MICROALGAL CULTIVATION UNDER LOWERED CO_2 CONDITIONS IN ORDER TO REDUCE THE CARBON DIOXIDE EMISSIONS IN THE ATMOSPHERE

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Abstract

In the last years, the problem with the increased carbon dioxide emissions influences all aspects of life, especially the environment. In this study was investigated what happens when microalgae are cultivated under lowered concentration of carbon dioxide, which is a novelty research. Our results show the positive effect of these conditions as they are connected with satisfactory biomass production without a release of carbon dioxide in the atmosphere. This cultivation approach also helps lowering the expenses for microalgal biomass production. Our results show the positive impact of these conditions, as they are related to satisfactory production of biomass with a balanced composition, without releasing carbon dioxide into the atmosphere.

Key words: *microalgae cultivation, lowered carbon dioxide, growth, biochemical composition, scenedesmus sp.*

1. INTRODUCTION

Human development and the anthropological activity have a huge impact on the environment and nature in our century, especially on the climate. Never before the world was so unanimous on their opinion on a certain problem. In the past 50 years the concentrations of carbon dioxide in the atmosphere have risen with about 18% (Taub 2010). On one hand, concerns for climate changes and related over-growth of greenhouse gases and global warming are a major topic of increasing scientific research, media and public debates. The aim of ecologists nowadays is to slow down this process by using different techniques. Many of the unwanted carbon dioxide emissions come from the mass productions, which necessitates their optimization. One of world mass productions is the algae biomass production. Microalgae are a large and diverse group of simple, fast growing photosynthetic microorganisms that can be in unicellular and simple multi cellular forms (Mostafa 2012). Microalgal biomass and its components receive a growing interest because of their wide field of applications (Makarevičienė 2011). They are currently used as food and dietary supplements; production of high-value natural bioactive substances (polyunsaturated fatty acids, carotenoids, phycobiliproteins and other bioactive compounds); bioremediation; bioassay and biomonitoring of environmental toxicants (Chu 2012). Microalgae perform a photosynthetic process similar to higher plants, but biological CO_2 fixation with microalgae is more efficient than terrestrial plants and microalgae can grow about 10 to 50 times faster (Dismukes et al. 2008; Williams and Laurens 2010). The biological fixation of carbon that they perform is an important natural mechanism to help reducing the greenhouse effect and global warming (Huntley & Redalje 2007). This positive effect of the photoautotrophic microalgae is diminished when it comes to mass cultivation where a bottled carbon dioxide is used, often in excess (Yang & Gao 2003), to ensure the growth. The increased concentration of CO_2 has a negative impact on the environment, since much of CO_2 is not assimilated by the culture and is emitted into the atmosphere. The green algae of genus *Scenedesmus*, commonly found in fresh waters (Borowitzka & Borowitzka 1998), are very suitable for both laboratory studies and mass intensive cultivation, since they have a high tolerance towards variation of the most important environmental factors (Ren et al. 2013). In our previous study, the newly isolated Bulgarian strain *Scenedesmus* sp. BGP was found to grow better in urea- containing than in ammonium nitrate + urea-containing medium (Vasileva, Marinova & Gigova 2015). In the present investigation this green alga was grown on the same two media, but supplied with different concentrations of carbon dioxide. The aim was to find out the

conditions providing simultaneously good growth, accumulation of useful metabolites, lower cost and mainly reduction of CO_2 emissions in the atmosphere.

