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INFLUENCE OF INORGANIC NUTRIENTS ON PARAMETERS OF BIOMASS PRODUCTION IN A LOCAL STRAIN OF THE MICROALGA *SCENEDESMUS ACUMINATUS*

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Abstract: Biotechnologies based on microalgal cell cultures have multiple applications in aquaculture, the food industry, health care, environmental management, and production of alternative energy sources. In the present study we investigated the influence of nutritional conditions on bioproductive properties of a local strain of the green microalga *Scenedesmus acuminatus*, grown in axenic laboratory cultures, in Bold's basal nutrient medium. Increment of nitrate concentration enhanced the biomass production, and increased protein content of the alga. Simultaneous supplement of reduced and oxidized form of inorganic nitrogen (as ammonium nitrate) did not result in an increased dry biomass, but the protein content of algal cells was significantly higher, while the carotenoid content decreased. Nitrogen deprivation increased the carotenoid pigment content of the dry algal biomass. 10 mM $KHCO_3$ as inorganic carbon source increased biomass production, decreased protein content and did not modify significantly the carotenoid content of the alga. A higher phosphate concentration in the medium induced an increment of dry biomass and carotenoid content, but had no relevant influence on protein content. Sulphate enrichment did not modify dry biomass and carotenoid content, but caused a moderate increment of protein amount. Photosynthetic light use efficiency and vitality index of photosynthetic apparatus, evaluated with the method of induced chlorophyll fluorescence, revealed a positive influence of ammonium nitrate and of elevated nitrate content, while nitrate starvation and an excessive phosphate and sulphate supply moderately decreased the functional efficiency of light reactions of photosynthesis.

Keywords: axenic cultures, carotenoids, nitrogen sources, photosynthetic efficiency, protein content, *Scenedesmus acuminatus*, vitality index

Introduction

Various strains of freshwater green microalgae have a high bioproductive potential if proper cultivation technologies are used. Microalgae cultivated under controlled conditions may be valuable alternative resources of dietary supplements [4, 33], fodder, pharmaceuticals [6, 11, 23] and even biofuel [30, 34]. Their broad adaptability based on metabolic plasticity allows manipulation of primary production of organic compounds by creating specific nutritional and developmental conditions. This can be achieved by optimization of nutrient media, of the light regime and of other external factors that enable an efficient photosynthesis, an intense growth and a reprogrammed metabolism [14, 16, 38].

Because they are photoautotrophic, algae do not require the presence of organic compounds in the nutrient media, and because they are aerobic organisms, they do not need anoxic growth conditions, as do many photosynthesizing bacteria. Under favourable conditions, many green microalgae can grow very fast, they have a high reproduction rate and their biomass has an especially high protein content (up to 50% of the dry weight), without possessing a

similarly high percentage of nucleic acids, as do bacteria. (A high level of nucleic acids makes the biomass unfavourable for regular consumption, because of risks of metabolic diseases.) The above mentioned characteristics make many green microalgae valuable resources of the so-called single-cell proteins (SCP, i.e. proteins from unicellular organisms), despite the costs of processing of raw algal material [18, 22, 23, 35].

Another considerable advantage of algal culture is that they do not compete for space with agricultural crop plants, while their cultivation technology is considered to be environmentally friendly [32]. Furthermore, the process of algal biomass production may be combined with waste water management and with decontamination of polluted aquatic habitats [2, 12, 15, 20, 24, 28, 29, 39]. The most recent and one of the most promising biotechnological applications of microalgal cultures is their use in the production of alternative, renewable energy sources, such as hydrogen gas, algoleum, and different algal oil derivatives as biofuels [7, 17, 21, 26].

As living organisms, microalgae combine useful properties of both microorganisms (e.g. high reproduction rate, wide adaptability) and plants (e.g. photoautotrophy, wide range of metabolites). Even in--- natural ecosystems, some species exhibit a very high growth rate and have a crucial impact on the dissolved oxygen regime of surface waters, especially when the concentration of dissolved phosphorus is elevated, the temperature is high and water transparency is pronounced [37]. Environmental conditions that lead to algal blooming can be reproduced in different types of algal cultures, enabling a high algal biomass production under controlled conditions [5, 13, 36]. Both in natural and artificial systems, the light regime has a determinant role in algal productivity [1, 9]. Besides light and temperature, salinity and different inorganic nutrients are also important in the regulation of algal metabolism and development. Especially nitrogen is known to have a strong influence on the metabolism of proteins in various microalgae. In addition, nitrogen is easy to manipulate and is low-cost compared with other external factors [2, 3, 6, 16, 22].

The aim of the present work is to investigate the influence of variations in nutritional conditions (different types and amounts of nitrogen sources, inorganic carbon, phosphorus and sulphur supply) on biomass production, protein content, photosynthetic light use efficiency and carotenoid pigment content of a local, metabolically active yet uncharacterized strain of the green microalga *Scenedesmus acuminatus*, in order to evaluate its bioproductive capacity and to optimize the culture conditions by changes in the composition of the inorganic nutrient medium. Optimization of culture conditions may lead to a more efficient biotechnological use of the algal strain.

