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Research Article

EFFECT OF DIFFERENT NITROGEN SOURCES ON THE PATTERN AND ACTIVITY OF CERTAIN METABOLIC ENZYMES OF *SCENEDESMUS* SP. BGP (CHLOROPHYCEAE)

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Summary: The impact of different nitrogen (N) sources (urea, ammonium nitrate and urea plus ammonium nitrate) in the cultivation medium on the growth rate as well as the pattern and the activity of glutamate synthase, NAD-dependent glutamate dehydrogenase, aspartate aminotransferase, NAD-malate dehydrogenase, esterases and proteases of a new Bulgarian green alga *Scenedesmus* sp. strain BGP was studied. The results showed that the media supplemented with urea or ammonium nitrate provided similar ($P > 0.05$), high growth rates (0.498 and 0.497 day^{-1}, respectively) and biomass accumulation (3.7 and 3.9 g L^{-1} DW, respectively) of the exponentially growing algal cultures. The simultaneous application of the two N compounds ensured equally high biomass yield, but the value of the specific growth rate was significantly lower ($P < 0.05$). At the beginning of the stationary phase, the growth rate and biomass dry weight were highest ($P < 0.05$) for urea-grown alga and lowest ($P < 0.05$) for alga cultivated in a medium with a mixture of the two N sources. The isoenzyme patterns of *Scenedesmus* sp. BGP did not change, except for esterases and proteases, but the relative total activity of the studied enzymes varied depending on the cultivation conditions. The different regulation of metabolic enzymes in response to the change in the N source, as well as the growth phase, ensured their effective functioning under all cultivation conditions, as evidenced by the good, albeit differing, algal growth. In large scale cultivation of *Scenedesmus* sp. BGP for the production of biomass for various useful applications, urea would be the most appropriate choice not only in terms of productivity but also of economic profitability as it is a cheaper source of N than ammonium nitrate.

Keywords: esterases; growth rate; nitrogen sources; nitrogen metabolizing enzymes; proteases; *Scenedesmus*

Abbreviations: AAT – aspartate aminotransferase; DW – dry weight; EST – esterase; GOGAT – glutamate synthase; N – nitrogen; NAD-GDH – NAD-dependent glutamate dehydrogenase; NAD-MDH – NAD-dependent malate dehydrogenase; PAGE – polyacrylamide gel electrophoresis.

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INTRODUCTION

Microalgae are photoautotrophic microorganisms that utilize solar energy and several essential nutrients (C, N, P, S, K, Fe etc.) in order to synthesize their biomass compounds and to multiply their cells. Some microalgae are presently cultivated to produce numerous high value products, such as proteins, lipids, fatty acids, polysaccharides, vitamins, pigments etc, which have different current and potential applications in animal or human nutrition, nutraceutics, pharmaceutics, cosmetics and bioenergy (Sharma and Sharma, 2017; Arica et al., 2017; Deniz et al., 2018). Nitrogen supply constitutes a major cost of large-scale algal cultivation and the selection of an appropriate, efficiently utilized N source may lead to more efficient and economical production of algal biomass for high-value products (Peccia et al., 2013; Sturm and Lamer, 2011). It is well known that most microalgae have the ability to use several inorganic forms of N, like nitrate, nitrite and ammonium, as well as urea (organic compound) as N sources for their photoautotrophic growth. These microorganisms have developed a diversity of pathways and regulatory mechanisms for conversion of each N source to ammonium inside the cells and for assimilation of ammonium into amino acids (Hodson and Thompson, 1969; Solomon and Glibert, 2008; Perez-Garcia et al., 2011). Despite the lower energy costs of ammonium uptake and assimilation, many algae grow better with alternative N sources (Sibi, 2015; Soni et al., 2017). Apart from its effect on growth, the source of N may also be responsible for important changes in the metabolic activity and biochemical composition of microalgal cells (Lourenço et al., 2002; El-Sheekh et al., 2004). There are a number of studies on the response of microalgal metabolic enzymes to the change of N source in the nutrient medium, all of which are focused on the replacement of nitrate with ammonium (Shatilov et al., 1978; Tischner and Lorenzen, 1980; Ahmad and Hellebust, 1984; Muñoz-Blanco and Cárdenas, 1989; Ahmad and Hellebust, 1993; Moyano et al., 1995; and references therein; Gérin et al., 2010) and rarely with urea (Muñoz-Blanco et al., 1988). *Chlorella* and *Chlamydomonas* species are the model algae in these studies, with their enzymes GS, GOGAT, GDH and AAT being best characterized for kinetic properties, changes in the isoenzyme patterns and activities. However, to our knowledge, the effects of different nitrogen sources on growth rate and metabolic enzymes pattern and activity of *Scenedesmus* have not been reported. The green algae of genus *Scenedesmus* are very suitable for both laboratory investigations and mass intensive cultivation, since they have 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). The purpose of this study was to investigate the changes in nitrogen and carbon metabolism in *Scenedesmus* sp. BGP in the presence of various sources of N in the culture medium, namely urea, ammonium nitrate and a mixture of both, focusing on the activity of the enzymes GOGAT, NAD-GDH, AAT and NAD-MDH.

