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INTERACTIVE EFFECTS OF HYPOXIA, LOW LIGHT STRESS AND DIFFERENT CARBON SOURCES ON PHOTOSYNTHETIC PARAMETERS OF THE GREEN ALGA *SCENEDESMUS INTERMEDIUS* CHOD.

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Abstract: The primary productivity in aquatic ecosystems depends strongly on the prevailing photosynthetic rates of algae, and the interaction of a great variety of environmental factors (such as carbon sources, oxygen, irradiance level) affects finally the biosynthesis of new organic compounds, on which the existence of all biological systems relies. Hypoxia acts synergically with higher levels of inorganic carbon sources in the improvement of the net photosynthetic efficiency of freshwater green microalgae, but the association of hypoxic conditions with low photon flux densities annihilates the positive influence of inorganic and organic carbon sources on the biomass production of *Scenedesmus intermedius*.

Introduction

In aquatic plants photosynthesis is often limited by the combined effects of low levels of irradiance, carbon source concentration and oxygen availability. *Scenedesmus intermedius* Chod. has been described previously as a highly productive freshwater microalga, with a pronounced metabolic plasticity. Its efficiency in converting light energy into chemical energy stored in newly synthesized organic compounds enables a qualitatively and quantitatively superior biomass production [1,9]. The main growth-limiting environmental factors of freshwater plants are the nutritional ones, and among them the carbon sources, the variable CO_2/O_2 ratios, as well as the photosynthetically active photon flux densities, have a central role in the regulation of the bioproductive potential of aquatic ecosystems [17,20,24].

Additive changes in environmental conditions may reduce or adversely change the plants' normal functions, constituting a driving force for transfer of energy or matter out of the organism, and leading to biological strain. Microalgae are usually able to acclimate easily to various environmental changes, to increase their resistance as a result of exposure to prior stress factors, and sometimes the resistance to one stress can be induced by acclimation to another one [3,4,16,22]. Rapid changes in light intensity are likely to occur frequently in the natural habitat of the plant. For microalgae, sources for short-term changes in the incident photon flux density include water circulation and waves, the movement of clouds and changes in the mutual shading [5,7,12]. All these phenomena can result in changes in the light intensity by one or two orders of magnitude within a few seconds. Taking into account that even a few minutes of sudden exposure to light can be sufficient for severe photoinhibitory damage, the importance of short-term acclimation is obvious [2,6,8]. The protective response of the algal cells becomes more complex when more stress factors act simultaneously and enhance or diminish each other's effect [13,18,23]. Oxidative stress caused by photoinhibition has been studied intensely in the last decade, but very few studies have been performed concerning low light stress, which imposes severe energetic limitations to all photoautotrophic organisms [3,25].

The aim of the present investigation is to reveal aspects of photosynthetic acclimation to the simultaneous action of low photon flux density, hypoxia and different carbon sources,

detectable in the cells of a freshwater green alga, which is widespread in polluted water ponds and has major contribution to the energy flow of aquatic ecosystems.

Material and Methods

The experiments were carried out with axenic monoalgal batch cultures of *Scenedesmus intermedius* Chod., grown in the Kuhl-Lorenzen nutrient medium (pH = 6.5) supplemented with 1.5 % (v/v) CO_2, 2 mM $KHCO_3$ or 2 mM glucose, and acclimated to a continuous illumination with 420 μmole photons $m^{-2}s^{-1}$ PAR provided by fluorescent lamps [10]. Hypoxic conditions were created by conducting the sterile air through a thick layer of pyrogallol before reaching the nutrient media [15]. Anoxia was prevented by the illuminated algal cells themselves, because they produce new amounts of oxygen. Low light stress was achieved by continuous illumination of cultures with 40 μmole photons $m^{-2}s^{-1}$. The initial cell density of all cultures was adjusted to 800 cells μl^{-1}, in order to avoid the reciprocal shading. Bubbling of the cultures with sterile air also ensured the homogenous distribution of the algae in the medium.