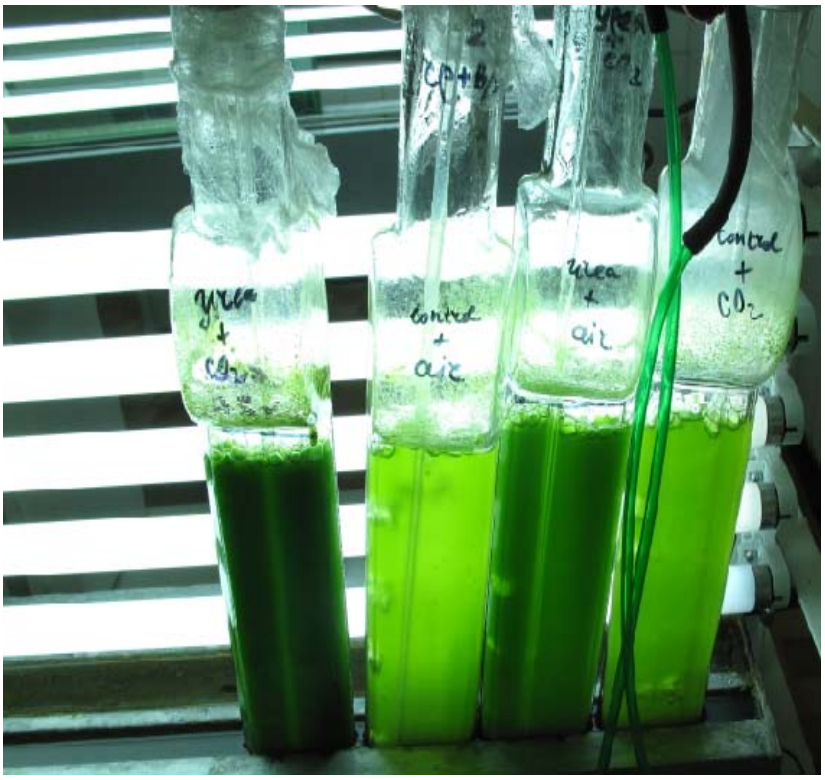

Photo 1. *Scenedesmus* sp. BGP cultures grown on different nutrient media and CO_2 concentrations – from left to the right: urea + 2% CO_2, control + 0.04% CO_2, urea + 0.04% CO_2, control + 2% CO_2

2. MATERIALS AND METHODS

2.1. Algal strain and cultivation conditions

Scenedesmus sp. strain BGP used in this study was isolated from a rainwater puddle (Sofia, Bulgaria). Monoalgal, non-axenic cultures of *Scenedesmus* sp. BGP are grown autotrophically on two different variations of the nutrient medium of Setlik (1967), modified by Georgiev et al. (1978) (standard recipe) and two carbon dioxide concentrations (2 and 0.04%).

2.2. *Nutrient media*

I. control medium - 1/4 from standard recipe: NH_4NO_3 (0.4 $g.L^{-1}$), $CO(NH_2)_2$ (0.3 $g.L^{-1}$), $MgSO_4.7H_2O$ (0.494 $g.L^{-1}$, KH_2PO_4 (0.17 $g.L^{-1}$), $NaHCO_3$ (2.0 $g.L^{-1}$), trace elements

II. medium with urea: NH_4NO_3 (0 $g.L^{-1}$), $CO(NH_2)_2$ (0.6 $g.L^{-1}$), $MgSO_4.7H_2O$ (0.494 $g.L^{-1}$), KH_2PO_4 (0.17 $g.L^{-1}$), $NaHCO_3$ (1 $g.L^{-1}$), trace elements

2.2.1. Variants

1. medium II supplied with 2% CO_2;
2. medium I supplied with 2% CO_2 (control);
3. medium II supplied with atmospheric air (0.04% CO_2);
4. medium I supplied medium with atmospheric air (0.04% CO_2).

The quantity of nitrogen in the urea-containing medium was kept the same as in the ¼x standard recipe. The experiments were carried out on a specially equipped block. The cultures, in 200 ml flasks, were continuously supplied with carbon dioxide (2%) or with atmospheric air (0.04 % CO_2) at constant temperature (about 24°C) and illumination (360 μmol m^{-2} s^{-1} photon flux density). An initial culture density of ~ 0.8 $g.L^{-1}$ was used for all treatments. To analyse the biochemical composition, experimental cultures were harvested in the early exponential phase of growth as well as in the beginning of stationary phase. Cells were collected by centrifugation (5000×g, 20 min), rinsed three times with distilled water, frozen, and stored at -20°C.

2.3. Measurements and analyses

The growth of *Scenedesmus* was evaluated by increase in biomass dry weight (DW). For this purpose, 10 ml of the algal suspension were centrifuged at 5000 g, 10 min (centrifuge rotofix 32 A). The cells were dried at 105°C for 16 h. The dry biomass concentration ($g.L^{-1}$) was calculated according to Makarevičienė et al. (2012). The protein content was measured according the method of Lowry (1951). The carbohydrates were quantified using the phenol-sulfuric acid method of Dubois et al. (1956). The lipid content was determined after extraction with hot ethanol (Petkov & Dilov, 1987).