MATERIALS AND METHODS

Strain and growth conditions

Scenedesmus sp. strain BGP used in this study was isolated from a rainwater puddle (Sofia, Bulgaria). The strain is deposited in the collection of the Laboratory of Experimental Algology, Institute of Plant Physiology and Genetics, BAS, Sofia, Bulgaria. Monoalgal, non-axenic cultures of *Scenedesmus* sp. BGP were grown autotrophically in the nutrient medium of Setlik (1967), modified by Georgiev et al. (1978) containing both urea and ammonium nitrate (conditionally called "standard medium"), or in the same medium, but with only urea and only ammonium nitrate as a source of N. Each of the three variants of N source had a final N concentration of 20 mM. The remaining cultivation conditions were as following: bubbling with air, enriched with 2% CO_2; 24°C; continuous lateral illumination with cool-white fluorescent lamps at a photon flux density of 132 μmol m^{-2} s^{-1} measured at the surface of 200 mL flasks. An initial culture density of 0.80-0.87g L^{-1} dry weight (DW) was used. The experimental cultures were harvested after 72 h (in an exponential growth phase, as determined by preliminary experiments). In separate experiments, all cultures were harvested after 192 h (at the early stationary phase). Cells were collected by centrifugation (5000×g, 20 min), rinsed three times with distilled water, frozen, and stored at -70°C until analyzed.

Analytical methods

For biomass DW determination, algal suspensions (3×5 mL each) were filtered through Whatman GF/C glass filters (Whatman International Ltd, Maidstone, UK), rinsed with tap water to eliminate salts and oven dried at 105°C to a constant weight. The growth of *Scenedesmus* was evaluated gravimetrically by the increase in biomass DW. The specific daily growth rate [μ] was calculated using the formula: $\mu=\ln(m_{t2}/m_{t1})/t_2-t_1$ (Levasseur et al., 1993), where m_{t1} and m_{t2} represent the DW at the starting day of the experiment (t_1) (t_1=0) and on the third or eight day of cultivation (t_2).

Preparation of cell extracts, polyacrylamide gel electrophoresis (PAGE) and activity staining of metabolic enzymes and proteases

The cells were mechanically homogenized using vibrations homogenisator VHG1 (Germany) in a 60 mM TE buffer (Tris base with 0.1 mM EDTA) (pH 7), at 4°C and centrifuged at 13000×g for 15 min. The concentration of soluble proteins in the supernatant was determined by the method of Bradford (1976), with BSA as a standard. Equal amounts (13 μg) of protein from cells grown under different conditions were subjected to discontinuous PAGE as described by Laemmli (1970), but under non-denaturing, non-reducing conditions (in the absence of SDS and βME). Electrophoretic separation was performed on 10% polyacrylamide gels at a constant current of 35 mA per gel for 3–4 h. Upon completion of the electrophoresis, separate gels were stained for the activities of glutamate synthase (NADH-GOGAT, EC 1.4.1.14) (Matoh et al., 1980), glutamate dehydrogenase (NAD-GDH, EC 1.4.1.2) (Nash and Davies, 1975),

malate dehydrogenase (NAD-MDH, EC 1.1.1.37) (Honold et al., 1966), aspartate aminotransferase (AAT, EC 2.6.1.1) (Griffith and Vance, 1989) and esterases (Murphy et al., 1996). Substrate gel electrophoresis and detection of protease activities were performed according to the method of Lodemel et al. (2004). Nonheated, nonreduced samples (9 μg protein each) were separated on gels, prepared by copolymerization of 10% acrylamide–0.1% gelatin (w/v) in the presence of 2% SDS. Electrophoresis took place for 30 min at 100 V followed by 75 min at 150 V. After electrophoresis, the proteins were re-natured in 2.5% (v/v) Triton X-100 for 15 min twice prior to incubation overnight in 50 mM Tris–HCl, pH 8, 10 mM $CaCl_2$ at 37°C. The gels were stained with 0.1% (w/v) Coomassie Brilliant Blue G250 in 30% ethanol and 10% (v/v) acetic acid. The reagents used for enzyme activity staining were purchased from Sigma (Sigma Inc., St. Louis, MO, USA). Gel patterns were photographed immediately after staining using the UVItec gel documentation system (Cambridge, UK). Image analysis of the gels was performed on a PC using Gel-Pro32 Analyzer software (Media Cybernetics, Bethesda, MD USA). The activity of each isoenzyme (band) was recorded as integrated optical density (IOD), in arbitrary units. When an enzyme had multiple bands, the sum of their IOD values was considered as a total enzyme activity for a particular experimental condition. For easier comparison, the values on the figures are presented relative to the total enzymatic activity of the exponential culture grown with urea, the value of which is conditionally assumed to be 100%.