The photosynthetic pigment content of the algal cells was determined spectrophotometrically after extraction with methanol and acetone, performed at 4°C in dim light, according to Kubin (1991) and to Nagy-Tóth *et al*. (1992) [14,17]. The net oxygen evolution of the cell suspensions was measured polarographically with a Clark-type electrode. The net H_2O_2-scavenging enzyme activity was determined titrimetrically on the base of the uncleaved H_2O_2 after 1 hour of incubation of the algal samples with 10 ml H_2O_2 3% [11]. All experiments were repeated 5 times, significance of the experimental data was evaluated with the Student test.

Results and Discussions

Environmental stress factors often occur simultaneously in anthropically affected aquatic ecosystems, and their interaction determines the efficiency of the algal cells in the use of light energy, as well as their capacity to protect themselves against functional damages.

Hypoxic conditions combined with an increased concentration of inorganic carbon source lead to an increased CO_2/O_2 ratio around the Rubisco molecules, and this result in a reduced rate of photorespiration and in an enhanced growth potential of the algal population [25]. This positive effect can be observed only in higher photon flux densities, when the light intensity does not become the main limiting factor of the photosynthetic biomass production (Fig. 1). The association of hypoxia with low light leads to the decline of algal population. Addition of 2 mM glucose to the algal cultures can support an intensified algal reproduction only for a limited period of time. This can be partly explained by the destruction of more and more thylakoid membranes by the overaccumulated starch grains in the chloroplast.

Interaction of hypoxia, which lowers the pH of the medium due to secretion of acidic compounds by the algal cells [4], with higher amounts of $KHCO_3$ that increase the alkalinity, results in a compensatory equilibration of the proton concentration. Considering that the enzymatic activity of catalase becomes inhibited when the pH value is higher than 8, hypoxic conditions ensure a normal H_2O_2-splitting enzyme activity even in the presence of higher amounts of bicarbonate, which means that cells can protect themselves more efficiently against oxidative damages caused by different other stress factors. Under hypoxic conditions the net H_2O_2-splitting enzyme activity increases significantly probably also because the ethanol produced during fermentation takes part in the sequence of reactions that follows when the oxidation of reduced iron triggers off the generation of superoxide, and finally of hydrogen peroxide [20].