Material and Methods

Microalgal strain and culture conditions

The green microalga *Scenedesmus acuminatus* (Lagerheim) Chodat (Chlorophyceae), strain AICB136, was isolated from a dam reservoir on the Tur rivulet (Cluj district, Romania), and axenic monoalgal cultures were obtained in the Biological Research Institute in Cluj-Napoca [8, 27]. Algal cultures were grown in Bold's basal nutrient medium [14], under a constant photon flux density of 96 μM m^{-2} s^{-1} provided by fluorescent lamps, and a temperature of 22 ºC. The control cultures were grown in unmodified Bold's medium, which contains as macronutrients 0.25 g l^{-1} $NaNO_3$ (2.9 mM), 0.075 g l^{-1} $K_2HPO_4 \cdot 3H_2O$ (0.4 mM), 0.175 g l^{-1} KH_2PO_4 (0.1 mM),

0.075 g l^{-1} $MgSO_4 \cdot 7H_2O$ (0.3 mM) and 0.085 g l^{-1} $CaCl_2 \cdot 2H_2O$ (0.2 mM). A nutrient deficient reference was represented by cultures without any nitrogen supply. The other experimental variants were: with 5-fold increased nitrate content (1.25 g l^{-1}), with 0.25 g l^{-1} NH_4NO_3 instead of $NaNO_3$, with addition of 10 mM (1 g l^{-1}) $KHCO_3$, with 5-fold increased potassium phosphate content, and with 5-fold increased magnesium sulphate content. The initial cell density of all cultures was set to 7.25 10^9 cells ml^{-1}, and during the 6 days of the experiment the cultures were stirred continuously on an orbital shaker at 180 rpm, in an algal growth chamber [9]. The pH value of all cultures was adjusted to 6.6. All experimental setups had 4 replicates.

Determination of cell density and of algal dry weight

The dynamics of algal cell divisions was evaluated cytometrically with a light microscope. Cell number counts were performed on samples of microalgal suspensions every 2 days, each time at the same hour. The dry weight of cultures was determined at the end of experiments, by filtering 100 ml of homogenized algal culture and drying the algae in an electric oven for 36 hours at 80 ºC [6, 36].

Determination of protein and carotenoid content of algal biomass

Protein content of algal biomass was determined using Bradford's method. Proteins were extracted from the ground dry algal biomass with 1.5 ml of 0.1 M potassium phosphate buffer (pH 7), containing 1 mM Na_2-EDTA, 1 mM ascorbic acid and 2% polyvinyl-pirrolidone (PVP). The extract was centrifuged at 4ºC for 15 min. with 9000 g, then 0.4 ml of supernatant was mixed with 2 ml of Bradford reagent, and after 5 min. the absorbance was determined at 595 nm. The blank consisted of 0.4 ml phosphate buffer mixed with 2 ml Bradford reagent. The standard curve for determination of protein concentration of extracts was obtained with known amounts (between 0 and 1 mg ml^{-1}) of bovine serum albumin [3, 12].

The carotenoid pigment content of algal cells was determined spectrophotometrically. Extraction of carotenoid pigments was performed in darkness with hot methanol. From each culture, 25 ml of algal cell suspension was centrifuged for 10 min. at 5000 g. The pellet was re-suspended in 3 ml methanol, transferred in heat-resistant test tube and heated in water bath at 70 ºC for 30 min. The extracts were centrifuged for 5 min. at 12000 g, and the supernatant was used for measurement of optical density at 470 nm, using methanol as blank [10, 11].

Measurement of in vivo *induced chlorophyll fluorescence*

Parameters of induced chlorophyll *a* fluorescence were measured with a pulse amplitude modulation chlorophyll fluorometer (PAM-FMS2, HANSATECH, UK). Algae were collected by low-pressure filtration, providing a uniform layer of cells on a 13 mm glass fiber filter. Samples were dark-adapted for 5 min. The modulated light was sufficiently weak (0.04 µM $m^{-2}s^{-1}$) so as not to produce any significant variable fluorescence. A single saturating flash (2000 µM $m^{-2}s^{-1}$ for 0.5 s) was applied to reach the maximal fluorescence Fm. After the decline of the signal, the actinic light was turned on (100 µM $m^{-2}s^{-1}$) to start the induction kinetics. The determined parameters were ground fluorescence F_0, maximal fluorescence Fm, the Fv/Fm ratio (Fv or variable fluorescence being the difference between the maximal and the ground fluorescence), the F_0/Fv ratio, modulated maximal fluorescence Fm', steady state fluorescence Fs, the effective quantum use efficiency (Φ) representing the ratio (Fm' – Fs)/Fm', as well as the vitality index (Rfd) expressed as the ratio (Fm – Fs)/Fs [3, 25, 31].

Statistical analysis

Each determination was made with 4 replicates, and then the means and standard errors were calculated. In data sets with normal distribution, significant differences between treatment means were determined using the post-ANOVA multiple comparison Tukey test, while in data sets with non-normal distribution, significant differences between means (at $P < 0.05$) were established with the Kruskal-Wallis test followed by the Mann-Whitney U-test.

Results and Discussion

Algal dry weight is an important indicator of biomass production achieved by the algal cell cultures under the given developmental conditions. Every external factor that impairs metabolism and growth of algae will result in a decreased net biomass production over a given time. By comparing the biomass production of parallel algal cultures grown under controlled conditions, one can evaluate the quality of the culture media and this allows optimization of algal production.