Statistical analysis

The experiments were conducted in triplicate. All data are presented as the means±standard deviation (SD). The significance of differences between the treatments was evaluated by one-way analysis of variance (ANOVA) and a Bonferroni post hoc test using InStat (GraphPad Software Inc., La Jolla, CA, USA). Values of $P < 0.05$ were considered significant.

RESULTS

Nitrogen source impact on the specific growth rate of *Scenedesmus* sp. BGP

The variations in the dry weight of biomass and the specific growth rate of *Scenedesmus* sp. BGP, depending on the nitrogen source in the medium were followed during the exponential and the early stationary growth phase (on the 3rd and the 8th day of cultivation). The obtained results showed that during the exponential growth phase, the media supplemented with only urea and only ammonium nitrate provided similar ($P > 0.05$), high growth rates and biomass accumulation (Table 1). The simultaneous administration of urea and ammonium nitrate also provided good algal growth, but the specific growth rate ($\mu/3$) was significantly lower ($P < 0.05$). At the 8th day of cultivation, the values of both parameters were highest ($P < 0.05$) for urea-grown algae and lowest ($P < 0.05$) for the algae cultivated in a medium with a mixture of the two N sources.

Response of metabolic enzymes of *Scenedesmus* sp. BGP to different nitrogen sources in the medium

Four main bands of GOGAT activity (designated a-d and arranged according

Table 1. Changes in the biomass dry weight (DW, g L^{-1}) and the cell growth rate [μ] of *Scenedesmus* sp. BGP in dependence of the nitrogen source and the cultivation time (t, days). Means for DW and [μ] with different letters are significantly different ($P < 0.05$) between N sources for a specific cultivation time.

Nitrogen source	Initial DW t=0	DW t=3	[μ] t_2=3	Initial DW t=0	DW t=8	[μ] t_2=8
Urea (U)	0.84±0.05	3.74±0.10[a]	0.498±0.009[a]	0.84±0.05	6.14±0.10[a]	0.248±0.002[a]
Ammonium nitrate (AN)	0.87±0.05	3.87±0.10[a]	0.498±0.009[a]	0.80±0.04	5.62±0.10[b]	0.244±0.002[b]
U+AN (standard medium)	0.87±0.04	3.67±0.10[a]	0.480±0.009[b]	0.80±0.04	5.30±0.10[c]	0.236±0.002[c]

to the increment in their migration mobility) were observed in *Scenedesmus* sp. BGP under all growth conditions (Fig. 1A). The algae grown in ammonium nitrate supplemented medium (AN) to exponential phase and in the standard medium (U+AN) to stationary phase showed a fifth (e), fast-moving but weak band. These were also the samples that had the highest relative total GOGAT activity (Fig. 1B). Cells cultured in media with urea (U) and with a mixed N source (U+AN) to the exponential growth phase showed the lowest enzyme activity.

Gels stained for NAD-dependent GDH activity revealed one band (Fig. 2A). In general, the intensity of this band was weak, indicating a low enzyme activity. A remarkably high was the activity of GDH in *Scenedesmus* sp. BGP grown in ammonium nitrate medium to the exponential phase (Fig. 2B).

One band of AAT activity was visible after native PAGE and subsequent in gel staining (Fig. 3A). The relative total AAT activity was highest in *Scenedesmus* sp. BGP, grown in ammonium nitrate-containing medium to the exponential growth phase (Fig. 3B). Prolonged growth of cells in the standard medium resulted in a significant increase (over 2 fold) of the enzyme activity compared to that in the exponential phase. All other cultivation conditions were associated with low AAT activity.