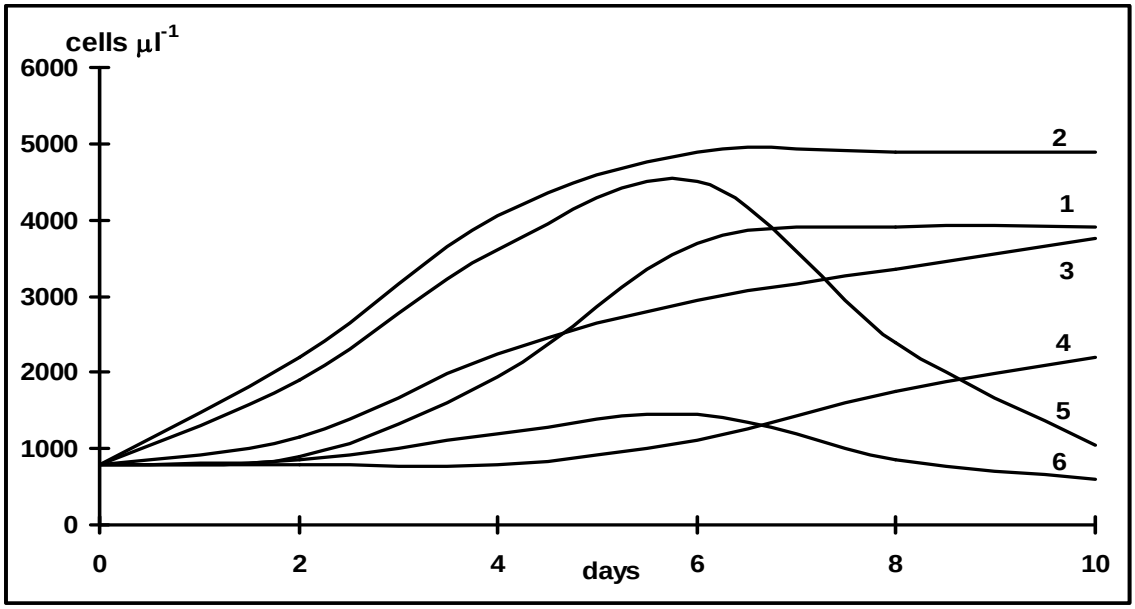

Fig. 1: Influence of hypoxia, low light stress and carbon sources on the dynamics of cell density in populations of *Scenedesmus intermedius* grown in the Kuhl-Lorenzen nutrient medium at 24°C. 1 – control; 2 – hypoxia + 2 mM $KHCO_3$; 3 – hypoxia + 1.5% CO_2; 4 – low light intensity; 5 – 2 mM glucose; 6 – hypoxia + low light intensity. Hypoxia combined with increased inorganic carbon supply favors the development of the algal cultures probably by reducing photorespiration, but the association of hypoxia with low light leads to the decline of the algal population.

It is also noticeable that an increased CO_2/O_2 ratio favors the anabolic processes and lowers the catalase and ascorbic peroxidase activity of the algal cells (Fig. 2.), in parallel with the limitation of oxidative metabolic processes [19].

