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Pretreated algal bloom as a substantial nutrient source for microalgae cultivation for biodiesel production

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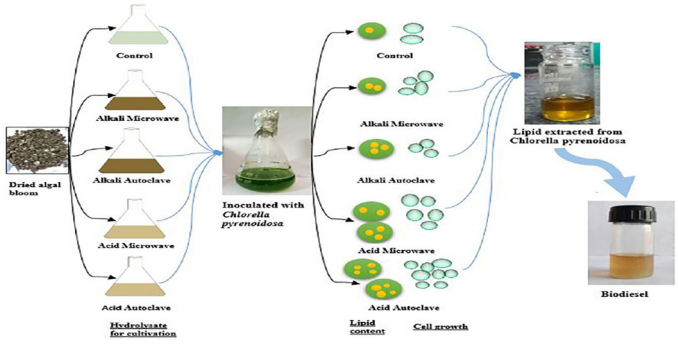
HIGHLIGHTS

- Toxic and copious algal bloom as a promising feedstock for *C. pyrenoidosa*.
- Acid autoclave hydrolysis showed maximum nitrogen, phosphate and carbon content.
- Maximum biomass productivity (436 mg/L/d) obtained in acid autoclave medium.
- High lipid content (43%) accumulated in acid autoclave hydrolysis medium.
- Acid autoclave hydrolysis had most appropriate FAME profile.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present investigation, toxic algal bloom, a copious and low-cost nutrient source was deployed for cultivating *Chlorella pyrenoidosa*. Various pre-treatment methods using combinations of acid/alkali and autoclave/microwave were tested for preparing hydrolysates and compared with minimal media (BG-11). Acid autoclave treatment resulted in maximum carbon, nitrogen and phosphorous content which substantially boosted the growth of the microalgal cells (4.36 g/L) as compared to rest of the media. The microalga grown in this media also showed enhanced lipid content (43.2%) and lipid productivity (188 mg/L/d) as compared to BG-11 (19.42 mg/L/d). The biochemical composition showed 1.6-fold declines in protein while 1.27 folds in carbohydrate content as compared to BG-11. The fatty acid profile revealed the presence of C14-C22 with increased amount of monounsaturated fatty acids as compared to BG-11. The results obtained showed that algal bloom can be used as a potential nutrient source for microalgae.

1. Introduction

The dependency on non-renewable fossil resources like coal, petroleum and natural gas are increasing as the world is moving towards modern civilization (Koh and Ghazi, 2011). This has caused serious threats to the world's energy security for future generation (Sanjid et al., 2013). The development of an alternate renewable energy source will not only reduce the environment pollution but preserve the non-renewable resources and inhibit the fluctuation in crude oil prices (Koh and Ghazi, 2011). In this regard, biodiesel is an attractive option which is biodegradable, sustainable and green fuel (Mostafa and El-Gendy, 2013). It

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consists of mono-alkyl esters of long chain fatty acids produced by transesterification of vegetable oil, animal fats or microbial lipids and can be directly used in conventional diesel engines (Kakkad et al., 2015).

Recently, biodiesel derived from microalgal lipids have gained immense interest as compared to plant-based oils due to their high growth rate, high lipid and reduced land requirements (Arora et al., 2016a). However, biodiesel is still not established on an industrial scale due to its high production cost (Han et al., 2015). Thus, in order to make microalgae financially and practically a viable feedstock, the focus should be towards decreasing the cost of feedstock, increasing the areal productivity, inducing the lipid content, increasing percentage extraction, improvising conversion of algal lipids to biodiesel (Moon et al., 2014). Among these, the feedstock contributes 70–80% of the total production cost which can be substantially reduced by utilization of waste, copious and cheaper raw materials.

To this end, toxic algal bloom could serve as an economical source of raw material for cultivating microalgae for biodiesel production. In recent years, its density has increased worldwide with the exponential increase in wastewater discharge which can be harnessed for growth enhancement in microalgal cultivation systems (Bharathiraja et al., 2015). Various initiatives to eradicate the algal bloom problem have been undertaken to clean up toxic algal blooms that occur in nitrogen and phosphorus-rich sewage wastewater and agricultural run-off streams (Van Dolah et al., 2016). As the algae die owing to their short lives, their decay process leads to an acute deficiency of oxygen in water bodies due to which marine life gets fatally affected (Petrowski et al., 2015). Additionally, the algal bloom leading to anoxic water bodies can amplify, transfer disease and parasites to wild fish populations. These unwholesome problems can be minimized by utilizing this nutrient-rich algal bloom as a key resource for cultivating microalgae for biodiesel production. Also, the residual algal bloom biomass after hydrolysis can be used in fertilizers or animal feeds to utilize the remaining nutrients.