Eight bands of NAD-dependent

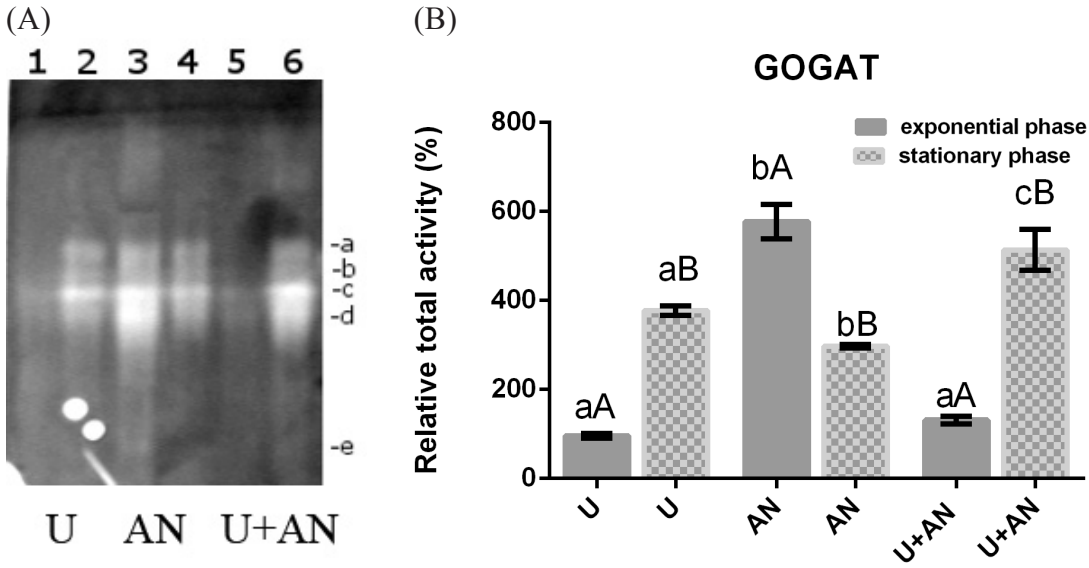

Figure 1. Isoenzyme pattern (A) and changes in total activity (B) of glutamate synthase (GOGAT) in *Scenedesmus* sp. BGP in response to nitrogen source in the medium and growth phase. In (A), letters (a–e) on the right indicate the bands of GOGAT activity in order of increasing electorphoretic mobility. Equal amounts (13 μg) of protein were loaded per well. Lanes 1 and 2 (U): urea; lanes 3 and 4 (AN): ammonium nitrate; lanes 5 and 6 (U+AN): urea plus ammonium nitrate; lanes 1, 3 and 5: exponential growth; lanes 2, 4 and 6: stationary phase. In (B), mean values with different lowercase letters are significantly different ($P < 0.05$) between N sources for a specific growth phase and different capital letters indicate significant differences for a specific N source between exponential and stationary phase.

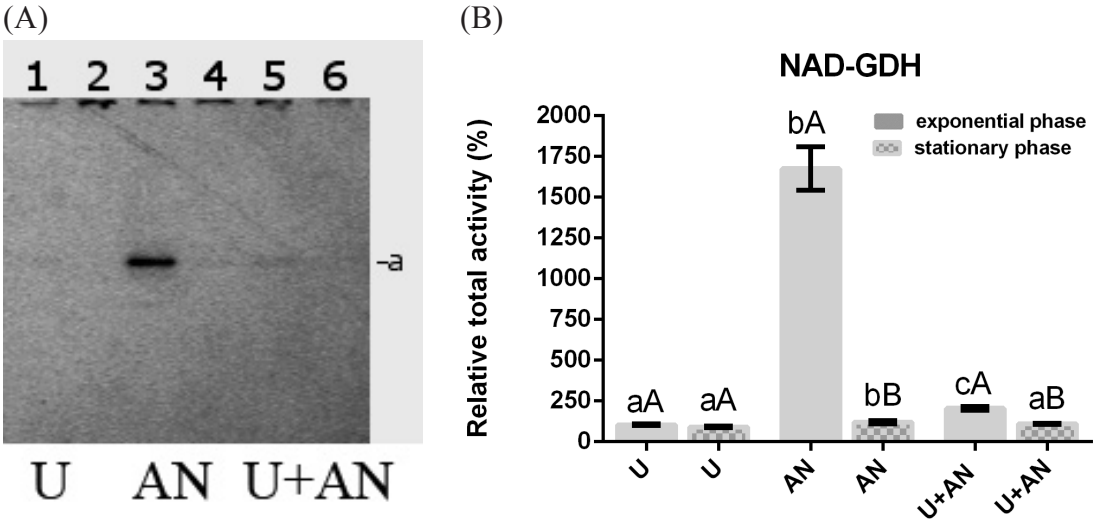

Figure 2. Changes in NAD-dependent glutamate dehydrogenase (NAD-GDH) activity of *Scenedesmus* sp. BGP in response to different nitrogen sources and the growth phase. A, isoenzyme patterns. Letter (a) on the right indicates the band of NAD-GDH activity. B, relative total NAD-GDH activity.