After six days of cultivation, the dry biomass of the algal cultures registered the highest values (an average of 244.5 mg l^{-1}) in the presence of 10 mM $KHCO_3$ as an inorganic carbon supply for photosynthetic assimilation. Similarly high dry weight was reached by the cultures having a 5 fold higher amount of sodium nitrate compared with the control, and the cultures supplemented with 5 fold higher amounts of potassium phosphate (Fig. 1). Application of a double nitrogen source in form of ammonium nitrate, and increment of the sulphate content of the medium did not result in a higher algal biomass production than the values registered in the control grown in unmodified Bold's medium. This reflects that the overall biomass production of this algal strain kept under the above mentioned conditions can be enhanced significantly by addition of bicarbonate (with a regular readjustment of pH which tends to increase), or by increasing the amounts of nitrate and phosphate of the nutrient medium. Metabolic changes induced by the different inorganic nutrient supplies cannot be reflected by the overall dry biomass production, but this parameter gives a general indication about growth conditions. In contradiction with what we have expected on the basis of other experiments with some microalgae [18, 22], the simultaneous use of ammonium and nitrate as inorganic nitrogen sources did not lead to a higher biomass production. This may be due to metabolic changes that are not clarified yet for this alga, or to a higher energy demand of processes induced by ammonium, even if by itself there is no cost for reduction as it is in the case of nitrate, which has to be reduced before assimilation into organic compounds [13].

Another unexpected result was that nitrogen starvation did not cause a serious decrease of biomass production during the 6 days of cultivation. It is generally accepted that limitation of nitrogen source is a nutritional stress factor for photoautotrophic organisms in aquatic environments, although the time scale of stress effects depends largely on previous reserves and on the prevailing growth conditions. Probably because the alga possesses an endogen nitrogen cycle and is capable of nitrogen mixotrophy, during a limited period of time it succeeds in surviving by reuse of nitrogen compounds released in the nutrient medium from the autolysed cells [36].

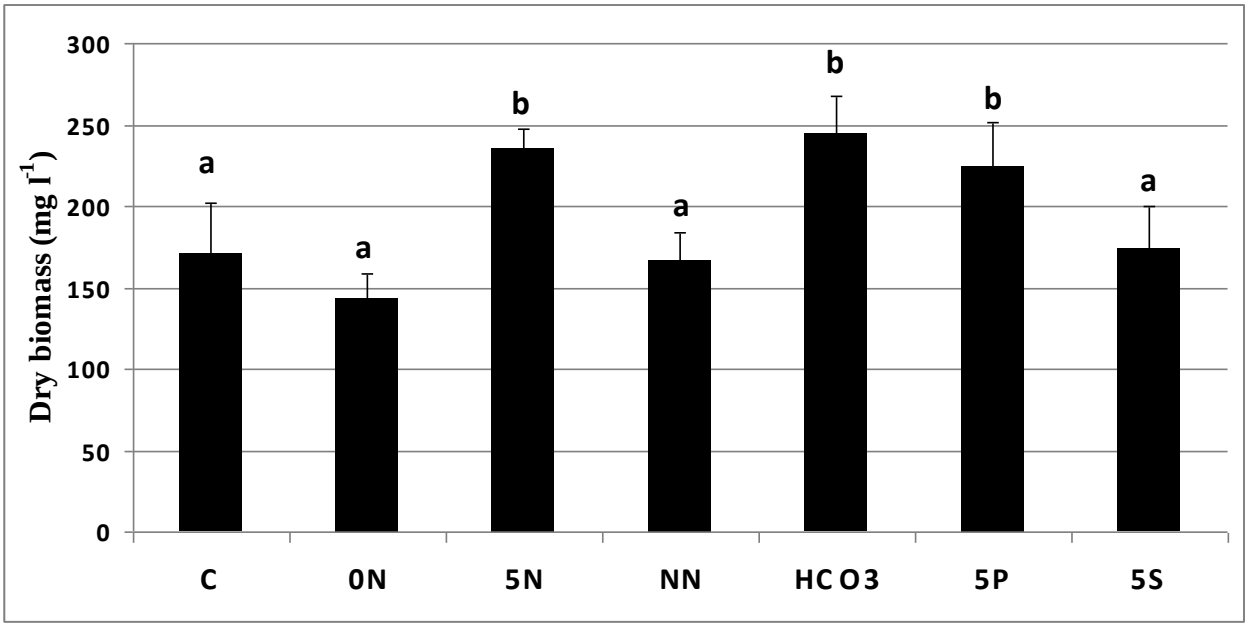

Fig. 1: Influence of different inorganic nutrient supplies on dry biomass production of *Scenedesmus acuminatus* on the 6th day of cultivation. C – control in Bold's nutrient medium; 0N – without nitrate; 5N – 5 fold increased amount of sodium nitrate; NN – with ammonium nitrate instead of sodium nitrate; HCO3 – supplemented with 10 mM potassium bicarbonate; 5P – 5 fold increased amount of potassium phosphate; 5S – 5 fold increased amount of magnesium sulphate. Bars represent standard errors from means (n = 4). Different letters represent significant differences at $P < 0.05$.

