microalgae strain (appropriate strain). The selection of appropriate algae strains is an important factor in the overall success of biofuel production from microalgae[19]. Micro algae were considered as a ideal candidates for bio diesel production because of their higher bio mass and inter cellular lipid content. The ultimate goal of bio diesel production from micro algae is to obtain maximum lipid yield by increasing bio mass and intra cellular lipid content. For economical production of biodiesel from microalgae, biomass and lipid content play an important role. Much of early research on biodiesel production from microalgae focused on the search for species with high lipid content.

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Oil productivity, that is the mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass [7]. Hence selection of microalgae and cultivation condition for biodiesel production is very important aspect for economical production. The aim of this study is to screen and select efficient microalgae strain based on high biomass and lipid production in their respective medium at indoor and outdoor condition.

MATERIALS AND METHODS

Microalgae Selection and Screening

The equal number of exponentially growing cells were inoculated in 2 L flask containing 1L of respective growth medium: (*C. vulgaris*, *D. olivaceus*, *P. autumnale* in BG 11 medium[12]; *N. palea* in DM medium[1]; *S. quadricaudus, C. turgidus, S. lividus* in Chu 10 media[6]; and incubated at 28°C for 28 days with 12:12h light dark cycle. The cultures were mixed twice in a day by shaking and illuminated (2500 lux maximum) at indoor lab condition. Simultaneously, the same procedure was repeated but incubated at outdoor condition exposed to sunlight for 28 days.

At all the three cultivation conditions, the growth of seven micro algal cultures (OD at 560nm) were observed at regular interval of 4 days for a period of 28 days. At 30th day, the biomass and lipid content were quantified as described later.

Estimation of Growth Value

The algal growth in 10 ml of culture sample was determined by measuring the Optical Density (OD) at 560nm using a Elico SL 177i spectrophotometer.

Determination of Total Biomass[9]

The biomass was harvested by flocculation using alum, centrifuged and was filtered. The dried petriplates were weighed (initial weight). The pellet was kept in dried petriplates and then it was allowed to dry at 60°C in hot air oven. The dried biomass in the petriplate was weighed (final weight)

Total biomass= Final weight-Initial weight.

Determination of Total Lipids[2]

The dried empty beaker was weighed (Initial weight). The dried algae from all the cultivation conditions were grounded separately in mortar and pestle. This was mixed with chloroform and methanol in the ratio of 2:1 ie (20 mL : 10 mL). This was kept in magnetic stirrer for 6 hours, sonicated for 30 seconds and centrifuged at 3000 rpm for 10 minutes. The upper layer was removed and collected in the preweighed beaker. The lower layer was transferred into a clear tube and the remaining solids were re-extracted with 4 mL of chloroform–methanol in 2:1 ratio. The extraction was repeated three times in a series to get possible saturation of the solvent mixture with lipids. Lipid content was determined separately for each extraction. Finally the collection of solvent (upper layer) was removed by evaporation after which the lipid content remain in the preweighed beaker was weighed (Final weight) and determined gravimetrically.

$$\text{Lipid content (\%)} = \frac{\text{final weight- initial weight x 100}}{\text{Volume of sample}}$$

Statistical Analysis

All the analysis was performed in triplicates. Data are expressed as mean values ± S.D. using Microsoft excel, graphs using Origin (Version 8.0). Two - way ANOVA methods were used for the analysis using MATLAB (7.8 Version).

RESULTS

Screening Of Highest Biomass And Lipid Producing Microalgae In Medium At Indoor And Outdoor Condition

The growth curve of seven microalgae in terms of optical density in their specific media at indoor condition was shown in **Fig 1.** The decline phase of strains differed from one strain to another. The growth curve of the

microalgae strains reached its maximum value/peak in 20 days of incubation except *Nitzschia palea*. (8th day) and *Phormidium autumnale* (12th day). The best growth was found in *Chlorella vulgaris* (OD 3.25±0.1**)** followed by *Scenedesmus quadricaudus* (OD 2.8±0.1) and poor growth was observed in *Nitzchia palea* (OD 0.204±0.06). *Chlorella vulgaris* showed significantly highest growth (P<0.001) rate than the other microalgae strains cultivated in media at indoor condition.

