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Evaluation of microalga for biodiesel using lipid and fatty acid as a marker – A central composite design approach

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ABSTRACT

Present study used central composite design (CCD) to evaluate algal strains for biodiesel by optimizing their harvesting time and pH. As a preliminary step, green alga *Chlorella vulgaris* MCRC A0001 and two cyanobacteria *Chroococcus turgidus* MCRC A0002 and *Spirulina platensis* MCRC A0005 have been explored for growth in terms of cell number, protein, chlorophyll-a, dry weight and pH from every 5^{th} day till 25^{th} day of growth. Furthermore, no hitherto report on CCD approach to inspect the impact of harvesting period and pH on algal lipid content. As evident from CCD, seemly candidate *C. vulgaris* MCRC A0001 exhibited high lipid content of 0.22 g dry weight^{-1} on day 20 compared to other strains. Under nitrogen stimulus, *C. vulgaris* MCRC A0001 grown under 0 g nitrogen/L and 27 °C showed 1.39 gL^{-1} biomass which marginally equal to control, and a significant increase in total lipid about 26% which is 4% high over control forum. It is noteworthy that, high biomass coupled with high lipid content was observed in nitrogen deprived and limited cells of *C. vulgaris* MCRC A0001. Additionally, robust methyl ester yield at 0.69 g g^{-1} was observed in 1:9 ratio of lipid-methanol and 3% NaOH, and ester yield was confirmed by FTIR spectra and gas chromatogram. Besides, gas chromatographic analysis revealed an increase in C16:0 −29.61%, C18:1−25.33%, C18:2−11.3% which are prerequisite for biodiesel production. Further, biodiesel was critically analyzed for Degree of unsaturation (DU-77.32) and Long chain saturation factor (LCSF-2.96) which accords the European standard.

1. Introduction

Worldwide at present, the fossil fuel reserves accomplish the majority of energy needs of humankind, and they are predicted to be exhausted in the future. They are determinate non-renewable resources concomitant with environmental hitches, and almost half of the world's energy reserves would be exhausted by 2025 if reliance on fossil fuel continues [6]. Thus, researchers eyeing for an alternative source that would be readily accessible, technically viable, and ecologically suitable. Indeed, biodiesel is one of the eminent sources to accomplish the energy demand of the world, and certainly avert the future catastrophe of fossil fuel usage. Biodiesel is a long chain methyl ester, non toxic, renewable, low carbon environmental friendly fuel produced from vegetable oils or animal fats and it undergoes oxidation and degenerate more quickly than mineral diesel [1]. Biodiesel noticeably releases less concentration of CO, CO_2, NO_X [11,13,22]. Initially, production of biodiesel from oil crop, waste cooking oil and animal fat has been attempted to swap fossil fuel usage [2], but they are not gratifying even a small fraction of the existing need of fuel. In this scenario, microalgae are the plausible and sustainable feedstock for biodiesel; milking their community for biodiesel production can be the apt solution since the microalgal solar energy conversion does not compete with food supply [24].

Microalgae are veritable miniature, sunlight-driven cell factories that mitigate carbon dioxide to bioactive compounds biofuels, foods, feeds and particularly biodiesel via esterification of microalgal oil. The first crucial point to be considered is to harvest the strain

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at its optimal growth phase to obtain a maximum oil yield. Evidently, period of harvesting with varied pH ranges play a vital role in improving microalgal lipid content as the metabolic machinery of algae can be altered during their life cycle. Hence, one of the objectives of this study is to develop an approach for better understanding of the relationships between the variables, (harvesting time and pH) and the response (lipid content) for biodiesel production. Therefore, central composite design (CCD) was used in this study for investigating the influence of harvesting/dewatering period, and pH on lipid content. In addition, physical parameters such temperatures, pH, light and nutrient composition are the key features that presumably change the microalgal biodiesel quality. From an economical viewpoint of biodiesel, growth medium need to be formulated with defined nitrogen and phosphorous concentrations for large scale cultivation. But the major obstacle often seen is inferior growth rate in nitrogen or phosphorous limitation. Therefore, a study has also been undertaken to obtain ample lipid content together with increased biomass yield under physical and chemical stress. Further, extracted lipid will be converted into fatty acid methyl ester (biodiesel) by the method transesterification/alcoholysis to minimize lipid viscosity characteristics. Suitability of biodiesel to use as a fuel is determined by various parameters such as cetane number, oxidative stability, Cold Filter Plugging Point (CFPP) and viscosity [15]. In our study, we used mathematical formulas to calculate the Degree of Unsaturation (DU), Long Chain Saturation Factor (LCSF) to determine the oxidative stability, cetane number and CFPP of algal biodiesel.

Hence, the aims of this current research were:

i) To use central composite design for exploring promising microalgae for ample lipid accumulation rate by the influence of harvesting time and pH,
ii) To obtain high lipid coupled with high biomass content under nitrogen and temperature stress,
iii) To analyze the fatty acid composition of algal biodiesel for fuel characteristics using DU and LCSF values.

2. Experimental

2.1. Sample collection and maintenance

Fresh water samples were collected in sterile plastic containers from the pond at Anna University, Chennai, India and brought to the laboratory for further processing. The collected samples were observed under the microscope to discern their morphological façade, and they were kept in Petri dishes enriched with suitable nutrients. At the end point of growth, unialgal strains were scaled up in Bold Basal Medium [5] in a thermostatically controlled environmental chamber illuminated with white fluorescent lamps with an intensity of 20 μmol photons m^{-2} s^{-1} at 27 ± 1 °C under 14: 10 h light–dark regime.

2.2. Growth kinetics

Culture growth was examined in terms of cell number, protein, chlorophyll and dry weight. The above mentioned growth parameters were estimated from every 5^{th} day up to 25^{th} day. Growth was recorded by counting the cells using a haemocytometer (Neubauer improved; made in India). The growth rate was obtained by using the formula.

$$Growth\ \ rate = \frac{\log N - \log N_0}{\log 2\ \ x\ t}$$

N – No. of cells per ml at the end of phase or mg/L
N_o – Initial count of cells per ml or mg/L
t – Duration of growth

2.3. Estimation of protein

Culture was centrifuged and the pellet was washed twice with Tris HCl buffer (pH–7.0) and resuspended in the same buffer. The suspension was sonicated for 15 min and centrifuged at 2000 rpm for 15 min. The supernatant was treated with 10% trichloro acetic acid (TCA) and it was neutralized with 2 N sodium hydroxide. Then, estimation of protein was carried out as described by [17].

2.4. Estimation of Chlorophyll-a

Culture pellet was suspended in 90% acetone and kept at dark for an hour. Thereafter, it was again centrifuged at 2000 rpm for 15 min and the clear supernatant was used for spectrophotometric estimation of chlorophyll-a using double beam UV-visible spectrophotometer [14].

2.5. Dry cell weight

Culture pellet was washed with distilled water. Then, the pellet was kept in preweighed plate and dried in hot air oven for 24 h or until constant cell weight was recorded.

2.6. pH measurement

During all the trials, pH was measured using a digital pH meter.