Hence, in present work, the feasibility of abundantly available algal bloom hydrolysate to cultivate *C. pyrenoidosa* as the model organism for biodiesel production was tested. To optimize the best method for extracting nutrients from the algal bloom, five different pretreatments using various concentration of acid/alkali in combination with autoclave/microwave were tested.

2. Materials and methods

2.1. Materials

Chlorella pyrenoidosa (NCIM2738) was procured from the National Centre of Industrial Microorganism (NCIM), Pune, India. All solvents and reagents used in this study were of HPLC grade. Nile red stain (9-diethylamino-5H-benzo[α]-phenoxazine-5-one), BG-11 medium, Triolein (standard for FTIR) were purchased from Hi-Media, India.

2.2. Algal strain and cultivation condition

The microalga strain was maintained in the BG-11 medium at 25 ± 2 °C with constant shaking at 130 rpm and 200 μmol m^{-2} s^{-1} illumination (white fluorescent lamps). The microalga was precultivated in BG-11 for a period of 4 days in the same growth conditions.

2.3. Algal hydrolysate preparation

Algal bloom was locally collected during Winter season (January-March 2016) having an average temperature of 20 °C from the nearby Paniyala (29°51′6″N 77°50′42″E) water pond, Roorkee, India and then washed thrice with distilled water followed by drying in hot air oven for 24 h. The dried algal bloom (DAB) was grounded into a fine powder before preparing its hydrolysates. Five different modified pre-treatments methods namely: acid autoclave (AA), acid microwave (AM), alkali autoclave (ALA), alkali microwave (ALM) and aqueous hydrolysis (AH) were used for preparing the hydrolysates. For AA hydrolysate and AM hydrolysate preparation, 5 g of DAB powder was treated with six different concentrations (0.25, 0.5, 0.75, 1, 1.25 and 1.5%) of 100 mL dilute H_2SO_4 and kept at 121 °C for 15 min in an autoclave and microwave digester (MULTIWAVE-PRO, ANTON PAAR) respectively. Similarly, for alkali autoclave hydrolysate and alkali microwave hydrolysate, 100 mL NaOH (0.25, 0.5, 0.75, 1, 1.25 and 1.5%) was used. As a comparative study to the autoclave/microwave and acid/alkali treatments aqueous hydrolysate (AH) was prepared by dissolving 5 g of DAB in 100 mL distilled water and heated at 121 °C for 15 min. Each of the hydrolysates was then cooled to room temperature before centrifuging at 10,000 rpm for 10 min to remove suspended algal bloom particles. The supernatant was then neutralized with phosphoric acid and calcium carbonate to attain pH = 7.4. The supernatant was passed through 0.22 μm filter paper to sterilize the hydrolysate before culturing the microalga strain.

2.4. Characterization of algal hydrolysate

The total organic carbon (TOC) and inorganic carbon (IC) were analyzed by using TOC analyzer (TOC-V CPH, SHIMADZU). The chemical oxygen demand (COD), total nitrogen (TN), and total phosphorous (TP) determination were done using water analysis kit and UV-spectrometer (DR5000, HACH). Total dissolved solids (TDS) and total suspended solids (TSS) were evaluated gravimetrically at 105–110 °C while the estimation of total nitrate, ammonium, magnesium, phosphate contents were done using Ion Chromatography (COMPACT IC plus 882, Metrohm).

2.5. Characterization of algal bloom before and after pretreatment

The elemental composition of algal bloom was analyzed before and after the pretreatments using CHNS elemental analyzer (Vario EL III, Elementar).The changes in the morphology of the algal bloom were recorded by field emission scanning electron microscopy (FE-SEM Quanta 200 FEG) with energy dispersion X-ray analysis (EDX) to determine the variation in constituents of algal bloom before and after treatment. Further, the functional groups present in algal bloom were identified by Fourier transform infrared spectroscopy (FT-IR 6700, NICOLET).

2.6. Cultivation of C. pyrenoidosa in algal bloom hydrolysates

The pre-cultivated culture of *C. pyrenoidosa* (0.2 g/L) was first adapted to all the algal bloom hydrolysates before starting the experiments. These adapted cultures (0.2 g/L) were then used to inoculate 250 mL of each algal hydrolysate (acid autoclave, acid microwave, alkali autoclave, alkali microwave, aqueous hydrolysis and BG-11). The flasks were kept at 25 ± 2 °C with constant shaking at 130 rpm and 200 μmol m^{-2} s^{-1} illumination for a period of 10 days. All the experiments were repeated thrice (n = 3) and the results are expressed as mean ± S.D.

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