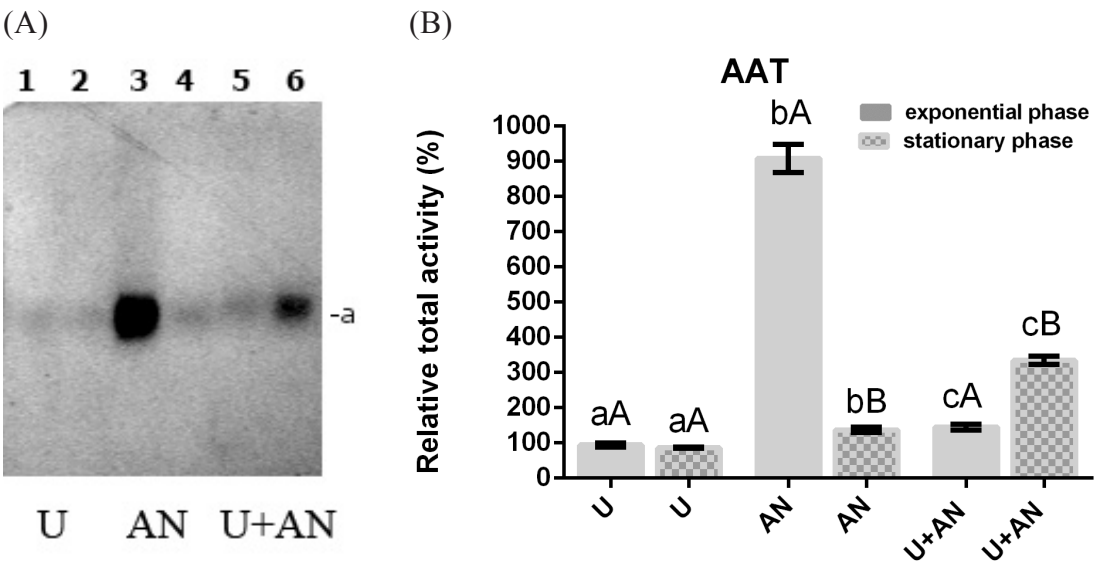

Figure 3. In gel activity staining (A) and relative total activity (B) of aspartate aminotransferase (AAT) of *Scenedesmus* sp. BGP in response to different nitrogen sources and the growth phase.

MDH activity (a-h) were detected in *Scenedesmus* sp. BGP under all treatments (Fig. 4A). The intensity of the bands varied between treatments and led to changes in total enzyme activity (Fig. 4B**)**. Cells grown in the standard medium showed the highest levels of enzyme activity due to the most intensive "c", "d" and "f" bands. In cells, grown in the urea medium, MDH activity was about 2 times higher in stationary than in exponential phase. The observed changes in enzyme activity with the growth phase were exactly opposite in the ammonium nitrate grown cells.

Gelatin zymograms revealed the presence of five constitutive proteases in *Scenedesmus* sp. BGP (Fig. 5A, b-f). In the culture grown in ammonium nitrate medium to the exponential phase, three new bands of protease activity (a, g and h) were observed and the relative total enzyme activity was highest (Figs. 5A and 5B). The total protease activity was higher in the younger than in the older cultures, grown with one N source only. The alga grown in the medium containing the two N sources showed the lowest protease activity during both grown phases.

The isoenzyme pattern and the changes in the esterase activity of *Scenedesmus* sp. BGP under different growth conditions are shown in Figure 6. Three clearly visible bands and six weaker bands of esterase activity were detected by gels analysis, indicated in a sequence by letters (Fig. 6A, a-i) starting from the anode, according to the increment in the negative charge. The relative total esterase activity (Fig. 6B) was highest in older cells grown in ammonium nitrate medium, due to the strongest intensity of bands "a", "b" and "c". In younger cells grown in the same medium, the total esterase activity was

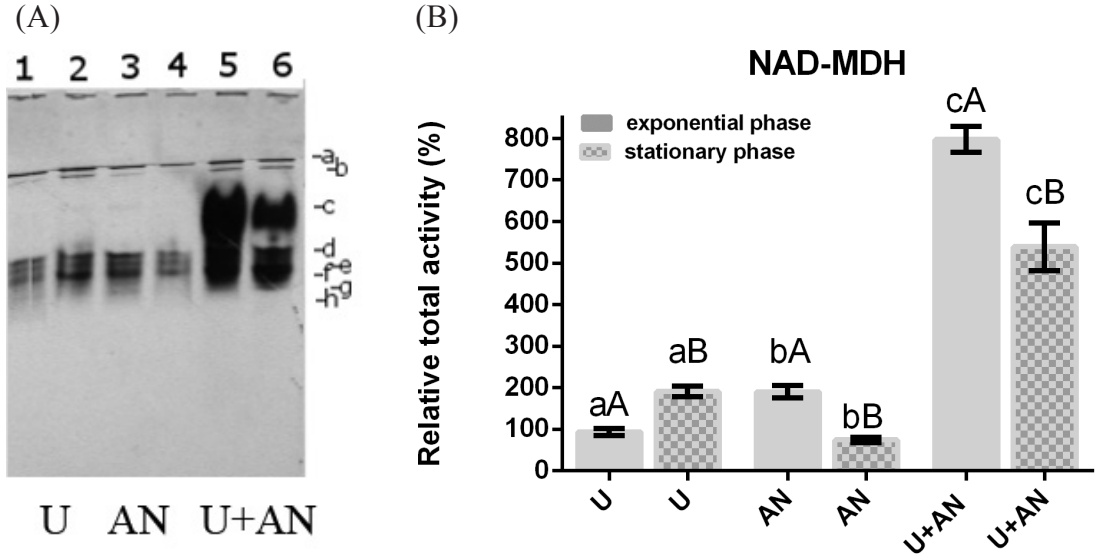

Figure 4. Isoenzyme pattern (A) and changes in total activity (B) of NAD-dependent malate dehydrogenase (NAD-MDH) of *Scenedesmus* sp. BGP in response to various nitrogen sources in the medium and the growth phase. In (A), letters (a–h) on the right indicate the bands of enzyme activity in order of increasing electorphoretic mobility.