The dynamics of cell density of algal cultures reflects the integrated effect of cell divisions leading to increased cell number, and of cell death resulting in a decline of algal cell density. Starting from similar cell densities, during the 6 days of the experiment there were obvious differences in the changes of cell density induced by different nutrient supplies. Nevertheless, none of the experimental variants exhibited a statistically significant increase in the final cell density of the cultures in comparison with the control. Differences between the final biomass and the final cell density of cultures result mainly from the fact that cell growth, accumulation of assimilation products and rate of cell division are not proportionally determined by the composition of nutrient media. Enrichment with inorganic nitrogen (both as sodium nitrate and as ammonium nitrate), as well as with potassium phosphate, resulted in an earlier establishment of the stationary phase of algal population growth. Nitrogen deprivation stimulated the growth of cell density during the first days of exposure, as a result of mobilization of reserves because of sensing the stress condition. This enhanced growth of algal cell number was transitory, being followed by a decline of population density. The presence of bicarbonate caused an initial decrease in cell density, probably because of inhibition of cell divisions during induction of bicarbonate uptake and conversion into carbon dioxide in order to be used in the Calvin cycle. In the presence of bicarbonate, the significant increase in dry biomass was associated with a decreased algal cell density of the cultures in comparison with the control. This indicates that supply of bicarbonate as inorganic carbon source stimulated growth of algal cell weight by accumulation of organic compounds, but it did not favor cell divisions. Sulphur supply had no significant positive influence on cell density of algal cultures (Fig. 2). Comparable results were obtained for another *Scenedesmus* species (*S. obliquus*) grown in the presence of different nitrate and phosphate concentrations [5].

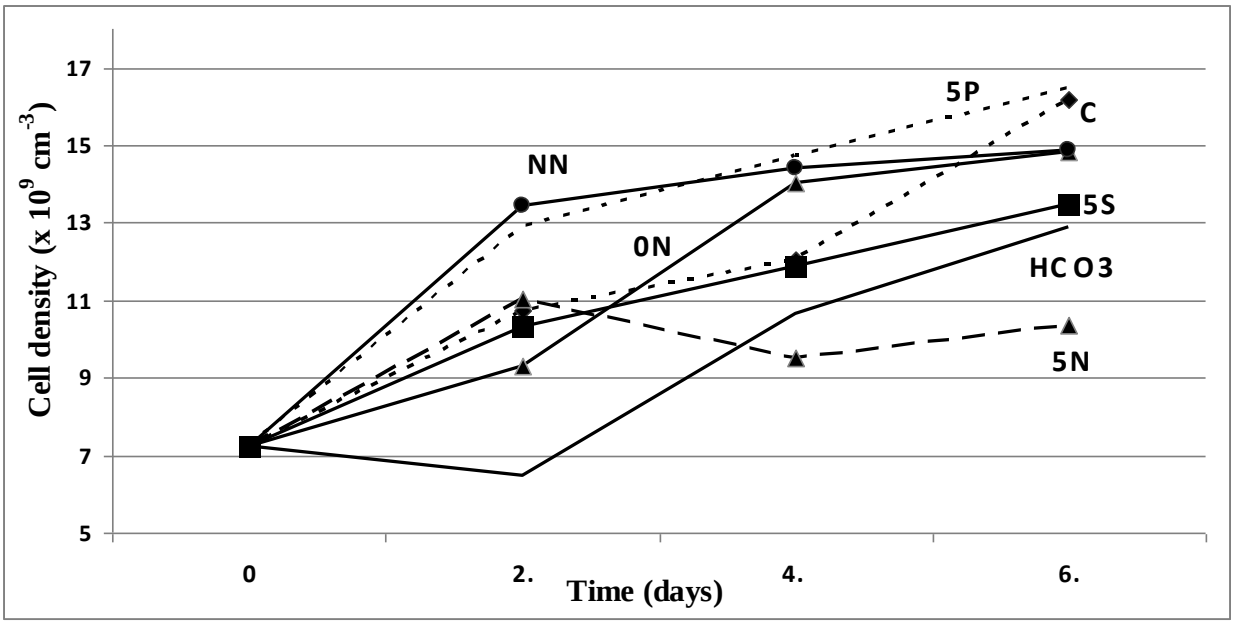

Fig. 2: Dynamics of cell density in the cultures of *Scenedesmus acuminatus* provided with different inorganic nutrients. C – control in Bold's nutrient medium; 0N – without nitrate; 5N – 5 fold increased amount of sodium nitrate; NN – with ammonium nitrate instead of sodium nitrate; HCO3 – supplemented with 10 mM potassium bicarbonate; 5P – 5 fold increased amount of potassium phosphate; 5S – 5 fold increased amount of magnesium sulphate.

Because many microalgae represent a promising source of single cell proteins as dietary supplements for humans and animals, protein content of algal cells has a well-defined biotechnological importance. In the present experiments, the highest protein content (an average of 440.5 mg protein per g dry weight) was achieved in the algal cultures supplied with 0.25 g l^{-1} of ammonium nitrate instead of the same amount of sodium nitrate in Bold's basal medium (Fig. 3). A statistically significant increase in protein content was also registered when 2.5 g l^{-1} of sodium nitrate was added to the medium instead of 0.5 g l^{-1} in the control. A moderate, but still significant improve of protein content was obtained when the magnesium sulfphate concentration of the nutrient medium was 5 times higher than in the control. Enhancement of potassium phosphate content did not result in an increased protein quantity, even if it stimulated the overall biomass growth. Bicarbonate supply decreased the protein content of algal cells. In connection with the fact that bicarbonate highly stimulated the biomass production of the algal cultures, one can conclude that this inorganic carbon source did not stimulate protein synthesis. Most probably, it induced accumulation of carbohydrate reserves, mainly in form of starch inclusions [13].

Carotenoids are accessory photosynthetic pigments also involved in the protection of organic biomolecules against oxidative damage. Many carotenoids (carotenes and xanthophylls) can directly scavenge singlet oxygen and hydroxyl radicals, being, along with vitamin E, the main lipophylic components of the non-enzymatic protective system against oxidative stress in living organisms. Because of their anti-oxidative properties, carotenoids are considered important alimentary components of a healthy diet. Several biotechnological procedures are intended to optimize carotenoid content of food and fodder by addition of algal extracts [11, 33].