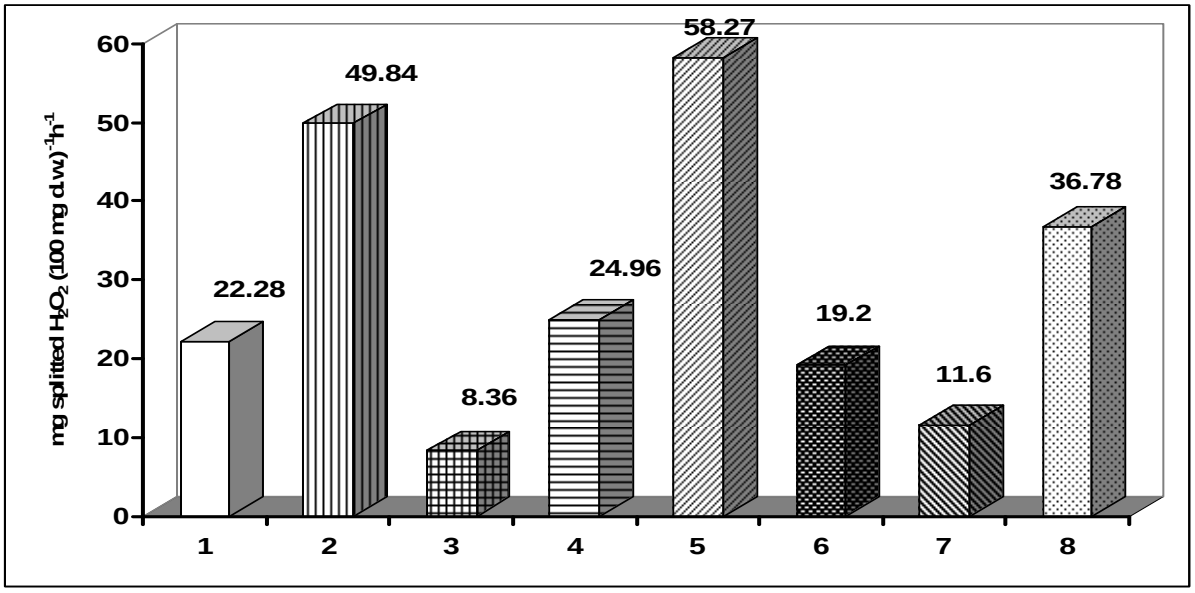

Fig. 2: Net H_2O_2-splitting enzyme activity in axenic cultures of *Scenedesmus intermedius* grown under different stress conditions: 1 – control; 2 – hypoxia; 3 – 2 mM $KHCO_3$; 4 – hypoxia + 2 mM $KHCO_3$; 5 – hypoxia + 2 mM glucose + low light; 6 – 1.5% CO_2; 7 – hypoxia + 1.5% CO_2; 8 – 1.5% CO_2 + low light. In each case $P < 0.0001$. $KHCO_3$ increases the pH of the medium, which leads to the inhibition of catalase. Hypoxia lowers the pH because the cells excrete acidic compounds, so it stimulates the catalase activity. An increased CO_2/O_2 ratio favors anabolic processes and lowers the overall H_2O_2-splitting enzyme activity.

Low light stress and glucose overdose compensate each other's influence on the photosynthetic pigment content of algal cells. When they are combined with hypoxic conditions, the chlorophyll *a*: chlorophyll *b* ratio decreases significantly (from 3.9 to 2.2) due to the overaccumulation of chlorophyll *b*, which is present mainly in the light-harvesting complex of photosystem II (Fig. 3). The carotenoid pigment content of the algal cells decreases in the presence of an insufficient illumination (because their photoprotective, heat-dissipating function is not needed under such conditions of energy deprivation), but it remains more stable than the chlorophyll content under the other nutritional conditions created in the algal cultures.

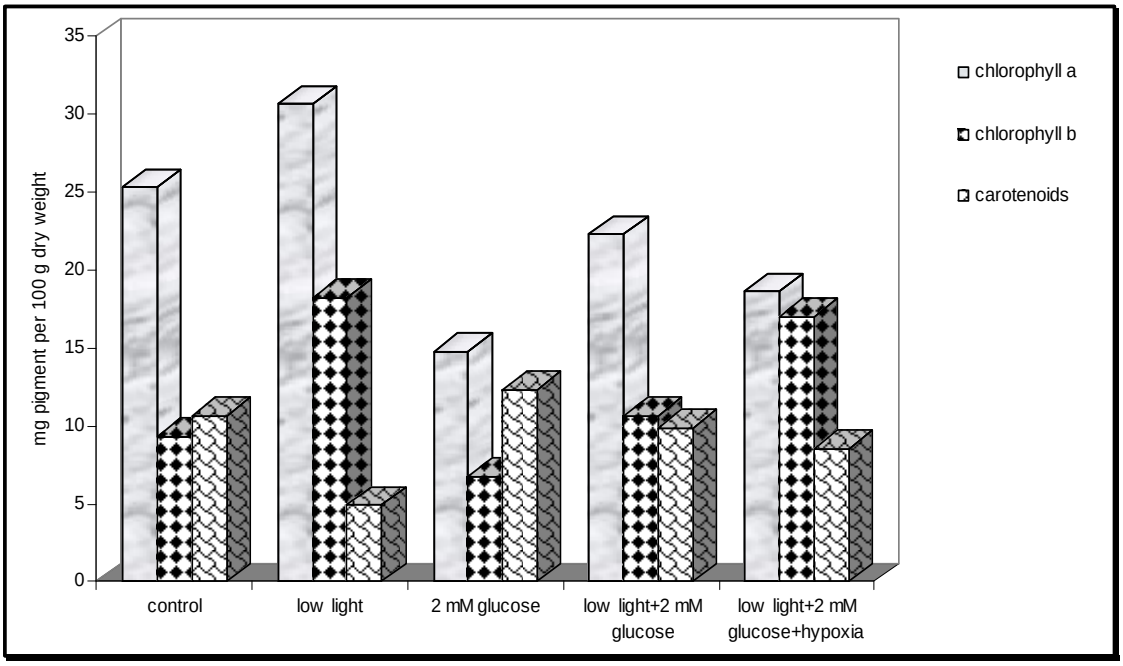

Fig. 3: Photosynthetic pigment content of *Scenedesmus intermedius* under the influence of abiotic stress factors. In each case P < 0.001. Low light stress and glucose overdose compensate each other's influence on the pigment content. When they are combined with hypoxia, the chlorophyll a/b ratio decreases significantly due to the accumulation of chlorophyll b, which is present mainly in the light-harvesting pigment-protein complex of the oxygen-evolving PS II.