The estimation of growth through OD in the seven strains in their specific media at outdoor condition showed different growth pattern **(Fig 2)** where growth rate of all the microalgae strains reached its peak of in 20 days of incubation except *Nitzschia palea* on 8th day and *Phormidium autumnale* (12th day). The growth was higher at *Chlorella vulgaris* (OD 3.29 ± 0.1**)** followed by *Scenedesmus quadricaudus* (OD 2.71 ± 0.07) and lower at *Nitzchia palea* (OD 0.184 ± 0.03). Among the seven strains, *Chlorella vulgaris* showed significant growth (P<0.001) than the other microalgae cultivated in medium at outdoor condition.

Total biomass of all algal strains produced at both indoor and outdoor conditions were shown in **Fig 3.**

The total biomass production of microalgae in medium at indoor condition indicated that *Chlorella vulgaris* recorded maximum biomass (1.62 ± 0.056 g L^{-1} dw) followed by *Scenedesmus quadricaudus* (1.5 ± 0.033 g L^{-1} dw) where as *Nitzschia palea* (0.524 ± 0.034 g L^{-1} dw) followed by *Synechococcus lividus* (0.55 ± 0.03 g L^{-1} dw) recorded minimum biomass. The biomass of eight microalgae was slightly differed when compared to their growth rate. The total biomass production of microalgae in medium at outdoor condition indicated that *Chlorella vulgaris* recorded maximum biomass (1.69 ± 0.045 g L^{-1} dw) followed by *Scenedesmus quadricaudus* (1.41 ± 0.023 g L^{-1} dw) where as minimum biomass was obtained in *Nitzschia palea* (0.47 ± 0.021 g L^{-1} dw) followed by *Synechococcus lividus* (0.61 ± 0.012 g L^{-1} dw).

Total lipid content of all algal strains produced at both indoor and outdoor conditions were shown in **Fig 4.** The total lipid percentage of seven strains at both these conditions were between: *Chlorella vulgaris* (21%-23%), *Nitzschia palea* (40%–41%), *Scenedesmus quadricaudus* (17%–19%), *Chroococcus turgidus* (10%), *Desmococcus olivaceous* (10%–11%), *Synechococcus lividus* (8%–9%) and *Phormidium autumnale* (14%–15%).

The lipid production of microalgae in medium at indoor condition revealed that highest lipid content (40.18 ± 0.44 %) was detected in *Nitzschia palea*. The moderate level of lipid content (23.8 ± 0.83 %) was obtained in *Chlorella vulgaris*. Blue green algae *Synechococcus lividus* (9.2 ± 0.38 %) followed by *Chroococcus turgidus* (10.1 ± 0.52 %) recorded lowest lipid content.

The lipid production of microalgae in medium at outdoor condition showed that *Nitzschia* sp. exhibited maximum lipid content (41.3 ± 0.66 %) among the seven microalgae *Chlorella vulgaris* has moderate lipid content of 21.1 ± 0.68 %. *Synechococcus lividus* showed the lowest total lipid percentage 8.9 ± 0.83% followed by *Chroococcus turgidus* and *Desmococcus olivaceous.*

From the results, the following details were observed: *Chlorella vulgaris* and *Synechococcus lividus* grown in their specific media at outdoor condition attained higher growth than these strains grown at indoor condition. The total biomass of *Chlorella vulgaris* and *Synechococcus lividus* produced at outdoor condition was significantly increased compared to their biomass production at indoor condition. The production of total lipid content in *Nitzschia pale*, *Scenedesmus quadricaudus*, *Chroococcus turgidus* and *Phormidium autumnale* at outdoor condition was more than the lipid content of these strains grown at indoor condition. At both the conditions, *Chlorella vulgaris* achieved maximum biomass and *Nitzschia palea* produced highest lipid content.

