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ScienceDirect
Bioresource Technology 98 (2007) 560–564
BIORESOURCE TECHNOLOGY

Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents

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Received 26 September 2005; received in revised form 23 January 2006; accepted 5 February 2006
Available online 19 June 2006

Abstract

Growth of *Botryococcus braunii* (race 'A') and production of its constituents viz, hydrocarbon, carbohydrate, fatty acid, and carotenoids were influenced by different levels of salinity. Under salinity at 34 mM and 85 mM, 1.7–2.25-fold increase in the relative proportion of palmitic acid and two fold increase in oleic acid were observed. A twofold increase in carotenoid content was noticed at 85 mM salinity with lutein (75% of total carotenoid) as the major carotenoid followed by β-carotene. The increase in biomass yields and changes in other constituents indicated the influence of salinity and the organism's adaptability to the tested levels of salinity (17 mM to 85 mM).

Keywords: *Botryococcus braunii*; Microalgae; Hydrocarbon; Biomass; Salinity; Carotenoids; Carbohydrates; Fatty acids

1. Introduction

Botryococcus braunii is a green colonial fresh water microalga and is recognized as one of the renewable resource for the production of liquid hydrocarbons. *B. braunii* is classified into A, B and L races depending on the type of hydrocarbons synthesized. Race A produces C_{23} to C_{33} odd numbered *n*-alkadienes, mono-, tri-, tetra-, and pentaenes, which are derived from fatty acids (Metzger et al., 1990). Race B produces C_{30} to C_{37} unsaturated hydrocarbons known as botryococcenes and small amounts of methyl branched squalenes (Metzger and Largeau, 2005), whereas race L, produces a single tetraterpenoid hydrocarbon known as lycopadiene (Metzger et al., 1990). Hydrocarbons extracted from the alga can be converted into fuel such as gasoline and diesel by catalytic cracking (Hillen et al., 1982). *B. braunii* (Races A and B) strains are also known to produce exopolysaccharides up to 250 g m^{-3}, whereas L race produce up to 1 kg m^{-3} (Banerjee et al., 2002). However, the amount of exopolysaccharides production varies with the strains and the culture conditions.

Algae differ in their adaptability to salinity and based on their tolerance extent they are grouped as halophilic (salt requiring for optimum growth) and halotolerant (having response mechanism that permits their existence in saline medium). In either case, the algae produce some metabolites to protect from salt injury and also to balance as per the surroundings osmotica (Richmond, 1986). *Dunaliella*, the unicellular green alga is an example for its ability to survive extreme salt stress and serve as a useful model to comprehend the strategies of cell response to high salt concentration. The present study focused on the adaptation of *B. braunii* (race A) to varied range of saline conditions and their effect on the growth, hydrocarbon, carotenoid and carbohydrate production.

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0960-8524/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2006.02.007

2. Methods

2.1. Algal culture

The strain *Botryococcus braunii* (LB 572) was obtained from University of Texas, USA. This organism was identified as race-A based on its hydrocarbon profile. Stock cultures were maintained regularly on both liquid and agar slants of modified Chu 13 medium (Largeau et al., 1980).

2.2. Media and culture conditions

A set of 500 ml Erlenmeyer conical flasks were taken and 200 ml of modified Chu 13 medium was distributed and sodium chloride was added in the range of 17 mM to 85 mM to the flasks and inoculated. Two weeks old culture of *B. braunii* LB 572 grown in modified Chu 13 was used as inoculum at 20% (v/v). The culture flasks were incubated at 26 ± 1 °C temperature under 1.2 ± 0.2 klux light intensity and 16:8 h light dark cycle. All the experiments were carried out in triplicates.

2.3. Analytical methods

2.3.1. Biomass, chlorophyll and carotenoid estimation

The cultures were harvested by centrifugation at 5000 rpm and the cells were washed twice with distilled water. Then the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight ($g\,L^{-1}$). To estimate chlorophyll, a known volume of *B. braunii* culture was centrifuged and the residue was extracted with methanol repeatedly. The chlorophyll content in the pooled extract was estimated spectrophotometrically by recording absorbance at 652 and 665 nm and quantified using the method of Lichtenthaler (1987). To estimate carotenoids, known amount of freeze dried algal biomass was extracted with acetone and absorbance was measured at 450 nm and the concentration of carotenoid was determined using Davies (1976) method.

2.3.1.1. HPLC analysis of carotenoids. The carotenoid profiles of stress induced cultures were analysed by HPLC using a reversed phase C18 column with an isocratic solvent system consisting of acetonitrile/methanol/dichloromethane (70:10:20) at a flow rate of 1.0 ml/min and detected at 450 nm. Lutein, β-carotene, violoxanthin and zeaxanthin were identified using authentic standards (Sigma).

2.3.2. Carbohydrate and protein estimation

Known amount of cell free (spent) medium was analysed for total carbohydrate by phenol–sulphuric acid method (Dubois et al., 1956). Protein content in the cell free (spent) medium was analysed by Bradford protein assay (Zor and Selinger, 1996).

2.3.3. Hydrocarbon extraction and analysis

The dry biomass was homogenised in mortar and pestle with *n*-hexane for 15 minutes and centrifuged. The extraction process was repeated twice and supernatant was transferred to pre-weighed glass vial and evaporated under the stream of nitrogen to complete dryness. The quantity of residue was measured gravimetrically and expressed as dry weight percentage (Dayananda et al., 2005). The crude extracts were purified by column chromatography on silica gel with *n*-hexane as an eluent. The eluent was concentrated and analysed by GC using BP-5 capillary column as described by Dayananda et al. (2005).

2.3.4. Fatty acid analysis

The lipids were extracted with chloroform–methanol (2:1) and quantified gravimetrically. The lipid sample was dissolved in benzene and 5% methanolic hydrogen chloride (95 mL chilled methanol + 5 mL of acetyl chloride) was added and shaken well. The mixture was refluxed for 2 h then 5% sodium chloride solution was added and the fatty acid methyl esters (FAME) were extracted with hexane. The hexane layer was washed with 2% potassium bicarbonate solution and dried over anhydrous sodium sulphate (Christie, 1982). FAME were analysed by GC–MS (Perkin-Elmer, Turbomass Gold, Mass Spectrometer) equipped with FID using SPB-1 (poly(dimethysiloxane)) capillary column (30 m × 0.32 mm ID × 0.25 μm film thickness) with a temperature programming 130 °C to 280 °C at a rate of 3 °C/min. The FAME were identified by comparing their fragmentation pattern with authentic standards (Sigma) and also with NIST library.

3. Results

3.1. Effect of sodium chloride on growth

B. braunii was able to grow in all the tested concentrations of sodium chloride (17 mM to 85 mM). The biomass yields increased with increasing concentration of sodium chloride and maximum biomass was achieved in 17 mM and 34 mM salinity (Fig. 1). The variation in pH from 8.5 to 9.5 observed during the experimental period was independent of sodium chloride concentration. It was evident from the data (Fig. 1) that the decrease in phosphate was due to its utilization by the alga, which was not influenced by salinity concentration.

3.2. Effect of salinity on metabolite production

Hydrocarbon content in *B. braunii* was similar to growth pattern. The content varied in the range of 12–28% in different salinities and maximum hydrocarbon content was observed in 51 mM and 68 mM of salinity (Fig. 2). The hydrocarbon profile as analysed by GC