The algal cells acclimated to hypoxia and to low photon flux densities are able to maintain a positive balance of net biomass production under the conditions of a low photosynthetic activity because they maintain an even lower level of respiratory metabolism. These algal cells exhibit a decreased rate of oxygen production, but their respiratory oxygen consumption (in complete darkness) is also very low (Fig. 4). It is also noticeable that hypoxia and addition of 2 mM $KHCO_3$ act synergically in the enhancement of net oxygen production, while glucose and low light stress lead to a decreased net oxygen evolution of the algal cells, because they impair the overall carbon assimilation in the chloroplasts. These results reflect that during the acclimation of algae to different unfavorable conditions, an integrated metabolic readjustment takes place, which enables the cells to maintain their overall physiological balance under the modified environmental situations. From the bioenergetic point of view, the main processes that have to be kept in equilibrium with each other, are photosynthetic carbon reduction, photorespiratory carbon dissipation and respiratory energy production from formerly deposited internal sources.

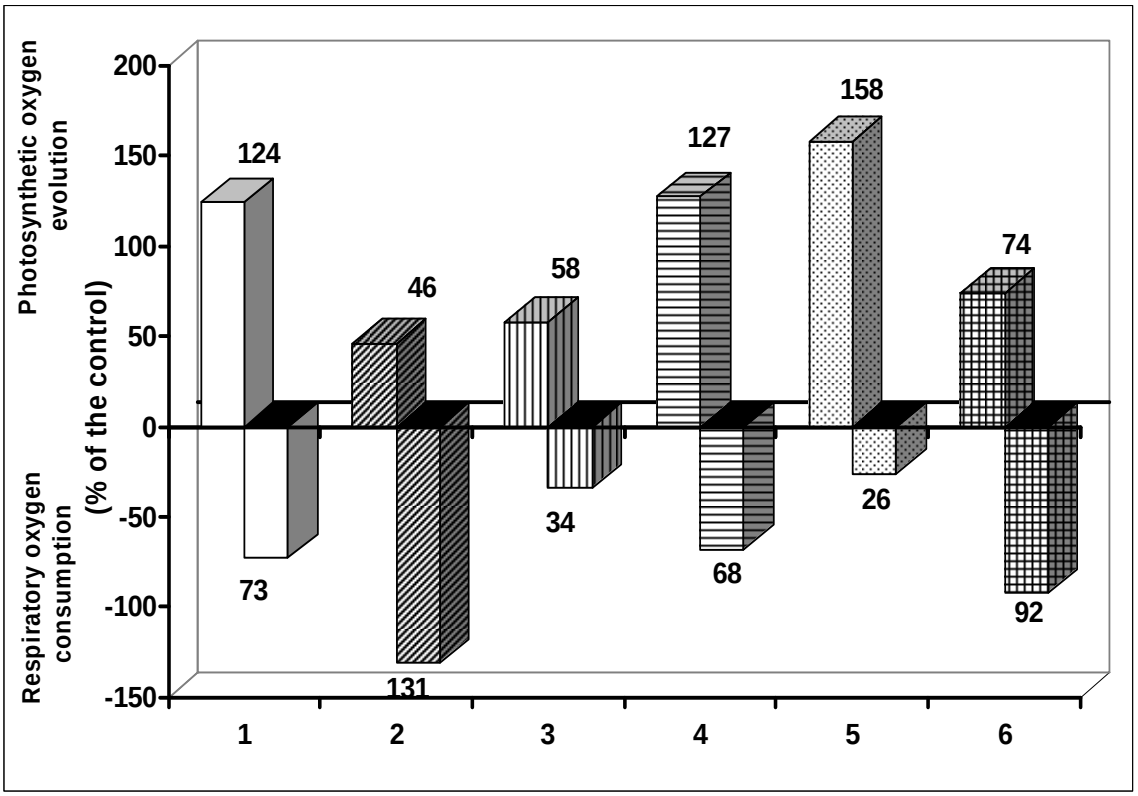

Fig. 4: Net oxygen exchange in % of the control of *Scenedesmus intermedius* in cultures exposed to the combined influence of different abiotic factors. 1 – hypoxia; 2 – 2 mM glucose + low light; 3 – hypoxia + low light; 4 – 2 mM $KHCO_3$; 5 – 2 mM $KHCO_3$ + hypoxia; 6 – 2 mM $KHCO_3$ + low light. Hypoxia and addition of $KHCO_3$ act synergically on the enhancement of net oxygen production. Glucose and low light stress lead to a decreased oxygen evolution.

Conclusions

It can be concluded that hypoxia by itself stimulates the photosynthetic oxygen production both in low and high light, and it lowers the pH of the medium, which has a positive effect in the presence of higher amounts of bicarbonates that create a high pH. Hypoxia and low light prevent the overaccumulation of stromal starch grains caused by additional organic and inorganic carbon sources. The association of hypoxia with low photon flux density also results in a low rate of cell divisions and of biomass accumulation. The overall activity of algal enzymes involved in protection against oxidative stress (ascorbic peroxidase, catalase, superoxide dismutase, glutathione reductase, etc.) is a good indicator of the additive or multiplicative interactions of different environmental factors that may induce a cross-resistance of algal cells in the anthropically affected aquatic ecosystems.

