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Cultivation of *Botryococcus braunii* strain in relation of its use for biodiesel production

ABSTRACT

Microalgae are reported as the potential resources to produce lipid from their biomass cell. The *Botryococcus braunii* strain was studied by using two media - BBM and 3N-BBM - and its potential for biodiesel production was established. The experiment was performed at room temperature, using fluorescent light at a photoperiod of 15/9h light and dark cycle. The duration of the experiment was 25 days. The biomass was evaluated by measuring dry weight, optical density, chlorophyll, carotenoids and total lipids. The received results showed that the maximum vegetative growth was reached after approximately 21 days of incubation. The maximum growth rate during this period was 1.84 g/l dry weight in 3N-BBM medium. The lipid content which we received from the examined strain was 25.2% in 3N-BBM medium.

Key words: *Botryococcus*, biomass, biofuel, media

Introduction

Mankind uses algae in many directions – such as food, for the production of useful components, such as biofilters for the removal of nutrients from the wastewater, in order to determine the quality of the water, as an indicator of changes in environmental and laboratory systems for research. In the recent years, the algae are cultivated and used for the extraction of biofuel and it was assumed that the best "donor" of biodiesel are the algae.

Biodiesel fuel is becoming more promising as it is produed from non toxic, biodegradable and renewable resources and its use leads to a decrease in the emission of harmful air pollutants (Gouveia & Oliveira, 2009). Microalgae are a group of fast growing unicellular or simple multicellular micro organisms, which have the ability to fix CO_2 while capturing solar energy with efficiency 10 to 50 times greater than that of terrestrial plants and higher biomass production compared to energy crops (Wang et al., 2008).

The main environmental factors influencing microalgal growth and chemical composition are light, nutrients, temperature and pH (Rousch et al., 2003). In the recent years microalgae cultivation has received much attention on account of their utility as a feasible CO_2 sequestration technology (Ono & Cuello, 2006). Microalgae have several advantages, including higher photosynthetic efficiency as well as higher growth rates and higher biomass production compared to other energy crops. Several microalgae strains have been reported to have the ability to accumulate large quantities of lipids.

To produce 100 mg of biomass, algae need approximately 183 mg of CO_2 (Frac et al., 2010). High lipid contents are usually produced under environmental stress, typical nutrient limitation, which is often associated with relatively low biomass productivity and, therefore, low overall lipid productivity (Li et al., 2008). The lipid content of microalgae could be increased by various cultivation strategies, such as nitrogen depletion (Li et al., 2008), phosphate limitation (Reitan et al., 1994), high salinity (Rao et al., 2007), and high iron concentration (Liu et al., 2008).

Algae strains used and cited as one of the best prospect for biofuel according to many scientists (Pulz & Gross, 2004; Rodolfi et al., 2009; Radakovits et al., 2011; Mc Donald, 2011) are as follows: *Phaeodactylum tricornutum*, *Nannochloropsis oculata*, *Botryococcus braunii*, *Scenedesmus dimorphus*, *Chlorella protothecoides*.

B. braunii is a green microalga that produces hydrocarbons up to 75% of its dry biomass and it has already been proposed as a future renewable source of fuel (Banerjee et al., 2002). Exceptionally, an oil content of 86% was reported in the brown resting state colonies of *B. braunii* by Brown et al. (1969).

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The purpose of this work is physiological characteristic of algae strain *Botryococcus braunii* (SKU: AC-1006) and identification of its potential as a producer of biodiesel.

Materials and Methods

Microalgae strain and medium

Botryococcus braunii (SKU: AC-1006) was purchased from Algae depot – USA (www.algaedepot.com). *B. braunii* was grown on two type media: BBM medium (http://www.ccap.ac.uk/media/recipes/BB.htm) and 3N-Bold's basal medium with added vitamins according to the recipe provided on the CCAP website:
http://www.ccap.ac.uk/media/documents/3N_BBM_V_000.pdf

Cultivation

The cells in exponential period were inoculated (10%, v/v) in a liquid medium. Cultivation was initiated in 500 mL Erlenmeyer flask containing 400 mL medium. The cultures were maintained at room temperature (25-27ºC) on a fluorescent light with a light dark photoperiod of 15h:9h. Sterile-air containing 2% (v/v) CO_2 was aerated into the flask through an air sparger at the bottom of the flask. The strains were checked for 25 days growth period. All experiments were conducted in duplicates (BBM medium – bb and bb1; 3N-BBM medium – 3N and 3N1).