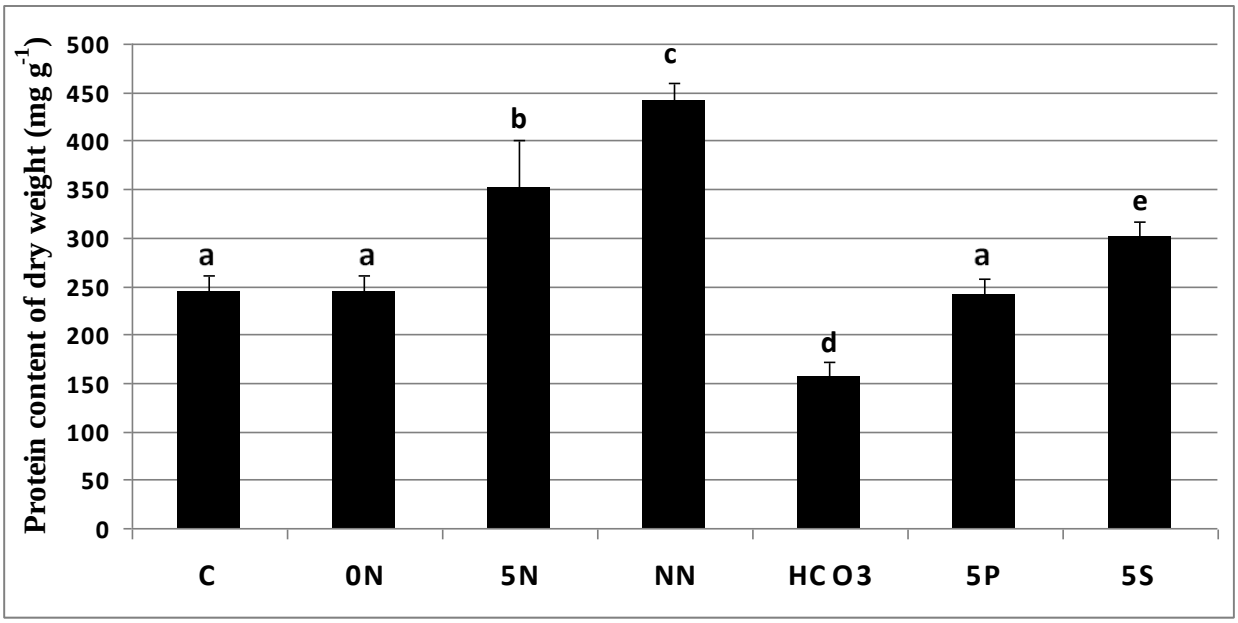

Fig. 3: Protein content of the algal cells in cultures of *Scenedesmus acuminatus* grown with different nutrient supplies. C – control in Bold's nutrient medium; 0N – without nitrate; 5N – 5 fold increased amount of sodium nitrate; NN – with ammonium nitrate instead of sodium nitrate; HCO3 – supplemented with 10 mM potassium bicarbonate; 5P – 5 fold increased amount of potassium phosphate; 5S – 5 fold increased amount of magnesium sulphate. Bars represent standard errors from means (n = 4). Different letters represent significant differences at $P < 0.05$.

Carotenoid content of algal cells was significantly increased by deprivation of nitrogen sources, as well as by elevation of the potassium phosphate amount in the nutrient medium (Fig. 4). Addition of bicarbonate and of sulphate did not have any significant influence on the quantity of carotenoid pigments in the dry algal biomass, while elevated nitrate concentration and ammonium nitrate caused a decrease in the quantity of this group of pigments with anti-oxidative properties. For another green microalgal species it was reported that a decreased nitrogen supply favours accumulation of ß-carotene [6], our findings being in accordance with these results. In addition, our local strain of *Scenedesmus acuminatus* also increases its carotenoid content when exposed to elevated concentration of potassium phosphate.

In vivo induced chlorophyll fluorescence is a sensitive, non-destructive tool for the study of environmental impacts on the primary energy-conversion processes of photosynthesis, on which the entire primary biomass production relies. From the different parameters of induced, non-modulated and pulse amplification modulated chlorophyll fluorescence, we have selected the potential quantum use efficiency of photosynthesis, reflected by the Fv/Fm ratio, and the relative fluorescence decrease (Rfd, expressed as the ratio (Fm – Fs)/Fs), also known as the vitality index. The value of this index depends on the difference between the temporary maximal fluorescence yield in dark-adapted samples and the steady state fluorescence level in illuminated algal cultures, and it is a very sensitive functional indicator of metabolic disturbances caused by stress conditions. Decrease of the value of Fv/Fm, as well as of the vitality index is directly related to the disturbed photochemical reactions that occur in photosystem II of thylakoid membranes in the chloroplast, leading to a less efficient photosynthetic use of the absorbed light energy [10, 25, 31].