2.4. Statistical analysis

All data are presented as mean ± standard deviation. The significance of differences between the treatments was evaluated by one-way analysis of variance (ANOVA). Values of $P \leq 0.05$ were considered significant.

3. RESULTS AND DISCUSSION

3.1. Growth

In this part of the study is observed the growth of *Scenedesmus sp. BGP* cultivated on two variations of the standard medium and two different concentrations of CO_2 to reveal which one provides the best ratio between good growth, lower cost and mainly reduction of CO_2 emissions in the atmosphere. The original recipe includes both urea and NH4NO3 as nitrogen sources. Both variations are based on the ¼x standard medium, but the first one contains only urea as a nitrogen source and the second – both urea and NH4NO3. All the cultures start their growth rapidly except the one control with air that has a longer adaptation phase and also shows the lowest growth (Figure 1). The best growth is observed when the algae is cultivated on urea medium with 2% CO_2, so the medium with urea is better for the growth of this certain strain, which was established during a previous study (Vasileva, Marinova, Gigova 2015). Here this data is not only confirmed, but it is visible that microalgae on a nutritive media with urea and without additional carbon dioxide microalga grows almost as good as the control with carbon dioxide (4.6 $g.L^{-1}$ vs 4.7 $g.L^{-1}$ for the same period of time). According to literature data, urea has a wider availability and lower cost per nitrogen unit when compared to ammonium nitrate (Kallenbach et al. 2005). That means that the cultivation on medium with urea + air is not only cheaper, but also gives better yield using one nitrogen source and no additional carbon dioxide which makes it quite environmentally friendly.

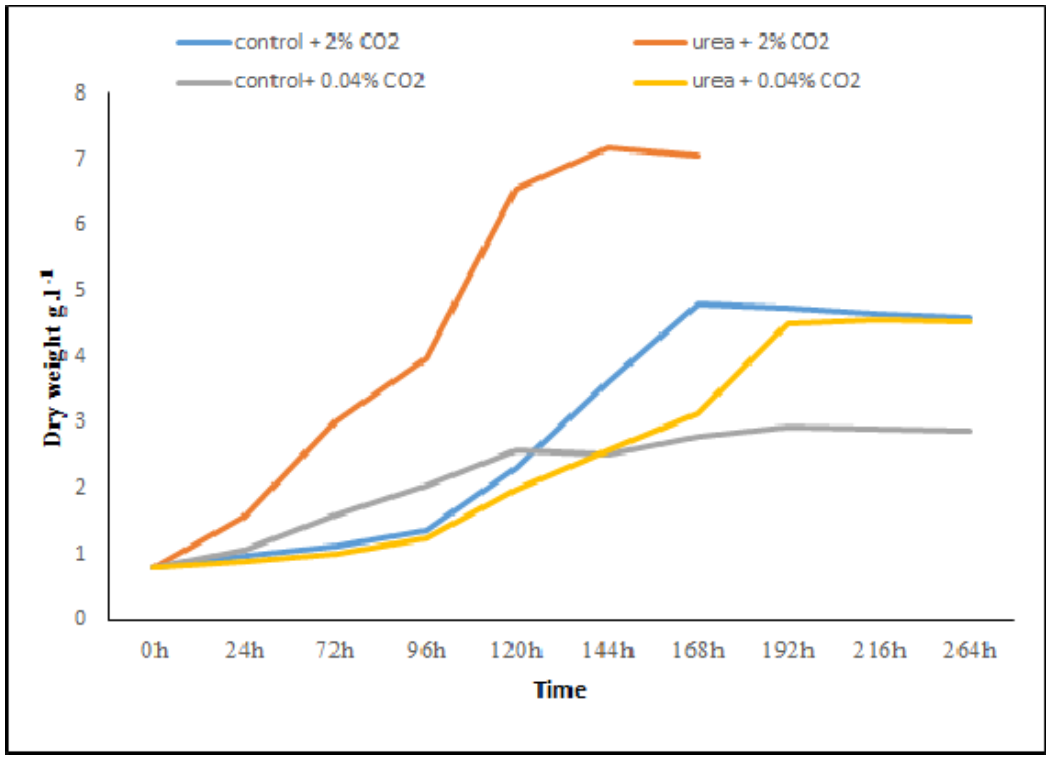

Figure 1. Growth curves of *Scenedesmus* sp. BGP, depending on the nutrient medium and CO_2 concentration.

