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Effect of nitrogen source on the growth and biochemical composition of a new Bulgarian isolate of *Scenedesmus* sp.

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ABSTRACT

The influence of different nitrogen sources (ammonium nitrate, urea and ammonium nitrate + urea) and different concentration of the medium, containing both nitrogen sources on the growth and the content of pigment, protein, carbohydrate and lipid of a newly isolated green alga *Scenedesmus* sp. BGP was studied. Even though the two nitrogen sources were present in the medium routinely used in the lab, the experiments showed that the use of medium with each one of them separately gave a better yield of algal biomass for the whole cultivation period. The best growth was observed in urea-containing medium, where the dry weight reached 9.0 g/l. Dilution of the standard medium (2-, 4- and 8- fold) had positive impact on the growth, in contrast to 2-fold concentrated. The total amount of pigments (chlorophyll a+b and carotenoids) correlated with the change of growth in the different media. As a whole, carbohydrates, followed by proteins and lipids, dominated the biochemical composition of Scenedesmus sp. BGP. The biomass of the alga, grown in urea- or ammonium nitrate-containing medium was characterized by stable qualitative content over the entire period of cultivation (about 29% proteins, 41% carbohydrates and 24% lipids). These results showed that the medium with urea was most suitable for cultivation of *Scenedesmus* sp. BGP, because as a nitrogen source, urea is more efficient in terms of productivity and is less expensive.

Key words: *Scenedesmus,* nutrient medium, growth, biochemical composition, urea

Introduction

Microalgae are a large group of aerobic photosynthetic microorganisms, found almost everywhere in nature (Falkowski & Raven, 1997). Due to their relatively simple morphological organization, fast growth, high productivity and enormous and diverse biosynthetic potential, as well as the possibility for laboratory and large-scale cultivation, microalgae have a significant role in different fields as ecology, agriculture, aquaculture, medicine, science, etc. (Priyadarshani & Rath, 2012; Buono et al., 2014). The wide range of current and potential applications of the biomass and algae-derived compounds can benefit from the targeted research on the algal physiology and biochemistry. The green algae of genus *Scenedesmus* are commonly found in fresh waters. They 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 such as light intensity, temperature, pH, as well as the content of the nutrient medium (Ren et al., 2013). Changes in the external environment are related to changes in cell growth and metabolism, which provide a possibility to explore and use the effects of cultivation conditions on biomass accumulation and its biochemical composition (Juneja et al., 2013; Gacheva & Gigova, 2014). This means that adequate beneficial production of valuable algal biomass can be achieved by optimizing the growth conditions. The usefulness of microalgae and the opportunity to manage their

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growth leads to increasing of the interest in and development of the algal biotechnology. Exploration and research of new algae strains, especially Bulgarian, are important in order to find a local, competitive, fast growing and highly productive strain. Therefore, in this study, the newly isolated strain *Scenedesmus* sp. BGP was grown in different nutrient media to find out which medium provided the best growth and accumulation of a high-quality algal biomass.

Materials and Methods

Algal material 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 were grown autotrophically on 8 different variations of a nutrient media: medium of Setlik (1967), modified by Georgiev et al. (1978) (standard recipe); 2x concentrated and ⅛x, ¼x, ½x diluted standard medium; variation with no nitrogen source; variations with only urea and only ammonium nitrate, as the quantity of the nitrogen 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 (2-3% in air) at constant temperature (about 24°C) and illumination (132 μmol m^{-2} s^{-1} photon flux density). An initial culture density of ~ 0.6g/l was used for all treatments. To analyze the biochemical composition, experimental cultures were harvested in both the middle exponential phase of growth and 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.

Measurements and analyses

The concentration of the dried algal biomass (dry weight, DW) was determined gravimetrically. The growth of *Scenedesmus* was evaluated by increase in biomass DW. 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). The pigments were measured spectrophotometrically after an extraction with boiling methanol and the quantity was calculated using the Mackiney coefficients (Mackiney, 1941).

All experiments were conducted at least in two repetitions and the means are presented.

