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Production and characterization of biodiesel from algae

Piyushi Nautiyal, K.A. Subramanian, M.G. Dastidar *

Centre for Energy Studies, Indian Institute of Technology, Delhi, Hauz Khas, New Delhi 110016, India

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ABSTRACT

The feasibility of biodiesel production from microalgae as third generation biodiesel feedstock was studied in the present investigation. The studies were conducted to evaluate the growth patterns of the algae species i.e. *Spirulina*, *Chlorella* and pond water algae. The oil was extracted from the algae biomass and then transesterified. Simultaneous extraction and transesterification were also studied using different solvents. Maximum biodiesel yield was obtained using simultaneous extraction and transesterification using hexane as a solvent. The systematic characterization of algae biomass, algae oil and algae biodiesel was carried out to establish the potential of microalgae for biodiesel production.

1. Introduction

The demand for alternative fuels has increased in the past several years. Several substitutes have come into existence in the recent years and many more are on their way to get established as a sustainable fuel alternative [1]. Biodiesel is gaining importance as one of the most important substitutes for the depleting fossil fuels. There are many advantages of using biodiesel as an alternative form of energy [2,3]. It can be used as such in the diesel engine without any engine modification indicating that it has comparable physical and chemical properties as conventional diesel. The combustion properties of biodiesel are also very close to those of petroleum diesel [4]. Biodiesel is highly biodegradable [5] and is nontoxic as well as renewable. The exhaust of biodiesel during combustion has lesser carbon monoxide, hydrocarbon, particulate matter and sulfur dioxide as compared to those of petro-diesel [6–9]. However, the problems like increased NO_x emissions [10,11], poor cold flow and poor oxidative stability [12] need to be solved. Therefore, a lot of research is focused on control of these NO_x emissions [13–16].

Biodiesel can be derived from edible oil seed crops such as sunflower, palm, rapeseed, soybean, coconut, etc. which are considered as first generation biodiesel feedstocks. However, use of such feedstocks for biodiesel production has faced problems as they disturb the overall worldwide balance of food reserves and safety. The non-edible seed crops of jatropha, karanja, jojoba, mahua and waste cooking oil, grease, animal fats, etc. have gained importance in the last few years as second generation feedstocks for biodiesel production. However, these second generation feedstocks are not sufficient to entirely substitute the present transportation needs.

Recent focus is on microalgae as the third generation feedstock. Using microalgae has several advantages like high photosynthetic efficiency and higher biomass production [17]. Microalgae do not compete for land and can grow anywhere, even in brackish saline water. The current research efforts have been concentrated on increasing lipid content in microalgae [18–20] and culturing of algae [21–24]. In order to establish the potential of microalgae biomass as an alternative for biodiesel production, more concentrated attempts are needed for detailed characterization of algae biomass, algae oil and algae biodiesel as very little information in literature is available on the same.

The present study dealt with three different species of algae i.e. *Chlorella*, *Spirulina* and pond water algae in order to assess their potential for biodiesel production. The natural pond water algae biomass is expected to be a cheaper feedstock for biodiesel production as compared to pure cultures of *Chlorella* and *Spirulina*. The growth patterns of the three algae species were studied with an aim to determine the maximum productivity of algae species. The algae biodiesel production was attempted via oil extraction and transesterification both in single stage and two stage reactor units in order to get the maximum biodiesel yield. The present work investigated the usefulness of techniques like FTIR, NMR, GC and proximate and elemental analyses to understand the chemical properties of algae biomass, algae oil and algae biodiesel. The fuel properties of algae biodiesel were also investigated. The results were then compared with that of karanja biodiesel and conventional diesel in order to establish the potential of algae biomass for biodiesel production.

* Corresponding author.
E-mail address: mgdastidar@gmail.com (M.G. Dastidar).

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2. Materials and methods

2.1. Microalgae, growth medium and conditions

Spirulina platensis and *Chlorella pyrenoidosa* used in the present study were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune-Maharashtra, India. The algae sample from a local pond was also collected from India gate, New Delhi, India. All the three samples were maintained in conical flasks containing 50 mL sterile BG11 media [25]. Proper shaking conditions were also maintained at 120 rpm and 24 °C for half an hour and then placed in the direct sunlight. Regular sub culturing was performed after every 15–18 days. In a growth chamber, the cultures were incubated under illumination (2000 lx) at 25 ± 1 °C with light: dark cycles of 12:12 h for 15 to 18 days with bubbling air at normal pressure required to maintain stirring of the cultures. With the concern for large scale cultivation of algae, growth was also attempted in tap water without BG 11 medium.

2.2. Growth evaluation

Growth of all the three species was monitored both in the presence and absence of BG 11 media for the total period of 27 days. Growth of the species was evaluated based on two parameters: (i) chlorophyll a content and (ii) dry cell weight.