3.2. Chemical composition

The variations in the content of the main biomass components of *Scenedesmus* sp. BGP during the exponential and stationary phase in relation to the nitrogen source and concentration of the carbon dioxide are compared.

3.2.1. Lipids

Lipid content of *Scenedesmus* sp. BGP falls within the normal range for the genus *Scenedesmus* (16-40 %, Becker, 1994). The quantity of lipids in the exponential phase of all the cultures is about 23-28% of the biomass DW (Figure 2). This means that the proposed conditions (urea + air) are not unfavorable for growth and do not cause significant stress for the culture. In the stationary phase, the quantity of lipids is around 22 % for algae grown with 2 % CO_2, but this percentage is rising up to 38 % for algae grown with air, meaning that the decreasing of CO2 concentration is a suitable manner for accumulation of lipids for production of polyunsaturated fatty acids. Similar results are received with *Chlorella vulgaris* under the same carbon dioxide concentration (Tsuzuki et al. 1990).

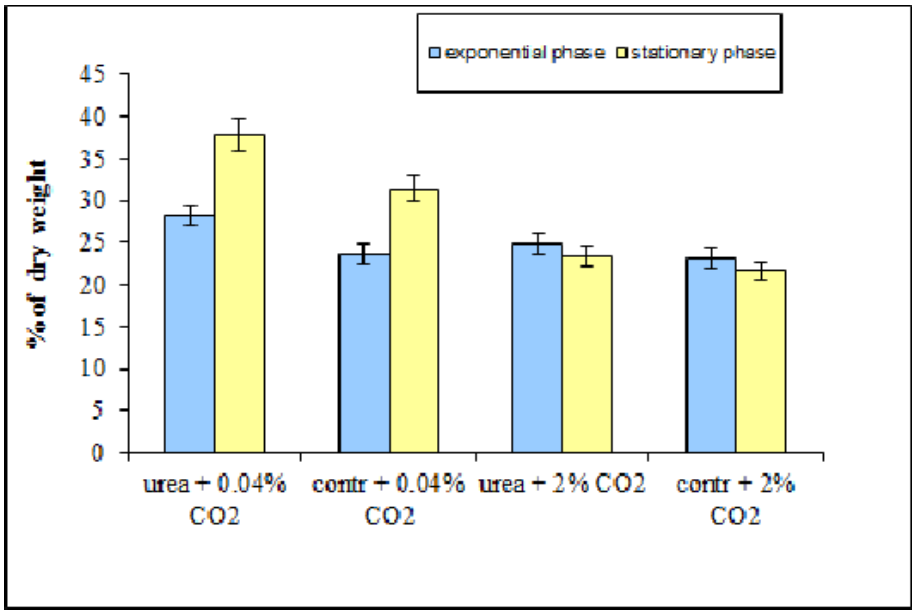

Figure 2. Lipid content in *Scendesmus* sp. BGP depending on the nutrient medium, concentration of CO_2 and growth phase.

3.2.2. Carbohydrates

As a whole, the carbohydrates are most abundant component of *Scenedesmus* sp. BGP biomass. The higher concentration of carbon dioxide induces the production of carbohydrates, reaching up to 45 % of DW (Figure 3). These values are higher than those of *Scenedesmus obliquus*, where the carbohydrate content is between 10-17% (Becker, 1994). Unlike the lipids, on the media with air the quantity of carbohydrates decreases with cultivation time (from 37- 45 % to 25 – 37 % of DW).