Results and Discussion

Growth

Scenedesmus sp. BGP was cultivated on 8 variations of the standard medium to reveal which one provided the best growth of the alga. The original recipe included both urea and NH_4NO_3 as nitrogen sources. Three of the variations were based on the ¼x standard medium, but contained only urea or NH_4NO_3 or no nitrogen source. Diluted or concentrated variants of the standard medium were also included. In 1x and 2x standard media and ¼x with no nitrogen, the weakest growth was observed (Figure 1). The weak and fleeting growth in the absence of external nitrogen is a response, observed in many algae (Gigova & Ivanova, 2015) since the nitrogen is an essential major element required for the synthesis of the vital amino acids, proteins, nucleic acids, coenzymes and chlorophylls. Although weak, the growth of *Scenedesmus* sp. BGP on 1x modified Setlik medium was better than those reported for *Scenedesmus dimorphus* on BBM, M4N, BG11, M8 and N8 media (Al-Shatri et al., 2014).

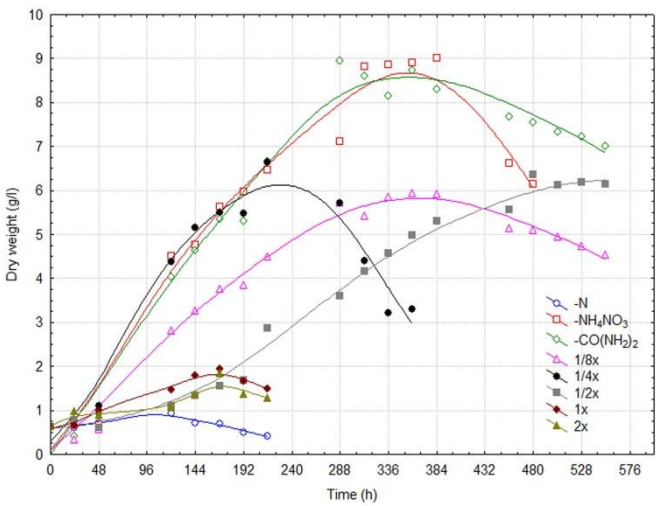

Figure 1. *Growth curves of Scenedesmus sp. BGP, cultivated in different nutrient media (the average data are shown)*

High concentration of the nutrients and the use of mixed nitrogen sources in 1x and 2x standard media also inhibited the growth of *Scenedesmus* sp. BGP which is probably due to substrate repression as NO_3^- and NH_4^+ (hydrolysis of urea also gives ammonia) are metabolized via a common pathway.

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Dilution of the standard medium had positive effect on the growth, especially the four-fold dilution, where the maximum yield of biomass was 6.6 g/l, reached at the 9th day of cultivation. The cultures at ⅛x and ½x medium grew more slowly, reaching almost the same maximum yield, but on the 14th and 20th day, respectively. Unlike *Scenedesmus* sp. BGP, the green alga *Chlorella* sp. strain R-06/2 grows better when the concentration of the same standard medium is higher (Gacheva & Pilarski, 2008). The use of media with only one nitrogen source gave best productivity of algal biomass for the whole cultivation period. In both, urea-containing and ammonium nitrate-containing media, dry weight of about 9.0 g/l was registered on the 13th day of cultivation. In these cases, the choice of nitrogen source of the medium for practical applications should be based on a cost-benefit. According to literature data, urea has wider availability and lower cost per nitrogen unit when compared to ammonium nitrate (Kallenbach & Massie, 2005).

Chemical composition

The variations in the content of pigments and the main biomass components of *Scenedesmus* sp. BGP during the exponential and stationary phase in relation to the nitrogen source and concentration of the nutrient media were compared.

Carbohydrates

As a whole, the carbohydrates were most abundant component of *Scenedesmus* sp. BGP biomass (ranging from 20 to 53% DW). These values were higher than those of *Scenedesmus obliquus*, where the carbohydrate content is between 10-17% (Becker, 1994). In cells, grown in the media containing only one nitrogen source and in the more diluted standard media (⅛x and ¼x), their values were in the range of 43-45% and showed a slight variation with each other, and between the exponential and stationary growth phase (Figure 2). In contrast, in the other media the percentage of carbohydrates was relatively low in exponentially growing cultures (20-28%), but in the stationary phase increased, reaching almost two times higher values.