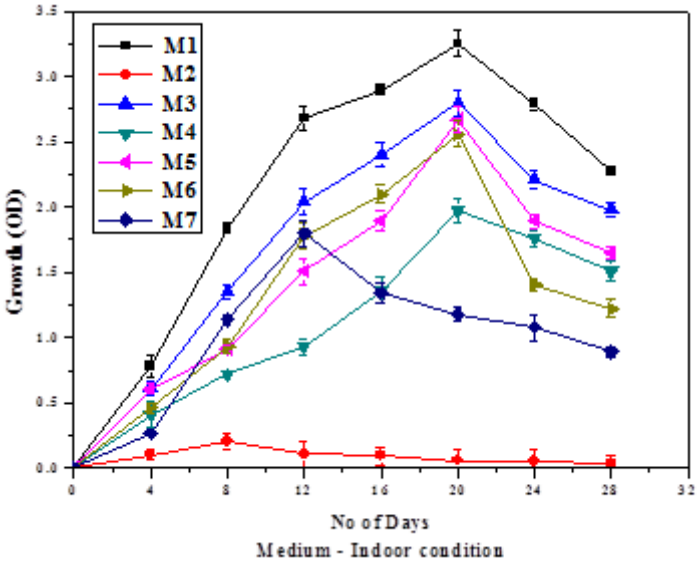

Fig. 1: Growth curve of microalgae in their respective medium at indoor condition

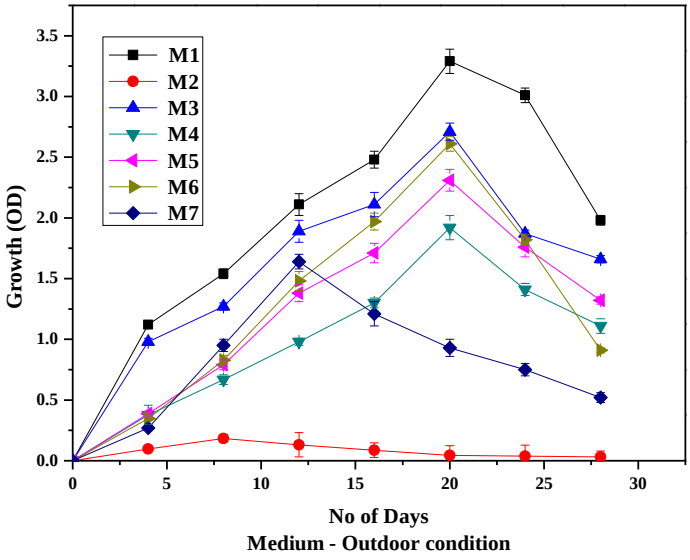

Fig. 2: Growth curve of microalgae in their respective medium at outdoor condition

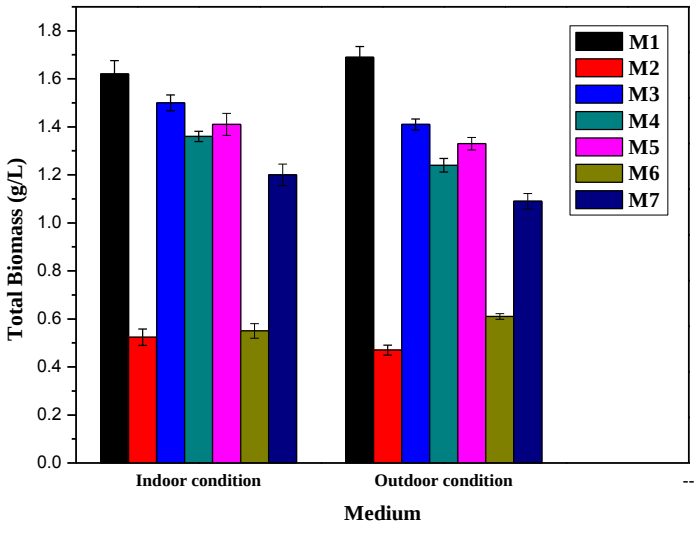

Fig 3: Total biomass of microalgae in their respective medium at indoor and outdoor condition

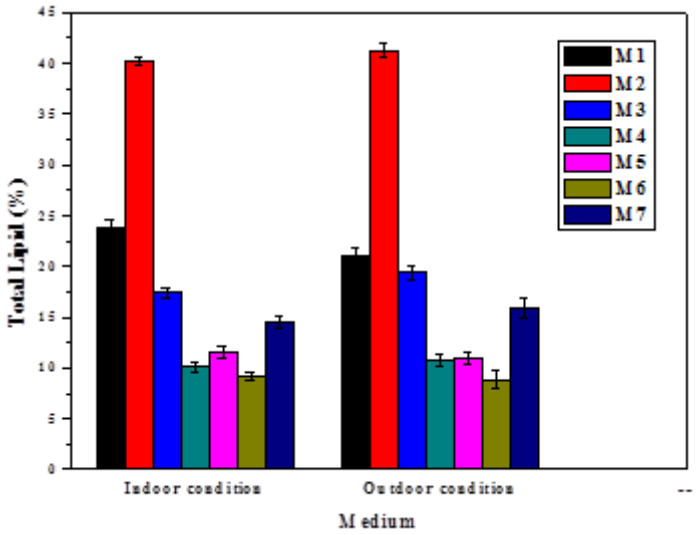

Fig. 4: Total lipid content of microalgae in their respective medium at indoor and outdoor condition