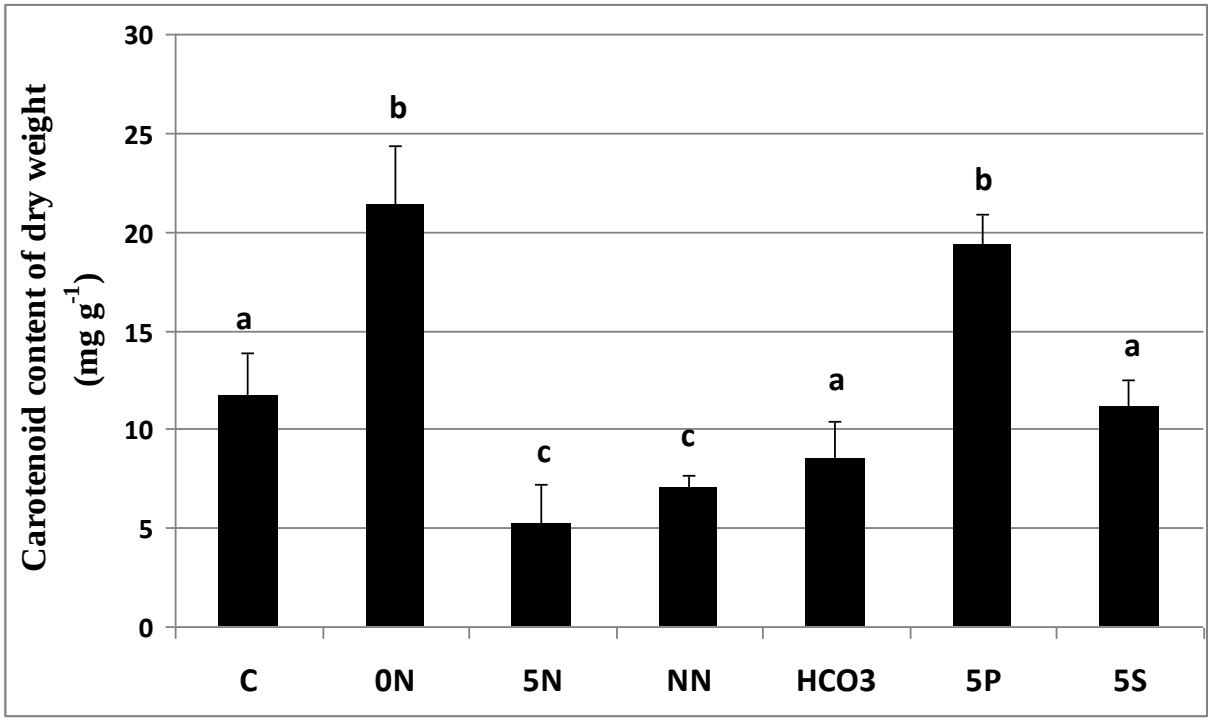

Fig. 4: Carotenoid pigment content of the algal cells in cultures of *Scenedesmus acuminatus* grown with different nutrient supplies. C – control in Bold's nutrient medium; 0N – without nitrate; 5N – 5 fold increased amount of sodium nitrate; NN – with ammonium nitrate instead of sodium nitrate; HCO3 – supplemented with 10 mM potassium bicarbonate; 5P – 5 fold increased amount of potassium phosphate; 5S – 5 fold increased amount of magnesium suphate. Bars represent standard errors from means (n = 4). Different letters represent significant differences at $P < 0.05$.

Increment of the nitrate content and substitution of sodium nitrate with ammonium nitrate ensured a high potential quantum use efficiency of photosystem II of the algal photosynthetic apparatus, with average values of the ratio between the variable and the maximal chlorophyll fluorescence higher than 0.85 (Fig. 5). The Fv/Fm ratio was decreased significantly only by nitrate deprivation and by addition of 0.375 g l^{-1} of magnesium sulphate instead of 0.075 g l^{-1} existing in the control. This reflects that photochemical reactions are disturbed, directly or indirectly, by nitrogen starvation stress and by high amounts of magnesium sulphate. The establishment of a connection between these nutrients and the light phase of photosynthesis needs further investigation.

Vitality index, reflected by the relative chlorophyll fluorescence decrease, exhibited a wider range of variation than other parameters of the induced chlorophyll fluorescence. The highest vitality index was recorded in the algal cultures supplied with ammonium nitrate, followed by those provided with increased amount of sodium nitrate. Cultures with elevated magnesium sulphate concentration also exhibited a higher vitality index than the control. Bicarbonate, phosphate enrichment and nitrate deprivation caused the reduction of vitality index of the algal photosynthetic apparatus, indicating an imbalance of the photochemical reactions (Fig. 6).