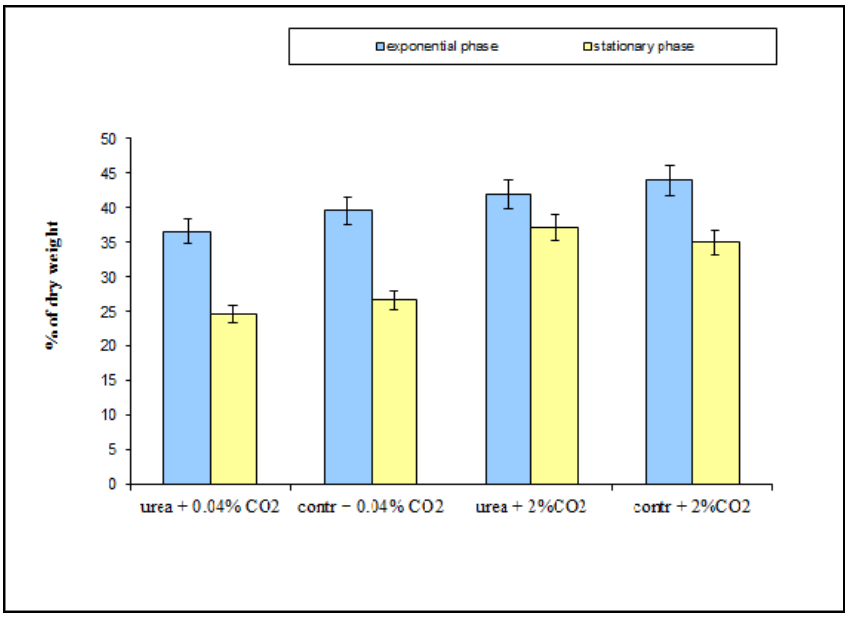

Figure 3. Carbohydrate content in *Scendesmus* sp. BGP depending on the nutrient medium, concentration of CO2 and growth phase.

3.2.3. Proteins

Protein content of *Scenedesmus* sp. BGP range between 23–39% (Figure 4) and is relatively lower in comparison with, for example, the widespread strain Scenedesmus incrassatulus R-83 having a protein content of 40.8-52.5% (Livansky, Kajan & Pilarski 1995). In the exponential phase of growth, the protein content of all cultures varies slightly in the range of 23-28 % DW. In stationary phase, the increased concentration of carbon dioxide stimulate the protein synthesis, as their levels rise up markedly (the range is from 31 to 39 %).

4. CONCLUSIONS

In conclusion, both the nitrogen source and the concentration of carbon dioxide have a substantial effect on the productivity and biochemical composition of the new Bulgarian strain *Scenedesmus* sp. BGP. The decrease in carbon dioxide concentration causes an enhancement in the biosynthesis and accumulation of lipids. The best growth is observed at the nutritive medium with urea + CO_2, but the case of urea + air has the advantage to give a satisfactory productivity of algal biomass with stable and balanced biochemical composition (about 37 % carbohydrates, 28 % lipids and 25 % proteins) and ultimately reduces both the cultivation cost and the unwanted carbon dioxide emissions in the atmosphere. The present work provides useful information for further studies on the application of this new Bulgarian strain for biotechnological purposes.

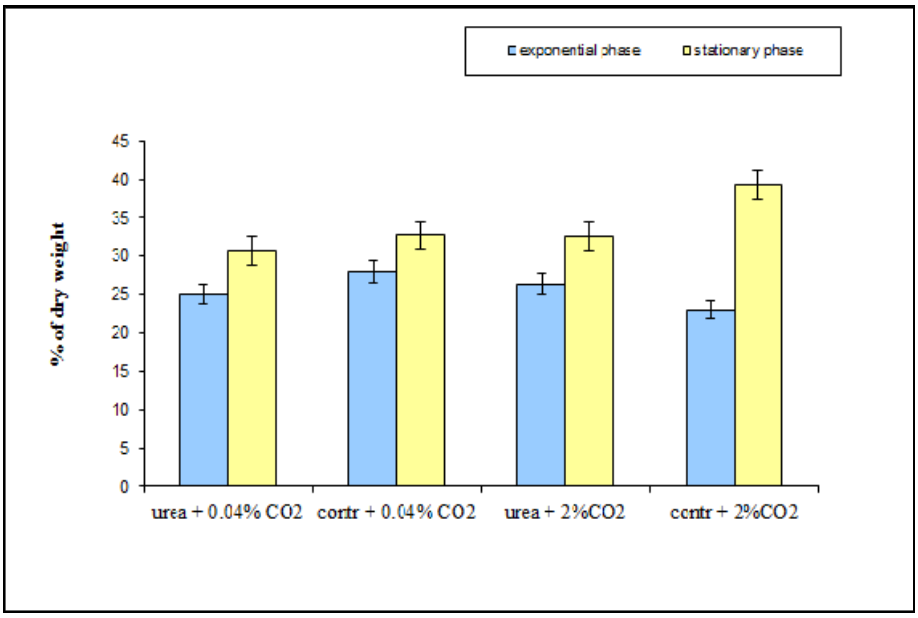

Figure 4. Protein content in *Scendesmus* sp. BGP depending on the nutrient medium, concentration of CO2 and growth phase.

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