Growth measurements

The growth of *B. braunii* was measured via spectrophotometry (DR 2800) and biomass dry weight. Optical density for biomass factor was determined at wavelength 550 nm. One ml of sample was appropriately diluted with deionized water and the absorbance of the sample was read at 550 nm.

The cultures were determined gravimetrically and growth was expressed in terms of dry weight (mg/L) (Rao et al., 2007). The cultures were harvested by centrifugation at 3000g for 10 min and the cells were washed 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).

Chlorophyll and carotenoid content

The isolation of pigments from algae cells included the folowing procedures: harvesting 2 ml of microalgae cells by centrifugation at 10000 rpm, two times for 3 min and discarding the supernatant, suspension of cells in 2 ml methanol/water 90:10 v/v and mixing of Vortex for 1 min., heating of the suspension for half an hour in a water bath at 60ºC, cooling of the samples at room temperature, centrifugating the suspension (10000 rpm for 3 min) and discarding the supernatant with dissolved pigments. The absorbance of the pigments extract (665, 652 nm for chlorophyll content (a+b) and 470, 666 nm for carotenoids content) was recorded by using spectrophotometer. The chlorophyll content was computed (mg/l) according Porra et al. (1989) and carotenoid content was computed (mg/l) according Lichtenthaler (1987).

Lipid content

The total lipids were extracted from microalgae biomass using a modified method of Bligh & Dyer, 1959. The lipids were extracted using a mixture of chloroform/methanol (1:2 v/v). The quantity of lipid residue was measured gravimetrically and expressed as dry weight percentage.

Results and Discussion

Like other microalgae, *B. braunii* culture requires water, light, CO_2, and inorganic nutrients. Culture productivity is affected by factors such as pH, CO_2, irradiance, salinity, and temperature (Banerjee et al., 2002). According Lupi et al. (1991) the optimum temperature for growth is 25°C. In our experiment the temperature was 25-27°C.

In our study as expected the cultures growing in the BBM medium have lower values of optical density, than the cultures growing in the medium with three times more nitrates (3N-BBM). The maximum values of the optical density at *B. braunii* grown in 3N-BBM medium is 2,23, while in BBM medium is 0.86 (Figure 1). The better results, when enriched medium with nitrates, confirmed their positive effect to the increase of the culture growth.

Although a deficiency of nitrogen favors lipid accumulation (Ben-Amotz et al., 1985), nitrogen is required for growth. Studies with nitrogen supplied as NO_3^-, NO_2^-, and NH_3 reveal that the primary factor regulating nitrogen metabolism in *B. braunii* is the nitrate uptake system. Nitrogen is generally supplied as nitrate salts. An initial NO_3^- concentration of ≥ 0.2 kg·m^{-3} favors hydrocarbon production (Casadevall et al., 1983).

The influence of the media constituents potassium nitrate, magnesium sulphate, dihydrogen potassium phosphate and ferric citrate on growth and hydrocarbon production in *B. braunii* (SAG 30.81) was investigated using response surface methodology (RSM) (Dayananda et al., 2005).

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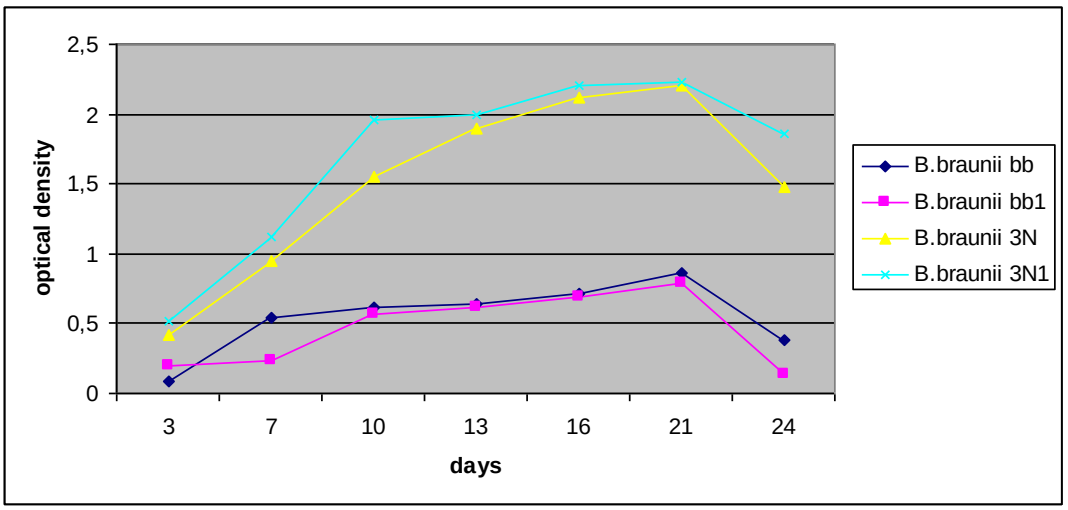