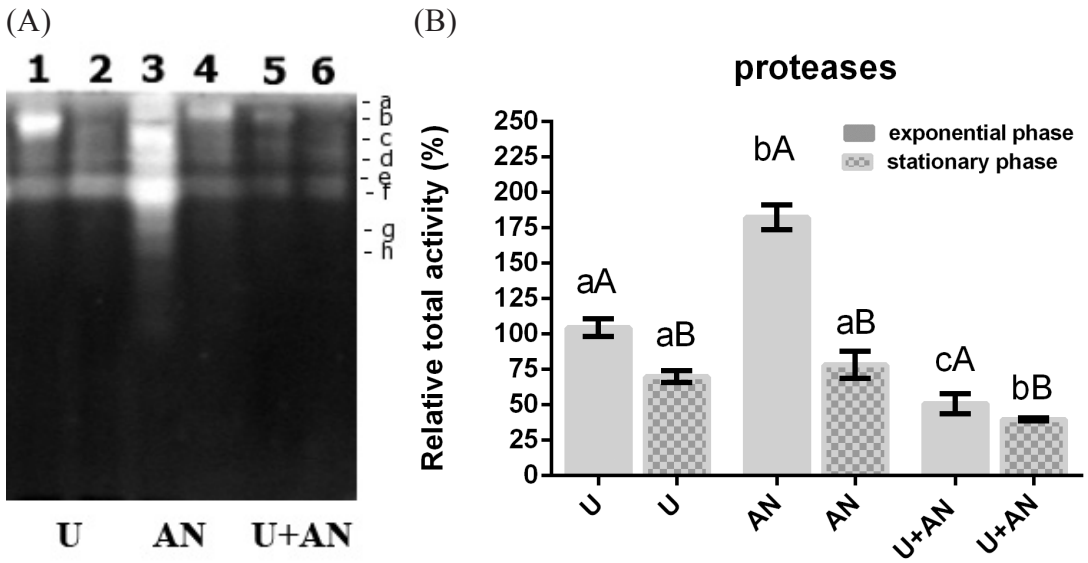

Figure 5. Changes in intracellular proteases of *Scenedesmus* sp. BGP in response to different nitrogen sources and the growth phase. A, gelatin zymograms of protease activities. Letters (a–h) on the right indicate the bands of protease activity in order of increasing electorphoretic mobility. Equal amounts (9 μg) of protein were loaded per well. B, relative total protease activity.

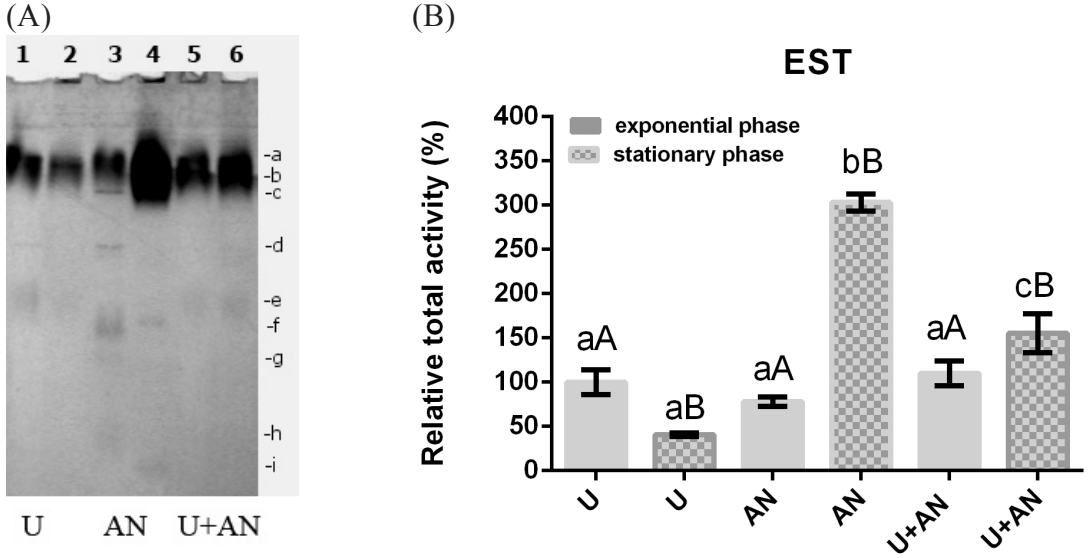

Figure 6. Changes in isoenzyme pattern (A) and relative total activity (B) of esterases of *Scenedesmus* sp. BGP in response to different nitrogen sources in the medium and the growth phase. In A, letters (a–i) on the right indicate the bands of esterase activity in order of increasing electorphoretic mobility.

about 3.5 times lower compared to the older cells, although the number of their esterase isoenzymes (8) was greatest. Cells cultured in the standard medium (U+AN) maintained a relatively high level of enzyme activity, being higher in the stationary than in the exponential growth phase. The culture grown with urea to the stationary phase showed the lowest esterase activity.

