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Production Systems

Cultivation of *Scenedesmus dimorphus* strain for biofuel production

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1Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

**Abstract.** Microalgae have several advantages, including higher photosynthetic efficiency as well as higher growth rates and higher biomass production compared to other energy crops. The *Scenedesmus dimorphus* strain was studied by using two media – BBM and 3N-BBM, and its potential for biofuel production was established. The temperature varied between 25 – 27ºC during the experiment. Fluorescent light was used to assure optimal light condition and a photoperiod of 15/9h light and dark cycle was maintained. The duration of the experiment was 25 days. Dry weight, optical density, chlorophyll, carotenoids and total lipids were measured for the biomass evaluation. The received results showed that the maximum vegetative growth was reached after approximately 16 days of incubation. The maximum growth rate during this period was 1.690 mg.l\(^{-1}\) dry weight in 3N-BBM medium, and in BBM medium – 0.960 mg.l\(^{-1}\). The lipid content which we received from the examined strain was 21.6% in BBM medium, and in 3N-BBM – 18.5%.

**Keywords:** biofuel, biomass, media, *Scenedesmus dimorphus*

**Introduction**

Biofuel can be produced from a variety of sources, including plants (bio-ethanol from corn, canola), bacteria (bio-hydrogen from microbial fuel cells using *Geobacter* sp.), microalgae (biodiesel, bio-oil and bio-hydrogen from *Chlorella, Scenedesmus* and *Botryococcus*) and solid wastes (biogas from anaerobic digestion). Among these, microalgae look promising as a versatile, environmentally friendly and economically sustainable solution (Hutchinson, 2007).

Microalgae can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others. Algae biodiesel contains no sulfur and performs as well as petroleum diesel, while reducing emissions of particulate matter, CO and hydrocarbons (Delucchi, 2003).

High lipid contents are usually produced under environmental stress, typical nutrient limitation, which is often associated with relatively low biomass productivities 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). Micro algae 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 (Pulz and Gross, 2004; Rodolfi et al., 2009; Radakovits et al., 2011; Mc Donald, 2011) to have the ability to accumulate large quantities of lipids: *Scenedesmus dimorphus, Botryococcus braunii, Nannochloropsis oculata, Phaeodactylum tricornutum, Chlorella protothecoides*. According to Becker (1994) *S. dimorphus* contains 16–40% lipid on dry weight basis. In light of this meaning, the potential biotechnological applications of microalgae growth and lipid production characteristics need to be explored. Different nutritional and environmental factors, cultivation conditions and growth phases may affect the fatty acid composition. The main effect on growth enhancement could be attributed to the initial content of some macro and micro-nutrients especially carbon and nitrogen.

The aim of our research was to compare the growth and lipid content of *Scenedesmus dimorphus* (SKU: AC-1002) strain cultivated in two nutrition media in connection of its use for biofuel production.

**Materials and methods**

Microalgae strain and medium

*Scenedesmus dimorphus* (SKU: AC-1002) was purchased from Algae depot – USA (www.algaedepot.com). *S. dimorphus* was grown on two types of media: BBM medium (http://www.ccap.ac.uk/media/documents/BB_000.pdf): NaNO\(_3\) – 10.0 g, MgSO\(_4\).7H\(_2\)O – 3.0 g, NaCl – 1.0 g, K\(_2\)HPO\(_4\) – 3.0 g, KH\(_2\)PO\(_4\) – 7.0 g, CaCl\(_2\).2H\(_2\)O – 1.0 g (stocks per 400 ml); ZnSO\(_4\).7H\(_2\)O – 8.82 g, MnCl\(_2\).4H\(_2\)O – 1.44 g, MoO\(_3\) – 0.71 g, CuSO\(_4\).5H\(_2\)O – 1.57 g, Co(NO\(_3\))\(_2\).6H\(_2\)O – 0.49 g (trace elements solution per litre); EDTANa\(_2\) – 5.0 g, FeSO\(_4\).7H\(_2\)O – 4.98 g.

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_BBmV_000.pdf): NaNO\(_3\) – 25.0 g, MgSO\(_4\).7H\(_2\)O – 7.5 g, NaCl – 1.0 g, K\(_2\)HPO\(_4\) – 17.5 g, NaCl – 2.5 g (stocks per 1000 ml); ZnCl\(_2\) – 5.0 mg, FeCl\(_3\).6H\(_2\)O – 97.0 mg, MnCl\(_2\).4H\(_2\)O – 41.0 mg, Na\(_2\)MoO\(_4\).2H\(_2\)O – 4. mg, CoCl\(_2\).6H\(_2\)O – 2.0 mg (trace elements solution per litre); EDTANa\(_2\) – 0.75 g, vitamins B\(_1\) – 0.12 g and B\(_6\) – 0.1 g.

**Cultivation**

The cells in exponential period were inoculated (10%, v/v) in a
liquid medium. Cultivation was initiated in a 500ml Erlenmeyer flask containing 400ml medium. The cultures were kept at room temperature (25–27°C) in fluorescent light with a light:dark photoperiod of 15 h: 9 h. Sterile-air containing 2% (v/v) CO₂ was aerated into the flask through an air sparger at the bottom of the flask. The cultures were kept at room temperature (25–27ºC) in fluorescent light with a light:dark photoperiod of 15 h: 9 h.

Sterile-air containing 2% (v/v) CO₂ 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).

**Lipid content**

The total lipids were extracted from microalgae biomass using a modified method of Bligh and 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.

Data analyses were conducted by using ANOVA (MS Office, 2010).