Proteins

Protein content of *Scenedesmus* sp. BGP ranged between 23–43% (Figure 3) 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 et al., 1995). In the exponential phase of cultures, grown in ¼x media regardless of the nitrogen source, the protein content was 23-27% DW. The increased concentration of the media (including the concentration of nitrogen) significantly stimulated the protein synthesis, as their levels rose up to around 40%. There were slight variations in the protein levels between stationary and exponential phase of all cultures, except for the cultures grown in the ⅛x and ¼x standard media, where an increase (from 23% to 32-39%, respectively) was registered. In the culture, grown without an external nitrogen supply the protein content decreased from 27% to almost undetectable, probably because the cells degraded the intracellular nitrogen compounds in order to maintain their vital metabolic functions (Pancha, 2014).

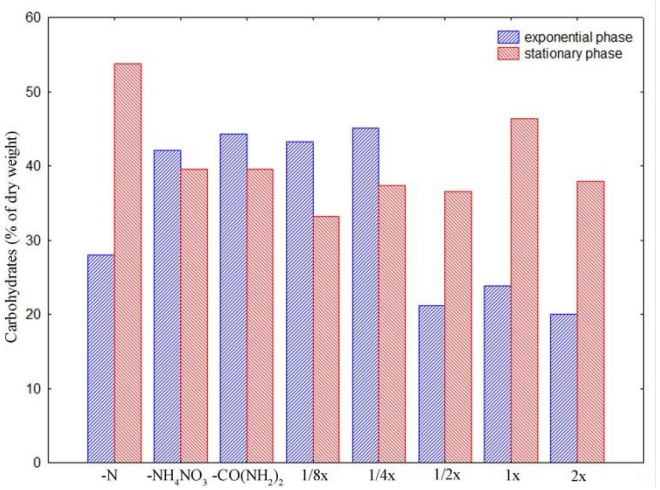

Figure 2. *Carbohydrate content in Scendesmus sp. BGP depending on the nutrient medium and growth phase (average data are shown)*

Lipids

Lipid content of *Scenedesmus* sp. BGP fell within the normal range for the genus *Scenedesmus* (16-40%, Becker, 1994). The quantity of lipids in the exponential phase of most cultures was about 24-25% of the biomass DW (Figure 4).

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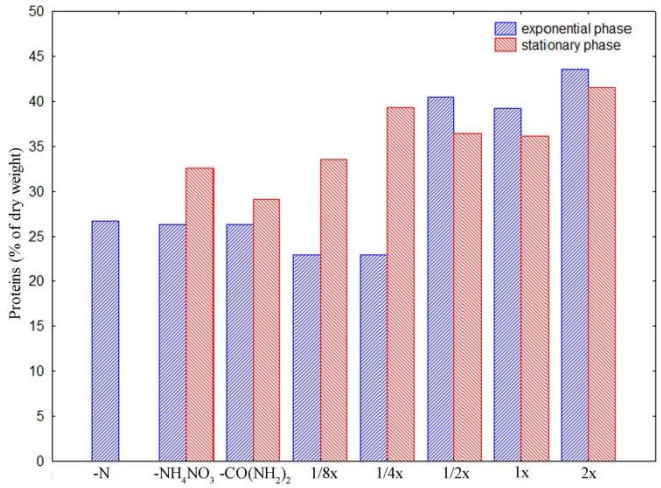

Figure 3. *Protein content in Scendesmus sp. BGP depending on the nutrient medium and growth phase (average data are shown).*