Figure 1. *Optical density of B. braunii (at 550nm) for 25 days of different media*

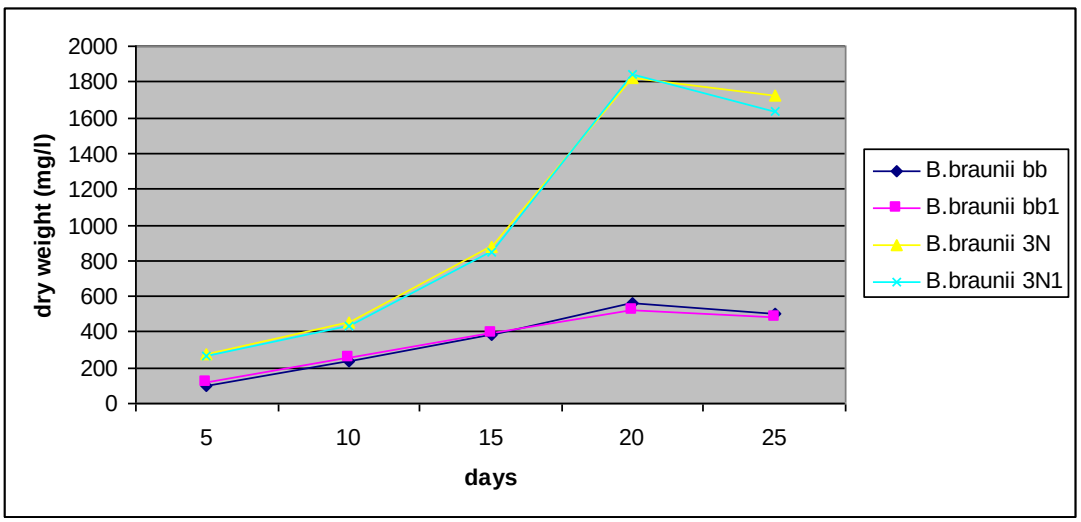

Figure 2. *Dry weight (mg/l) of B. braunii for 25 days grown of different media*

In our studies of *Botryococcus braunii* maximum dry biomass of 1.84 g/L was obtained on the enriched with nitrates medium (Figure 2).

Dayananda et al., 2007 reported the biomass yield of 2.0 and 2.8 g/l in *B. braunii* culture (SAG 30.81 and LB-572) treaded with different levels of BG11 media. The increase in the biomass yield of *B. braunii* under light and dark conditions was reported by Tanoi et al. (2010). Furthermore, the biomass of *B. braunii* was increased with the rise of sodium chloride concentration and maximum biomass was achieved in 17 mM and 34 mM salinity, while phosphate decrease was observed due to its utilization by the algae (Ranga Rao et al., 2007). According to Ge et al. (2010) the maximum biomass of *B. braunii* is 2.3 g/l. Shen et al. (2008) reported dry biomass concentration of up to 2.543 g L^{-1} for *B. braunii*.

In the accounting for chlorophyll of *B. braunii* again higher values (9.8) occurred in cultures grown in 3N-BBM medium (Figure 3).