(i) *Chlorophyll a content*: Chlorophyll a was extracted and estimated using the procedure used by Chinnaswamy et al. [26]. For this, a known volume of algae biomass was suspended in the medium and centrifuged at 8000 rpm for 10 min. Biomass collected after centrifugation was again suspended in a known volume of methanol. This methanol and algae biomass suspension was then immersed in the water bath for half an hour at 60 °C in order to extract the chlorophyll from the biomass. After the stipulated time, the chlorophyll concentration in the above suspension was spectrophotometrically determined using UV visible spectrophotometer (Systronics117). The absorbance values were then substituted in Eq. (1) used for chlorophyll estimation [27]:

$$\text{Chl a}\left(\frac{\mu g}{ml}\right) = 16.29\left(A^{665.2} - A^{750}\right) - 8.54\left(A^{652} - A^{750}\right) \quad (1)$$

where A^{750}, $A^{665.2}$, A^{652} are referred as the absorbance of algae biomass-chlorophyll suspension in methanol at 750, 665.2 and 652 nm, respectively.

(ii) *Dry cell weight*: In order to evaluate the growth with respect to dry cell weight, a known volume of algae biomass growing in the suspension was taken and centrifuged at 8000 rpm for 10 min. The collected biomass was washed with distilled water to remove the salts present. This biomass was then filtered on the pre-weighed filter paper and dried overnight at 60 °C and then weighed.

2.3. Oil extraction and biodiesel production

The acid values of *Spirulina* algae oil and pond water algae oil obtained in the present study were 9.42 mg KOH/g and 8.86 mg KOH/g respectively. The acid values were quite high and thus acid catalyzed transesterification was done in the present investigation. Acid based transesterification of algae oils also has been reported effective for biodiesel production [28].

(i) *Oil extraction followed by transesterification* (*two stage process*): The dried algae biomass after pulverization was subjected to Soxhlet extraction using hexane as a solvent at 56 °C. The solvent phase was separated from oil phase by distillation.

The lipids separated from the solvent were then subjected to transesterification process for biodiesel production. For transesterification, lipids were treated with methanol along with conc. H_2SO_4 at 60 °C for 1 h. Proper mixing was maintained in the reactor during the process. After the completion of the reaction, products were allowed to cool at room temperature. Water was then added to the reactor and the mixture was transferred to a separating funnel. Phase separation was observed. Lower phase of biodiesel was collected and washed with distilled water. The percentage yield of biodiesel was calculated using the Eq. (2):

$$\text{Yield of biodiesel } (\%) = \frac{\text{Grams of biodiesel produced}}{\text{Grams of the oil used}} * 100 \quad 2$$

(ii) *Simultaneous oil extraction and transesterification* (*single stage process*): The dried algae biomass was added to the reactor after pulverization. The solvent and methanol along with the catalyst (conc. H_2SO_4) were also added to the reactor. The temperature was maintained at 60 °C for 1 h with mixing. After the reaction was completed, the products were allowed to cool down to room temperature and distilled water was added to the mixture. The products were transferred to the separating funnel which immediately resulted in the formation of two layers. The upper solvent layer with biodiesel was separated and subjected to distillation for biodiesel recovery. Percentage yield of biodiesel was then determined using the above Eq. (2). The effect of different solvents (chloroform, hexane, and no solvent) on biodiesel yield was also studied using the similar procedure.

2.4. Characterization

2.4.1. Proximate analysis

The moisture, volatile matter and ash content of the dried algal biomass of the three species were determined according to ASTM protocol.

2.4.2. CHNS analysis

The elemental analysis of carbon, hydrogen, nitrogen and sulfur of dried algae biomass, algae oil and biodiesel was carried out using CHNS analyzer (Elementar Vario El Cube) and the oxygen content was calculated by difference.

2.4.3. Proton nuclear magnetic resonance (^{1}H NMR)

The ^{1}H NMR analysis of algae oil, algae biodiesel, karanja biodiesel and diesel was conducted using Bruken Spectrospin 300 operating at 300 MHz. The samples were prepared in $CDCl_3$ with the ratio of 1:1 by volume in 5 mm NMR tube and TMS (tetramethylsilane) was used as an internal standard.

2.4.4. ^{13}C nuclear magnetic resonance (^{13}C NMR)

The ^{13}C NMR analysis of algae oil, algae biodiesel, karanja biodiesel and diesel was performed on a Bruken Spectrospin 300 operating at 300 MHz. The samples were prepared in $CDCl_3$ with the ratio of 1:1 by volume in 5 mm NMR tube and TMS (tetramethylsilane) was used as an internal standard.

2.4.5. Fourier transform-infrared (FTIR) spectroscopy

The FTIR spectra of dried algal biomass, algae oil and biodiesel were obtained using FTIR spectrometer (Nicolet 6700) at room temperature. The dried algal powder was mixed with potassium