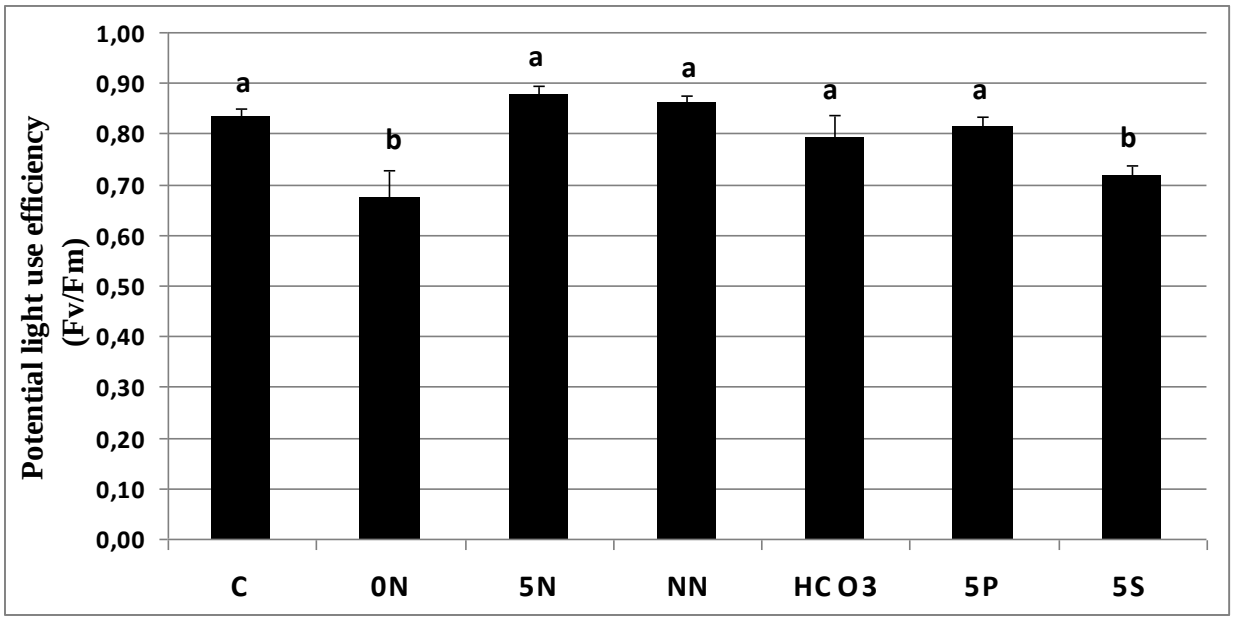

Fig. 5: Influence of different inorganic nutrient supplies on the potential photosynthetic light energy use efficiency of *Scenedesmus acuminatus*, based on induced chlorophyll fluorescence parameters. C – control in Bold's nutrient medium; 0N – without nitrate; 5N – 5 fold increased amount of sodium nitrate; NN – with ammonium nitrate instead of sodium nitrate; HCO3 – supplemented with 10 mM potassium bicarbonate; 5P – 5 fold increased amount of potassium phosphate; 5S – 5 fold increased amount of magnesium sulphate; Fm – maximal fluorescence; Fv – variable fluorescence. Bars represent standard errors from means (n = 4). Different letters represent significant differences at $P < 0.05$.

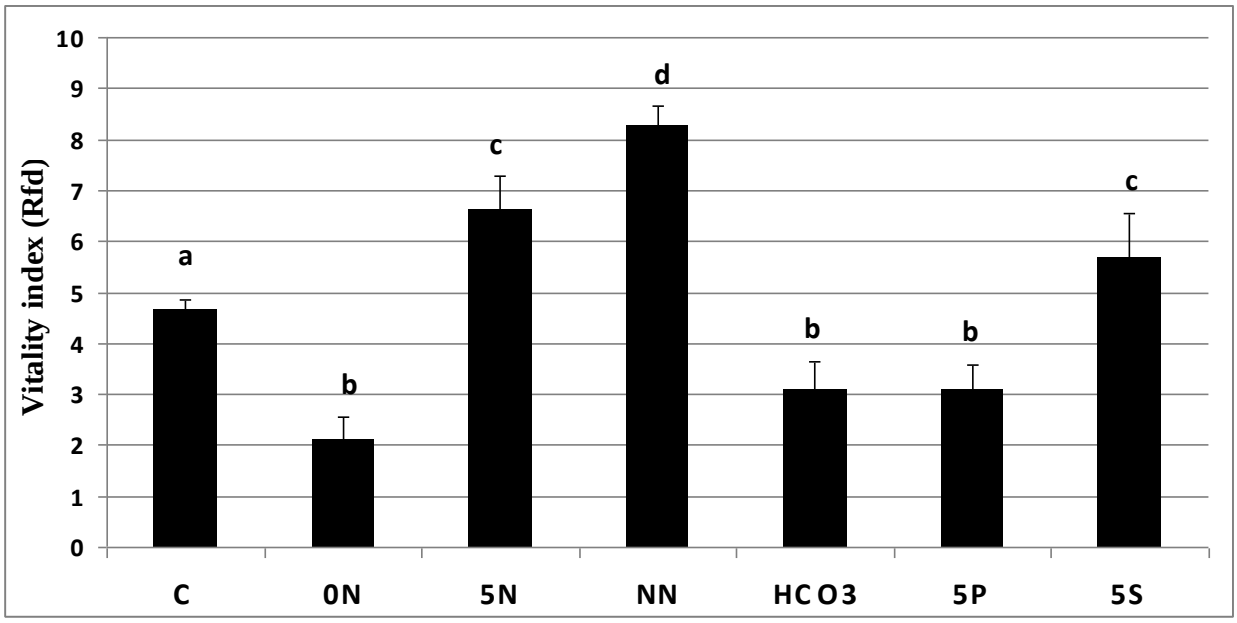

Fig. 6: Vitality index based on induced chlorophyll fluorescence parameters in cultures of *Scenedesmus acuminatus* grown with different nutrient supplies. C – control in Bold's nutrient medium; 0N – without nitrate; 5N – 5 fold increased amount of sodium nitrate; NN – with ammonium nitrate instead of sodium nitrate; HCO3 – supplemented with 10 mM potassium bicarbonate; 5P – 5 fold increased amount of potassium phosphate; 5S – 5 fold increased amount of magnesium sulphate; Rfd – relative fluorescence decrease. Bars represent standard errors from means (n = 4). Different letters represent significant differences at $P < 0.05$.