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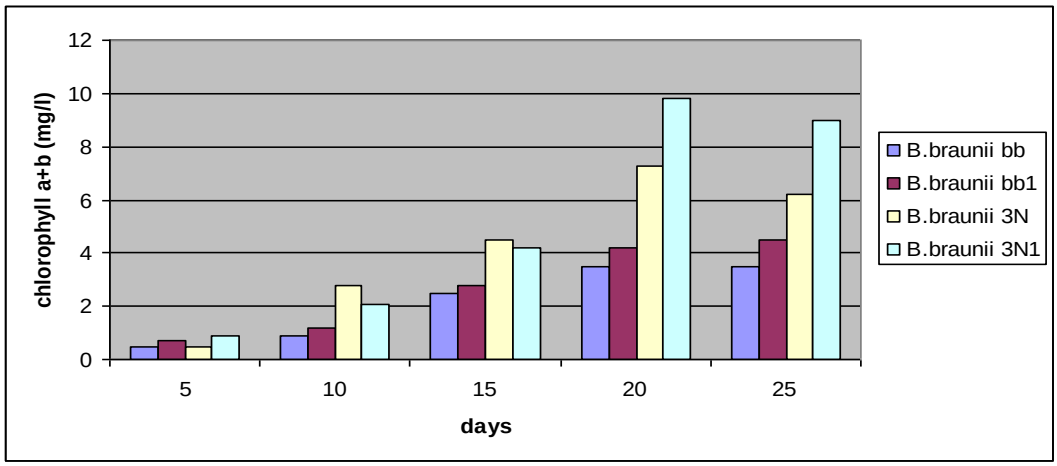

Figure 3. *Chlorophyll (mg/l) of B. braunii for 25 days grown of different media*

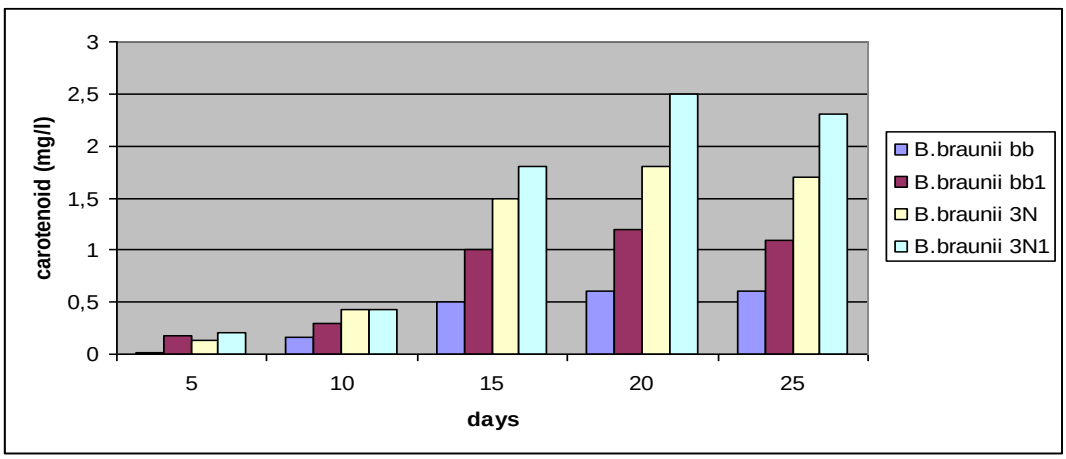

Figure 4. *Carotenoid (mg/l) of B. braunii for 25 days grown of different media*

Dayananda et al. (2007a) researching *Botryococcus* in Chu 13 media and 2% CO_2 receives similar results in terms of chlorophyll (9.8) and carotenoids (1.8). Anitha et al. (2009) reveals that at decreasing concentration of nitrogen sources there was a decreased growth, chlorophyll and biomass. Nitrogen starvation also triggered a rapid decline in nitrogen containing compound such as photosynthetic pigments, causing complete loss of photosynthetic efficiency. Rao et al. (2007) also receive close to our values of chlorophyll (10.6) and carotenoids (2.0).

A high intensity of light increases the carotenoid-to-chlorophyll ratio, and this affects the color of algal colonies (Wolf et al., 1985). In our culture there was a strong dark green color. Taking into account the carotenoids of *B. braunii* again higher values (2.5) occurred in cultures grown in enriched with nitrates medium (Figure 4). Lutein is the major carotenoid among the total carotenoids from *B. braunii* as reported by Ranga Rao et al. (2006).

The green algae *B. braunii* has received much attention because it contained unusually high levels of hydrocarbons ranging from 15-75% of dry wt (Sawayama et al., 1994).

Our results about total lipids were: for *B. braunii* grown in BBM– 20.3%; B.braunii grown in 3N-BBM – 25.2%. The lower rates of lipids in our cultures are likely due to the high concentration of nitrates, which interferes with the production of hydrocarbons (Brenckman et al., 1989).

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Conclusion

The obtained results show that the research strain of *B. braunii* develops better in 3N-BBM, as larger values are observed in the biomass and in the percentage of lipids. Chlorophyll content in all cultures follows the dynamics of variation of the curves of growth. Carotenoid content has the same character, and it is three times less than chlorophyll.

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