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Dual uses of microalgal biomass: An integrative approach for biohydrogen and biodiesel production

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HIGHLIGHTS

- *Chlorella* sp. NBRI029 and *Scenedesmus* sp. NBRI012 shows high biomass productivity.
- *Scenedesmus* sp. NBRI012 shows maximum H_2 evolution in 6th day of fermentation.
- Residual biomass after H_2 production contains high lipid content.
- Lipid extracted from the residual biomass fulfills various biodiesel properties.

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ABSTRACT

Dual application of biomass for biohydrogen and biodiesel production could be considered a feasible option for economic and sustainable energy production from microalgae. In this study, after a large screening of fresh water microalgal isolates, *Scenedesmus* sp. NBRI012 and *Chlorella* sp. NBRI029 have exhibited high biomass (1.31 ± 0.11 and 2.62 ± 0.13 g/L respectively) and lipid (244.44 ± 12.3 and 587.38 ± 20.2 mg/L respectively) yield with an organic carbon (acetate) source. *Scenedesmus* sp. NBRI012 has shown the highest H_2 (maximum evolution of 17.72% v/v H_2 of total gases) production; it produced H_2 continuously for seven days in sulfur-deprived TAP media. Sulfur deprivation during the H_2 production was found to increase the lipid content (410.03 ± 18.5 mg/L) of the residual biomass. Fatty acid profile of the lipid extracted from the residual biomass of *Scenedesmus* sp. NBRI012 has showed abundance of fatty acids with a carbon chain length of C16 and C18. Cetane number, iodine value, and saponification value of biodiesel were found suitable according to the range given by the Indian standard (IS 15607), Brazilian National Petroleum Agency (ANP255) and the European biodiesel standard EN14214.

1. Introduction

There is an urgent need for a renewable, clean, carbon-free, alternative energy source due to global threat of fuel shortage in the near future. Research effort has been aimed at microalgae as a suitable biomass-producing organism with high photosynthetic efficiency and faster growth as compared with any other energy crop [1]. Microalgae can be grown in ponds, photobioreactor [2,3] using waste water also [4,5]. During photoautotrophic conditions microalgae are able to reduce net carbon dioxide (CO_2) from the environment [6,7]. It has the ability to provide several bioenergy carriers, including biodiesel from stored lipids as well as alcohols and hydrogen (H_2) from fermentation of reserve carbohydrates, particularly in anoxic condition [8]. In stress conditions, some microalgae can produce approximately 80% lipid of their dry cell weight [9,10]. It has been reported that deprivation of nutrient generates stress for the algae and could increase the lipid accumulation [11]. Algal biodiesel has a higher flashpoint, faster biodegradation, and greater lubricity as compared with traditional diesel fuel [12]. An integrative approach to use the algal biomass for H_2 and biodiesel production can make this technology more economic. There are several start-up companies that are attempting to commercialize algal fuels [13].

A few microalgae, in scarcity of oxygen (O_2), could switch to anoxic (fermentative) metabolism and produce H_2 [14]. Gaffron and his co-researchers first observed that in anoxia, unicellular green alga, *Scenedesmus obliquus*, generates H_2 in the presence of light [15]. After this discovery, an extensive research on H_2 metabolism in algae was performed. Among all the microalgae,

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Chlamydomonas reinhardtii is mostly explored due to its relatively high hydrogenase activity [16]. Melis showed that sulfur (S) deprivation in the media could generate anoxia, required for H_2 evolution [16]. The reducing equivalents produced during catabolism of reserve carbohydrates are used for the production of H_2 by the enzyme hydrogenase [16]. Hydrogen gas provides high gravimetric energy content (142 kJ/g), and its combustion produces only water [17]. After the H_2 production, the residual algal biomass may contain large amounts of valuable components that may be extracted for pharmaceutical, biodiesel, and other industrial purposes, which hence may have a considerable commercial and financial value [18]. Several reports are available for an increase in lipid and Triacylglyceride (TAG) contents in nitrogen starvation [11,19]. However, Cakmak et al. demonstrated that S deprivation increases lipid and TAG contents even better than does nitrogen starvation [20].

In this study, screening of the potentiality of fresh water microalgae isolates was done for their biomass productivity, lipid and carbohydrate content. They were studied for H_2 production and efficacy of the residual biomass after H_2 production for lipid accumulation with an aim at establishing the dual application of the same biomass to make it economic. Determination of fatty acid profile and various biodiesel properties such as cetane number, iodine value, and saponification was done to establish the potentiality of the lipid extracted from the residual biomass as a source of biodiesel.