Conclusions

The local strain AICB136 of the green microalga *Scenedesmus acuminatus* (Lagerheim) Chodat exhibits distinctive developmental and metabolic reactions to changes of inorganic macronutrient content of the culture medium, and it has the potential for being used in biotechnologies for production of dietary supplements and for water management. Addition of 10 mM potassium bicarbonate and increment of the nitrate amount of the nutrient medium from 0.25 g l^{-1} to 1.25 g l^{-1} result in a significant increase of the algal biomass production. Substitution of sodium nitrate with ammonium nitrate leads to an increased protein content of the algal cells. Enrichment of the nutrient media with potassium phosphate and reduction of nitrate content induce the accumulation of carotenoid pigments. Increased nitrate and phosphate concentrations, and the presence of ammonium nitrate do not exert a negative influence on photosynthetic light use efficiency of the alga. Elevation of magnesium sulphate and sodium nitrate content, as well as application of ammonium nitrate instead of sodium nitrate, results in an increased vitality index of the photosynthetic apparatus. The results demonstrate that by changes in the inorganic nutrient content of the growth medium it is possible to optimize biomass production, protein content and carotenoid pigment content of the alga *Scenedesmus acuminatus*, according to the main purpose of its biotechnological application.

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INFLUENŢA UNOR NUTRIENŢI ANORGANICI ASUPRA UNOR PARAMETRI AI PRODUCŢIEI DE BIOMASĂ LA O TULPINĂ LOCALĂ A MICROALGEI *SCENEDESMUS ACUMINATUS*

(Rezumat)

Variabilitatea intraspecifică a algelor este încă puţin studiată şi exploatată în scopuri biotehnologice, deşi populaţiile locale pot prezenta proprietăţi metabolice care le conferă un potenţial bioproductiv crescut în anumite condiţii de dezvoltare. În acest context, având în vedere tendinţele actuale de utilizare a algelor în epurarea apelor poluate, în combinaţie cu folosirea biomasei microalgale ca resursă de supliment alimentar, de furaj şi de biocombustibil, prezentul studiu îşi propune evaluarea efectelor modificării condiţiilor de nutriţie minerală asupra capacităţii fotosintetice, a dinamicii de creştere populaţională, a cantităţii de proteine şi de carotenoizi cu proprietăţi antioxidative din biomasa algală. Modificarea cantităţii şi a naturii sursei anorganice de azot, suplimentarea cu sursă anorganică de carbon, fosfor, sulf şi macroelemente metalice (potasiu şi magneziu) au ca efect o modulare metabolică care rezultă în modificări cantitative şi calitative ale biomasei algale. Pentru experimentele realizate în condiţii axenice de laborator, în culturi unialgale statice realizate în mediul nutritiv Bold, s-a utilizat o tulpină locală a microalgei dulcicole *Scenedesmus acuminatus* (Lagerheim) Chodat, provenită dintr-un mic lac de acumulare de pe pârâul Tur. În condiţii de iluminare şi temperatură constantă, s-a constatat că suplimentarea mediului cu azotat, cu bicarbonat şi cu fosfat duce la o acumulare pronunţată de biomasă uscată într-un interval de 6 zile de cultură, alga fiind predispusă la creştere în mediu acvatic eutrof. În cazul bicarbonatului şi în cel al suplimentării cu azotat, creşterea biomasei nu se datorează unei densităţi celulare mărite a culturilor algale, ci creşterii dimensiunii celulelor prin acumulare de substanţe organice. Suplimentarea mediului cu azotat, precum şi înlocuirea azotatului de sodiu cu azotat de amoniu, duce la o creştere statistic semnificativă a conţinutului de proteine din biomasa algală uscată, ceea ce reprezintă un avantaj în cazul folosirii algelor ca resursă nutritivă în acvacultura şi în creşterea animalelor. O creştere moderată a conţinutului proteic se poate obţine şi prin mărirea concentraţiei sulfatului de magneziu în mediul nutritiv. În schimb, suplimentarea cu bicarbonat ca sursă anorganică de carbon are ca efect scăderea proporţiei proteinelor din biomasa uscată totală. Lipsa sursei externe de azot nu afectează semnificativ producţia de biomasă într-un interval de timp mai scurt de o săptămână. În schimb, deficienţa azotului induce o creştere semnificativă a conţinutului de carotenoizi cu proprietăţi antioxidative, acelaşi efect rezultând şi din suplimentarea culturilor cu fosfat. Abundenţa azotului anorganic în mediul nutritiv reduce cantitatea pigmenţilor carotenoidici din substanţa uscată algală. Înlocuirea azotatului de sodiu cu azotatul de amoniu, precum şi creşterea cantităţii de azotat din mediul nutritiv au un efect benefic asupra vitalităţii în ansamblu a aparatului fotosintetic care asigură conversia fotochimică a energiei fotonice absorbite de clorofile. Carenţa de azot, în paralel cu mărirea conţinutului de pigmenţi carotenoidici fotoprotectivi, reduce randamentul cuantic al proceselor fotochimice, ceea ce se manifestă prin scăderea sub valoarea de 0,7 a raportului Fv/Fm în cursul inducerii fluorescenţei clorofiliene. Rezultatele arată că metabolismul tulpinii studiate a algei *Scenedesmus acuminatus* poate fi influenţat prin modificarea condiţiilor de nutriţie minerală, în funcţie de scopul cultivării pentru o posibilă utilizare biotehnologică a biomasei algale.