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Raceway pond cultivation of a marine microalga of Indian origin for biomass and lipid production: A case study

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ABSTRACT

Microalgal biodiesel has emerged as the third generation biofuel with lots of business promise and environmental benefits. Marine microalgae as a source of lipid feedstock for biodiesel are preferred due to negligible requirements of incubation time, landmass and fresh water as compared with other energy crops and also due to their resistance to contamination in relatively inexpensive open pond cultivation system. In this study, the growth kinetics and lipid accumulation of a marine microalga, *Chlorella variabilis* (PTA-ATCC no. 12198) of Indian marine origin were studied in a 400 L raceway pond with 150 L working volume. The culture showed an average growth rate (μ) of 0.36 day^{-1} with consequent total lipid content of 10% on dry cell weight basis. An average biomass productivity of 5.78 g^{-2} day^{-1} was obtained in the raceway pond cultivation. Maximum biomass productivity of 8.1 g^{-2} day^{-1} was achieved during summer. The effect of seasonal variations on biomass productivity of the selected marine microalga was studied throughout the year at IIT Kharagpur campus, West Bengal, India. The lipid was extracted by using hexane as solvent and the fatty acid composition was determined by using GC and MALDI-ToF analyses to check for its suitability for biodiesel production.

1. Introduction

The global concern over energy and environmental security has thrown many technological challenges before the world scientific community for deriving sustainable solutions through the discovery and development of feedstock for alternative green energy. According to annual world primary energy consumption report in 2008, fossil fuels accounted for about 88% of the total primary energy consumption in which oil and coal as the major participating fuels contributed to around 65% [1]. Consumption of fossil fuel resources generates greenhouse gases (GHGs) which eventually lead to global warming and climatic changes. With the advent of modern industrialization and ever increasing demand of energy, the paradigm has shifted toward the production of carbon neutral energy sources [2]. Thus, the technology development initiatives are being directed toward mitigating environmental pollution through the sustainable production of biofuels [3]. For a country like India where the public transport has a strong presence, biodiesel attracts considerable attention as an alternative to petroleum diesel.

Microalgae have significant advantage for biodiesel production over other terrestrial oil crops; due to their rapid biomass production rate, high photosynthetic efficiency and the ability to convert light energy into storage lipid reserves [4]. On an average, microalgae produce 10–20 times higher biodiesel than energy crops and oleaginous yeasts [5]. Aquatic species program (1976–1998) indicates that microalgae can produce 5000 to 15,000 gal of biodiesel per year per acre in an open pond culture system. Sustainable and feasible production of biodiesel from microalgal origin necessitates the large scale cultivation of biomass to meet the ever increasing energy demand [6]. Several arrays of limitations and influencing environmental factors on biomass productivity of microalgal cultures have been studied meticulously [7]. Major environmental factors such as temperature, light and humidity affect outdoor cultivation of a particular microalgal species for biofuel application. In this context, the Indian scenario satisfies the criteria for outdoor cultivation of microalgal biomass. It is essential that the organisms be of Indian origin as they are best adapted to the environmental factors. Hence, the dire need to evaluate their annual productivity in concurrence with the environmental factors on outdoor cultures is shown in the present work. In this study we also focused on the lipid composition of the marine microalga indicating its suitability for biodiesel application.

2. Materials and methods

2.1. Isolation and identification of the microalgal strain

The microalgal strain used in this study was isolated from the Jalandhar beach of Diu (a Union territory) India (located between

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20° 57.584' latitude and 70° 16.759' longitude). Sea water sample was taken in 50 mL sterile bottles and brought to lab in cold condition in cool pack. Isolation of unicellular microalgae was done by serial dilution (10^{-1} to 10^{-5}) and then spreading it on ASN-III agar plate. The plates were incubated for two weeks under low light (100 lx) for growth at 25 ± 2 °C in the algal culture room. The green color colonies grow on solid agar plates were picked up and grown in fresh ASN-III medium. The cultures were axenic and free from other contaminants. Based on morphology it was identified as *Chlorella* sp. Further, through 18s it is identified as a *Chlorella variabilis* and gene sequence is submitted to NCBI (accession no. KF849475). This strain was deposited with ATCC under the strain designation 12198.

2.2. Growing of algae

C. variabilis (PTA-ATCC no. 12198) of Indian marine origin was grown in Zarrouk's medium [8] with a pH value of 10 ± 0.1. The marine culture was cultivated outdoors at Kharagpur (22.3302° N, 87.3237° E) in a 450 L raceway pond with 150 L working volume. The raceway pond was 2.5 m long and had a channel width of 0.32 m. The average depth of the working volume (0.15 m^3) was 0.1 m. It was agitated for 24 h by a paddle wheel such that the linear velocity of the culture was 0.3 m/s. The raceway pond was operated in Summer, Monsoon, Autumn, and Winter (throughout the year) in batch mode with each batch lasting 15 days. Inoculum preparation was done using a 30 L airlift photobiorector placed indoor with artificial light (light intensity 4000–5000 lx), for a period of 7–8 days and was added to provide 10% of the working. Zarrouk's medium was used for inoculum development as well as for raceway cultivation. Evaporation losses were at the rate of 1 to 2 cm/day and were replenished with water every morning. Fresh water was used to replace evaporative losses. Thus, salinity was maintained after fresh water addition. Solar intensities varied between 102 lx (early morning and late evening) and 30,000 lx (mid-day). The light intensity was measured by a digital light meter (HTC, India; Model no. 102).