2. Methods

2.1. Isolation, identification, and FT-IR analysis of collected algae

Fresh water algae have been collected from ecological niches, including lakes, rivers, and fresh water bodies of India, purified, and identified [21] under a microscope (Leica DM500). They were grown autotrophically in batch culture using Bold's basal medium (BBM) in 1000 ml Erlenmeyer flask in uniform growth condition at a temperature of 27 °C ± 0.5 °C, a photoperiod of 14:10 h light/dark cycle and fluorescent illumination of 3000 lux. During stationary phase, the dry biomass was obtained by centrifugation followed by lyophilization. Rapid screening of carbohydrate and lipid content of isolates was done by Fourier transform infrared spectroscopy (FT-IR). Analysis was done by mixing 2 mg of dry algal biomass with 198 mg of IR spectroscopy grade potassium bromide (KBr). Each sample was analyzed by OMNIC software by collecting 100 scans at 4 cm^{-1} resolution using an FT-IR spectrophotometer (Nicolet 6700, Thermo Scientific). Specific bands were identified using published algal FT-IR spectra in relation to specific molecular groups [22,23].

2.2. Growth analysis, biomass, and carbohydrate content of the isolates

Among sixteen algal isolates, eight were selected for further study on the basis of FT-IR analysis. They were grown autotrophically in 200 ml BG-11, BBM as well as with an organic carbon source in TAP medium in batch culture. The growth patterns of the respective cultures were observed by measuring the optical density at 680 nm using a UV–VIS spectrophotometer (Spectrascan UV 2700, Thermo scientific). Biomass productivity was calculated by filtering 10 ml of culture through pre-weighed, 0.45-μm nylon positive zeta membrane filters (Pall life sciences, India) followed by drying at 55 °C in a hot air oven for one to two days till constant weight was achieved. Biomass content (*BC*) of the cultures was obtained by the total weight of the dry biomass in grams per liter of culture.

Biomass productivity (*BP*) was calculated by the formula given by Griffiths and Harrison [24]:

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

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

Carbohydrate content of the dry biomass estimated by the Anthrone method was suitably adapted for estimation of total carbohydrate [25]. Calibration curve has been obtained by using D-glucose. Percentage of carbohydrate (*TC*) in dry biomass was calculated by the following formula:

$$TC = OD \times \text{slope value of calibration curve} \times 100 \quad (2)$$

Carbohydrate content (*CC*) was calculated by the following formula:

$$CC = BC \times TC \quad (3)$$

2.3. H_2 production from the isolates

Suitable fermentation method has been set up at laboratory scale to screen the efficiency of isolated strains for H_2 production. Cultures were grown in 1000 ml TAP media (with S) till the late log phase with same culture conditions mentioned earlier. An indigenous photobioreactor has been developed for H_2 production in batch fermentation. A rectangular glass reactor with 2 L capacity, having three openings fitted with probes for H_2 (HY-OPTIMA-700 H2scan, Valencia, CA), pH and dissolved oxygen (dO_2) (ORION Star A329, Thermo Scientific) placed on a magnetic stirring platform. The evolved gas mixture was allowed to pass through 40% KOH solution for selective absorption of CO_2. The H_2 gas was collected in a glass cylinder by water displacement system. All the media and glass wares were autoclaved.

Biomass of the late log phase was collected by centrifugation and transferred to 1000 ml TAP (S free) media in the photobioreactor with same culture conditions. Argon gas was sparged in culture medium to create anaerobic condition within the reactor. Initial pH of the fermentation was 7.2. Real-time H_2 production (percentage of H_2 v/v in total evolved gas) has been measured with an H_2 sensor, which determines the online percentage of H_2 in total evolved gas in the headspace by the hyper-terminal in the computer.

2.4. Lipid extraction and estimation

Lipid has been estimated by Folch extraction method [11,26]. Percentage of total lipid (*TL*) in dry biomass was calculated by the following formula:

$$TL = \text{weight (extracted lipid)/weight (biomass taken)} \times 100 \quad (4)$$

The lipid content (*LC*) was calculated by the following formula:

$$LC = TL \times BC \quad (5)$$

The lipid productivity (*LP*) was calculated by the formula given by Griffiths and Harrison [24] expressed in mg/L/d:

$$LP = BP \times TL \quad (6)$$

2.5. Fatty acid analysis

The derivatization of lipid fraction and gas chromatographic analysis was done by the method given by Mandotra et al. [11].