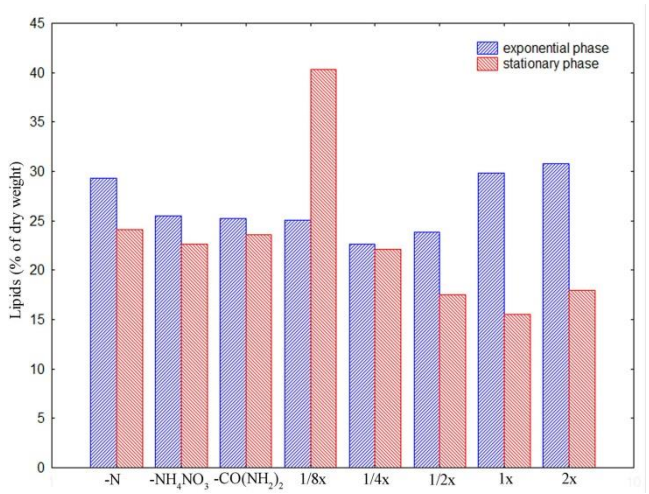

Figure 4. *Lipid content in Scendesmus sp. BGP depending on the nutrient medium and growth phase (average data are shown)*

Exceptions are those grown on the medium without a nitrogen source as well as in the concentrated media (1x and 2x), where the lipid production was increased to 29-30%, which could probably be associated with the culture conditions unfavorable for growth (stress). Increasing the lipid content is often associated with lower biomass productivity (Rodolfi et al., 2008) and is widely accepted as a stress marker (Yilanchoglu et al., 2014). In the stationary phase, the level of lipids decreased only in the concentrated media (from 29-30% to 15.5–18%), while in the ⅛x medium it reached the highest value (around 40%).

Pigments

In the exponentially growing cultures, the total pigment content (chlorophyll a + chlorophyll b + carotenoids) was in the range of 0.5 to 2.2% of DW, which corresponded to the data concerning *Scenedesmus incrassatulus* (Livansky et al, 1995). Pigment values depended on the nitrogen source, but were much more dependent on the concentration of the culture medium, including that of nitrogen (Figure 5). In the stationary phase, the cultures showed either pigment content higher than in the exponential phase, or there were no pigments at all (in the cases of 1x, 2x and medium without nitrogen). Only the cells grown in ¼x medium maintained the amount of their pigments almost the same during the two growth phases.

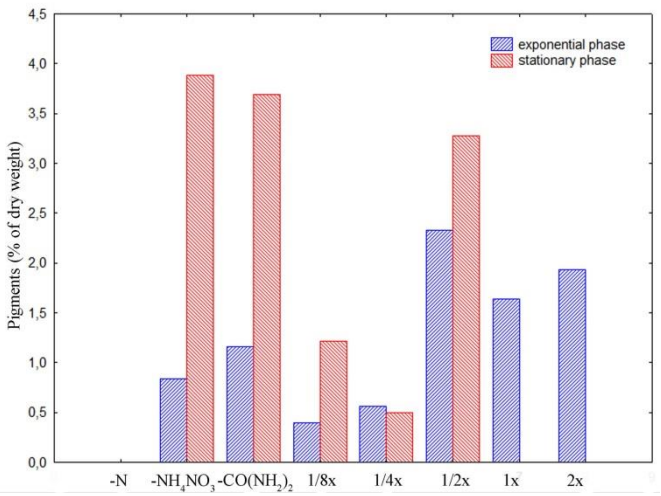

Figure 5. *Pigment content in Scendesmus sp. BGP depending on the nutrient medium and growth phase (average data are shown).*

Conclusion

Both the nitrogen source and the concentration of the nutrient medium had substantial effect on the productivity and biochemical composition of the new Bulgarian strain *Scenedesmus* sp. BGP. In various microalgae, nitrogen starvation or limitation conditions are shown to enhance the biosynthesis and accumulation of lipids or carbohydrates or both (Hu et al, 2004; Pancha et al., 2014). In the nitrogen-deprived cultures of *Scenedesmus* sp. BGP the carbohydrates were accumulated, but the biomass yield was reduced, while

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in the nitrogen-limited (⅛x medium) culture lipid biosynthesis was dominant over carbohydrate production, accompanied by a satisfactory growth. Compared to the mixed nitrogen sources based media, the use of media with only one nitrogen source had the advantage to give a better productivity of algal biomass, with stable and balanced biochemical composition (about 41% carbohydrates, 29% proteins and 24% lipids) and ultimately reduced cultivation cost.

The present work provided useful information for further studies on the application of this new Bulgarian strain for biotechnological purposes.

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