DISCUSSION

In our previous study, we showed that the choice of N source had a substantial effect on the growth of the new Bulgarian strain *Scenedesmus* sp. BGP. The use of media with either urea or ammonium nitrate alone had the advantage to give a better biomass yield with a stable and balanced biochemical composition (about 41% carbohydrates, 29% proteins and 24% lipids), compared to the medium containing a mixture of both N sources (Vasileva et al., 2015). The results from the present work showed that not only the DW but also the specific growth rate of the alga supplied simultaneously with urea and ammonium nitrate were lowest during both growth phases (Table 1). This pattern could be a consequence of the less efficient assimilation of a mixture of two N-containing compounds than of a single one. The growth rate of *Scenedesmus* sp. BGP fed with ammonium nitrate was as high as with urea during the first 3 days, but decreased on the 8th day of cultivation. Thus, urea appeared to be the preferred N source for this green alga. Our results are consistent with the data of Kim et al. (2016) on the growth of the green microalga *Tetraselmis* sp. which was weaker when grown with ammonium nitrate than with urea. Another study showed that the highest yield could be achieved by using mixed N sources (urea plus sodium nitrate). It was about *Scenedesmus rubescens* like microalga cultured under the abovementioned conditions and at the end of the 17-days culture period it had the highest biomass DW (4.146 ± 0.375 g L^{-1}) (Lin and Lin, 2011). Urea was found to be more effective than sodium nitrate and ammonium chloride for *Scenedesmus obliquus* and *Chlorella kessleri* due to its stimulatory effect on the cell number and the biomass DW (El-Sheekh et al., 2004). For its growth, *Chlorella pyrenoidosa* preferred potassium nitrate, while *Chlorella* sp. preferred urea (Soni et al., 2017). The specific growth rate and the biomass concentration of *Scenedesmus obliquus* were significantly higher in the presence of sodium nitrate and potassium nitrate than in urea-, ammonium nitrate- and ammonium chloride-based media (Sibi, 2015). In general, the growth responses to different N sources are strongly species-specific. Therefore, for increasing the biomass yield, it is important to choose the proper nitrogen source for each microalgal species.

In order to get better understanding of the metabolic responses of *Scenedesmus* sp. BGP to various N sources, the changes in the isoenzyme patterns and relative activities of six selected enzymes were tracked during the exponential and the stationary phase of the algal growth. GOGAT, AAT, NAD-GDH and NAD-MDH are enzymes that link nitrogen and carbon (energy) metabolism. Proteases contribute to the total cellular amino acid pool. Intracellular esterases were studied as indicators of overall

metabolic activity. Glutamate synthase is a partner in the glutamine synthetase-glutamate synthase (GS-GOGAT) cycle essential for the biosynthesis of glutamate (Scanlan and Post, 2008). Glutamate is one of the central players in the nitrogen metabolism since it is capable of both receiving and donating N group (Dagenais-Bellefeuille and Morse, 2013). AAT catalyzes the interconversion of glutamate and oxaloacetate to aspartate and alpha ketoglutarate. The amino group transfer from glutamate or aspartate to the respective keto acids is crucial in both amino acid biosynthesis and degradation. In our study, the activity of AAT was analyzed in a glutamate producing direction. NAD-dependent glutamate dehydrogenase catalyzes the deamination of glutamate to alpha ketoglutarate. This biochemical reaction provides an oxidizable carbon source (alpha ketoglutarate) used for the production of energy as well as a reduced electron carrier (NADH), thus linking amino acid metabolism to the tricarboxylic acid (TCA) cycle (Berges and Mulholland, 2008). Using NAD as a cofactor, malate dehydrogenase catalyzes the conversion of malate to oxaloacetate (Minárik et al., 2002), a reaction that is part of many metabolic pathways, including the TCA cycle.