**Results and discussion**

Like other microalgae, *S. dimorphus* culture requires water, light, CO₂, and inorganic nutrients. Culture productivity is affected by factors such as pH, CO₂, irradiance, salinity, and temperature (Banerjee et al., 2002). According to Lupi et al. (1991) the optimum temperature for growth is 25°C. The temperature was held between 25–27°C during the experiment.

In our study as we expected the cultures grown in the BBM medium have lower values of optical density than the cultures grown in the medium with three times more nitrates (3N-BBM). The maximum values of the optical density at *S. dimorphus* grown in 3N-BBM medium was 1.89 compared with BBM medium where it was 1.53 (Figure 1). The medium enriched with higher nitrates content showed better algae’s culture growth compared with BBM. According to Ilavarasi et al. (2011) *Scenedesmus* sp. (NTAI03) showed maximum growth in Bold’s Basal medium, based on the optical density measurement.

Maximum dry biomass (1.690 mg.l⁻¹) of *S. dimorphus* was obtained in medium enriched with nitrates (Figure 2), in comparison with its dry weight in BBM medium (0.960 mg.l⁻¹) (Table 1). The received results showed that the maximum vegetative growth was reached after approximately 16 days of incubation. According to Goswami and Kailita (2011) the maximum increase in biomass per day for *Scenedesmus dimorphus* was found to be 1.523 mg/l/day. Varsharani and Getta (2011) reported dry biomass concentration of 1.200 mg.l⁻¹.

**Growth measurements**

The growth of *S. dimorphus* was measured via spectrophotometry (DR 2800) and biomass dry weight. Optical density for biomass factor was determined at wavelength 550 nm. One ml of the 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 3,000 x g 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 (mg.l⁻¹).

**Chlorophyll and carotenoid content**

The isolation of pigments from algae cells included the following 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 60°C, cooling the samples at room temperature, centrifugating the suspension (10000 rpm for 3 min) and discarding the supernatant with dissolved pigments. The absorbance of the pigment extract (665, 652 nm for chlorophyll content (a+b) and 470, 666nm for carotenoids content) was recorded by using a spectrophotometer. The chlorophyll content was computed (mg.l⁻¹) according to Porra et al. (1989) and carotenoid content was computed (mg.l⁻¹) according to Lichtenthaler (1987).

![Figure 1. Growth response of S. dimorphus (at 550nm) for 25 days under different media](image)

**Table 1.** Growth, chlorophyll, and carotenoid content of *S. dimorphus* grown in different media.
In the accounting for chlorophyll of *S. dimorphus* again higher values (9.6 mg.l⁻¹) occurred in cultures grown in 3N-BBM medium (Figure 3) (Table 1). Varsharani and Geeta (2011) reported chlorophyll (a+b) concentration - 11.5 mg.l⁻¹ in *S. dimorphus*. Prabakaran and Ravindran (2012) received higher values of chlorophyll in *S. dimorphus* grown in nitrogen sources and carbon source in different concentrations.

Different hypothesis on carotenoid accumulation by green microalgae were mentioned by scientists. Previous studies on carotenoid accumulation by *Scenedesmus*, *Chlorella* and *Haematococcus* showed that the addition of at least 10% of nutrients mainly nitrogen are required to overcome the dry weight accumulation failure (El-Shafey et al., 1999). The main reason could be ascribed to the presence of organic carbon that allows the fast carotenoid accumulation (El-Sayed, 2010). In our study the carotenoids of *S. dimorphus* were again with higher values (2.3 mg.l⁻¹) in cultures grown in medium enriched with nitrates (Figure 4).

According to Goswami and Kalita (2011) the maximum increase

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**Table 1.** Optical density, dry weight, chlorophyll and carotenoid of *S. dimorphus* grown of different media (BBM, 3N-BBM)

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<th>Parameters</th>
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<th><em>S. dimorphus</em> 3N-BBM</th>
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<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>MEAN ± SEM</td>
<td>Min</td>
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<tr>
<td>Optical density</td>
<td>0.23</td>
<td>1.53</td>
<td>0.96 ± 0.15</td>
<td>0.21</td>
</tr>
<tr>
<td>Dry weight (mg.l⁻¹)</td>
<td>116</td>
<td>920</td>
<td>558.22 ± 9.12</td>
<td>260</td>
</tr>
<tr>
<td>Chlorophyll a+b (mg.l⁻¹)</td>
<td>0.40</td>
<td>5.50</td>
<td>2.91 ± 3.49</td>
<td>0.45</td>
</tr>
<tr>
<td>Carotenoid (mg.l⁻¹)</td>
<td>0.12</td>
<td>1.60</td>
<td>0.74 ± 0.26</td>
<td>0.10</td>
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*P<0.05
in biomass per day and lipid content for *S. dimorphus* was found to be 1.523 mg/l/day and 34%. Our results about total lipids were: for *S. dimorphus* grown in BBM – 21.6%; *S. dimorphus* grown in 3N-BBM – 18.5%. This was probably due to the fact that in these media there was depletion of nutrients at much earlier stage compared to the media enriched with nitrogen. The cells stopped dividing and began to accumulate spare products in the form of fatty acids and glycerol, which were connected by triglyceride. In the 3N-BBM medium nutrients were still not the limiting factor for cell division, and the energy and building blocks were redirected in the direction of growth and division of cells rather than the accumulation of spare products.

![Figure 4. Carotenoid (mg.l⁻¹) of *S. dimorphus* for 25 days grown in different media](image)

**Conclusion**

The obtained results showed that the researched strain of *S. dimorphus* developed better in 3N-BBM, as larger values were observed in the biomass, but the percentage of lipids was better in BBM. Chlorophyll content in all cultures followed the dynamics of variation of the curves of growth. Carotenoid content had the same character, and it was five times less than chlorophyll.

**References**


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