DISCUSSION

For microalgal lipids to be cost competitive as a liquid fuel source, a feedstock organism must be capable of high photosynthetic efficiencies (12–16%) and large lipid yields (50–60% of biomass weight in the form of lipids) [18].

The yield of biodiesel from microalgae depends up on both the biomass concentration of the cultures and the oil content of individual cells[17]. The cultivation time differed from one strain to another depending on the optimum growth rate till reaching stationary phase which always ranged between (15-20) days[20]. Days of log growth phase was long (16 to 20 days) in algal strains except *N. palea* and *P. autumnale* which showed short log phase (8 to 12 days).

Seven native cyanobacterial isolates and one native green alga was evaluated for their nutrient removal, biomass accumulation and lipid production capacities. The native green alga demonstrated significantly greater neutral lipid accumulation than the cyanobacteria under the conditions studied [10]. Similarly, the growth and biomass was higher at *C.vulgaris* followed by *S. quadricauda* and *D. olivaceus* in medium both at indoor and outdoor condition. Also, compared among the green algae, *C. vulgaris* showed maximum biomass and oil content than the cyanobacteria. The green microalgae of the genus *Chlorella* are among the potential candidates of biofuel or biodiesel production due to inherent characteristics of large biomass production and oil accumulation with minimal nutrient requirements[3].

A few diatom species (for example, *T. weissflogii* CCMP 1010 or *Cyclotella cryptica* CCMP 331) accounted lipids for almost 40% of the total organic matter, whereas the other microalgae, including the non-diatoms, had lipid levels usually below 30% of the total biomass. Similarly the diatom (*N. palea*.) produced highest lipid content both in medium at indoor and outdoor condition compared to the other non diatom (cyanobacteria and green algae). The green alga *Scenedesmus quadricauda* have total lipid content 16% and *Chroococcus turgidus* have the percentage of total lipid is 10% [20]. *Scenedesmus quadricauda* has a total lipid of 19.9% [16]. The total lipid of *Phormidium autumnale* KNUA026 was approximately 14% of dry weight [13]. In *C. humicola*, protein is major organic constituent (26%) followed by lipid and carbohydrates (14.26% and 11.4% respectively), whereas for *D.olivaceus* protein is major constituent (21%), with lipid (10.31%) and carbohydrate (11.86%). Here the exponential phase was persisted with increasing growth till 15 days[21]. The lipid content of *Lyngbya* sp. and *Synechococcus* sp. were 6.7 and 9.0% respectively[14].

However, there are two categories of microalgae that used for lipid production such as: 1) High lipid content but low growth rate, for example *Botryococcus braunii* with lipid content of 50% but had low biomass productivity of 28 mg/L/day. High growth rate but low lipid content, such as, *Chlorella vulgaris* [15,8,11]. In present study, *C. vulgaris* produced highest biomass with optimum lipid content. Compared to the microalgae (*P. autumnale* and *S. lividus*), *N. palea* produced moderate biomass with high lipid content.

CONCLUSION

For biodiesel production, the economic feasibility of microalgal mass culture have to be taken into consideration, the searching of microalgal species with high lipid content and high cell growth is a great importance. At both the cultivation conditions, *C. vulgaris* showed maximum biomass followed by *S. quadricaudus* and *D. olivaceus*; whereas the maximum lipid content was found in *N. palea* followed by *C. vulgaris*. The total biomass of *C. vulgaris* and total lipid content of *N. palea* was further increased in medium at outdoor condition. Hence these two microalgae: *C. vulgaris* (green algae) and *N. palea* (diatom) could also be cultivated at outdoor conditions and thereby effective for commercial large scale production. Thus these two microalgae can be selected and used as efficient strains for biodiesel production.

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