2.3. Chemical and reagents

Standard fatty acid methyl ester (FAME) mixtures were obtained from Sigma-Aldrich. Organic solvents such as hexane, chloroform and methanol were of analytical grade and were obtained from Merck, India. Olive oil and sulfuric acid were purchased from SRL, India. Analytical grade brucine reagent used for nitrate estimation was obtained from SRL, India. Samples prepared for MALDI-ToF MS (2,5-dihydroxybenzoic acid (DHB) and trifluoroacetic acid (TFA)) were of analytical grade with the highest commercially available purity and were purchased from Sigma-Aldrich. Medium constituents and sodium sulfate were obtained from Merck, India.

2.4. Biochemical procedures

The algal cultures were sampled at exactly 24 h interval from the time of inoculum addition for each batch. In case of each season sampling was almost done (±1 h during winter season) at the same point in the diurnal cycle (900 h IST) Biomass and lipid were analyzed in terms of concentration on dry cell weight basis (g L^{-1}) and on % (ww-1) Dry Cell Weight basis respectively. All biochemical analyses were done at room temperature (≈25 °C). Methods for individual estimation of biomass, nitrate and lipid are described below.

2.4.1. Biomass measurement, growth rate and biomass productivity calculation

Biomass was estimated in terms of dry cell weight (DCW) gravimetrically and along with the gravimetric method, optical density of the samples was also estimated by measuring the O.D value at 720 and 750 nm with a UV–visible spectrophotometer (Perkin-Elmer, MA, USA) [9] (not shown in this script). Cell counting was done using a hemocytometer (Rohem instruments, India) and a compound microscope (Olympus, India).

Growth rate of each culture was calculated based on the following relationship (Eq. (1))

$$\ln \frac{x}{x_0} = \mu t, \tag{1}$$

where, x_0 = initial biomass concentration, at time; x = final biomass concentration, and μ = specific growth rate of the culture (day^{-1}).

Biomass productivity calculation (g^{-2} day^{-1}) (Eq. (2)):

$$\text{Aerial productivity(for carpet area occupied)} = \frac{(\text{Final culture density} - \text{Initial culture density}) \times \text{Working volume}}{\text{Batch run time} \times \text{Carpet area occupied}}. \tag{2}$$

2.4.2. Nitrate estimation

Total nitrate consumption by the microalgal cells was estimated by using the method described in [10].

2.4.3. Total lipid content determination

Total lipid content was determined following a well established method of lipid extraction [11]. Lipid content was then calculated by the equation below (Eq. (3)):

$$\text{lipidcontent}(\%\ \text{DCW}) = \frac{\text{lipid wt}(g)}{\text{biomass wt}(\text{DCWg})} \times 100. \tag{3}$$

2.5. Dewatering (bulk water removal and drying)

Bulk water was removed by employing the method of flocculation [12]. The effect of culture pH on the flocculation efficiency of the flocculant was carried out in subsequent experiments by adding different dosage of alum [potassium aluminum sulfate, $KAl(SO_4)_2 \cdot 12H_2O$)] (MERCK, India) at different pH values (4,6,8 and 10). Dosage of alum (140–220 mg·L^{-1}) and flocculation time of 48 h were studied at 25–27 °C. After a settling period, supernatant was taken specifically from the 20 mL suspension culture and was centrifuged for biomass dry weight (g). Percentage flocculation was then calculated with the help of the following equation (Eq. (4)):

$$\%\ \text{Flocculated} = \left\{ \text{Dry cell wt in supernatant (DCW g)} \Big/ \text{Total wt of biomass (DCW g)} \right\} \times 100. \tag{4}$$

For flocculation:

(i) Initial biomass concentration before flocculation = 0.81 ± 0.06 g L^{-1};
(ii) Flocculation conditions: volume of the broth = 20 mL; temp. 25 °C;
(iii) Mixing time and agitation: the flocculant was initially added at a specific dosage and pH condition under stirring speed of 250 rpm using a magnetic stirrer for 10 min. After 10 min, the stirring was stopped and the culture with the flocculant was kept under the room temperature. Every hour the 1 mL supernatant was collected and analyzed for biomass dry weight (via O.D vs dry weight calibration plot). Until the flocculant showed almost 80% sedimentation.
(iv) Flocculation time and sedimentation time: initial flocculant formation was observed after 8 h of incubation of the test with respect to alum dosage and change in pH. Initial sedimentation of the microalgal suspension was observed after 10 h and it

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