The GOGAT, NAD-GDH, AAT, NAD-MDH, esterase and protease isoforms of *Scenedesmus* sp. BGP were visualized using the excellent capabilities of the methods for separation of native proteins by PAGE and subsequent in gel enzyme activity staining. Being determined in crude cell extracts with identical total protein content, the images on each gel also allowed a comparison of the enzyme activities. It was found that the isoenzyme patterns of *Scenedesmus* sp. BGP did not change, except for esterases and proteases, but the intensity of some bands varied depending on the N source in the culture medium and the growth phase, resulting in changes in the relative total activity of the respective enzyme. In cells cultured in ammonium nitrate-containing medium to the exponential phase, the relative total activities of glutamate-producing (GOGAT and AAT) and glutamate-catabolizing (NAD-GDH) enzymes were highest, with a medium NAD-MDH activity level as compared to the exponentially growing cultures in the urea and standard media. The use of urea as a N source instead of ammonium nitrate led to a decrease in the activity of these enzymes. Despite the observed differences in the enzyme responses to urea versus ammonium nitrate, both N sources provided almost the same growth rate ($P > 0.05$, Table 1). *Scenedesmus* sp. BGP cells supplied simultaneously with both ammonium nitrate and urea showed the most active NAD-MDH due mainly to the up-regulation of “c”, “d”, and “f” enzyme isoforms (Fig. 4A), coupled with intermediary activities of NAD-GDH and AAT. The activity of GOGAT in these cells was slightly higher than that of the urea grown culture, but not significantly different ($P > 0.05$). This pattern of enzyme activity was related to the same biomass yield but a lower growth rate ($P < 0.05$) than the other two exponentially growing cultures. In contrast to enzymes associated with nitrogen/carbon metabolism, the activity of esterases as a measure of the total metabolic activity was not significantly affected by the change of the N source in

the medium.

The regulation of the metabolic enzymes in dependence of the N source during the stationary growth phase differed from that in the exponential phase. Among all older cultures, the cells grown with ammonium nitrate plus urea exhibited the highest total GOGAT, AAT and NAD-MDH activities with NAD-GDH and esterases being less active than in ammonium nitrate grown cells ($P < 0.05$), but more active than in urea grown cells. It should be noted that although the difference was not significant ($P > 0.05$), the activity of NAD-GDH was by about 20% higher in the standard medium than in the urea medium. Despite the up-regulation of enzyme activities in the standard medium, the specific growth rate and the biomass yield of this culture were lowest ($P < 0.05$). *Scenedesmus* sp. BGP grown with ammonium nitrate to stationary phase had the most active NAD-GDH and esterases, intermediary level of AAT activity, coupled with the lowest GOGAT and NAD-MDH activity. Compared to ammonium nitrate and ammonium nitrate plus urea, the use of urea alone resulted in medium levels of GOGAT and NAD-MDH activity in the older cells, and suppressed activities of AAT, NAD-GDH and esterases. These levels of metabolic enzyme activities were sufficient to provide the highest specific growth rate.

The relatively high activity of GOGAT in the exponentially growing culture fed with ammonium nitrate as well as in the two cultures grown in urea and ammonium nitrate media to the stationary phase coincided with increased protease activity in these cells (Fig. 5B), suggesting a role for the NADH-GOGAT of *Scenedesmus* sp. BGP in the re-assimilation of ammonium, produced by protein degradation, as has been reported for *Chlamydomonas reinhardtii* (Marquez et al., 1986; Vega et al., 1987). It is noteworthy that the activity of NAD-MDH was highest in the cultures grown with mixed N sources (urea plus ammonium nitrate) during the two growth phases and this was probably due to the greater energy needs of these cells.

The activities of NADH-GOGAT, NAD-GDH, AAT and NAD-MDH of *Scenedesmus* sp. BGP varied in response to the N source, which may be related to the interactions between the applied different chemical forms of N, namely $CO(NH_2)_2$, NH_4 and NO_3. It is well known that in the green microalgae, as with many algae, ammonium (Ricketts, 1988; Cannons and Shiflett, 2001; De Montaigu et al., 2010) and urea (Molloy and Syrett, 1988) inhibit the nitrate transport and suppress the nitrate-reducing enzymes nitrate reductase and nitrite reductase. Further, in *Chlamydomonas reinhardii*, ammonium is shown to act as an inhibitor of the urea transport activity (Williams and Hodson, 1977) and a repressor of the synthesis of ATP-urea amidolyase (Hodson et al., 1975). ATP-urea amidolyase is the urea hydrolyzing enzyme in this and some other green microalgae (Bekheet and Syrett, 1977). The differences in the size of the endogenous cellular pools of ammonium and the intermediary metabolites such as glutamate and alpha ketoglutarate depending on the N source as well as the growth phase may also have a considerable impact on the activity of the metabolic enzymes studied.

The results of the present study showed the metabolic flexibility of the

Bulgarian strain *Scenedesmus* sp. BGP when changing the N source of the nutrient medium. Its ability to employ various regulatory mechanisms in response to urea, ammonium nitrate or a mix of both as well as the cultivation time ensured the maintenance of an efficient action of the metabolic enzymes at each of the cultivation conditions studied and ultimately led to good algal growth. The strain utilized urea most effectively, resulting in the highest growth rate and the largest amount of final biomass. In large scale cultivation of *Scenedesmus* sp. BGP for the production of biomass for various useful applications, urea would be the more appropriate choice also in terms of economic profitability as it is a cheaper source of N than ammonium nitrate.

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