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EFECTE INTERACTIVE ALE HIPOXIEI, STRESULUI DE FLUX FOTONIC SCĂZUT ŞI DIFERITELOR SURSE DE CARBON LA NIVELUL UNOR PARAMETRI FOTOSINTETICI AI ALGEI VERZI *SCENEDESMUS INTERMEDIUS* CHOD.

(Rezumat)

În lucrarea de faţă s-a studiat interacţiunea unor factori ambientali care limitează frecvent producţia primară de biomasă şi fluxul energetic în ecosisteme acvatice aflate în stare naturală sau supuse diferitelor influenţe antropice. În culturi monoalgale axenice s-a combinat influenţa fluxului fotonic scăzut cu carenţa de oxigen şi cu suplimentarea mediului nutritiv cu surse anorganice şi organice de carbon. În prezenţa a 2 mM $KHCO_3$, condiţiile de hipoxie favorizează creşterea populaţiilor algale şi producţia netă de biomasă, deoarece raportul molar dintre carbon şi oxigen creşte considerabil în jurul enzimei Rubisco care poate cataliza atât asimilaţia carbonului cât şi fotorespiraţia. Reducerea intensităţii acesteia din urmă duce la creşterea randamentului fotosintetic net. Acest efect benefic al condiţiilor de hipoxia nu se poate manifesta în condiţiile unei iluminări deficitare, deoarece sursa

insuficientă de energie devine factor limitativ primordial al biosintezei noilor compuşi organici, chiar dacă raportul CO_2/O_2 ar permite o asimilaţie sporită. În acelaşi timp, se modifică şi condiţiile de pH, deoarece $KHCO_3$ măreşte alcalinitatea, iar hipoxia induce acidoză. În aceste condiţii se modifică activitatea enzimatică a catalazei şi a altor enzime implicate în protecţia împotriva stresului oxidativ, ceea ce modifică susceptibilitatea celulelor algale la alţi factori ambientali perturbatori cu acţiune aditivă sau multiplicativă. Pe de altă parte, fluxul fotonic scăzut şi adăugarea glucozei ca sursă organică de carbon îşi compensează reciproc acţiunile exercitate la nivelul modificărilor cantitative ale principalilor pigmenţi fotosintetici. Cantitatea clorofilelor creşte în urma acomodării algelor la lumina insuficientă, respectiv scade când datorită condiţiilor de heterotrofie nu este necesară captarea energiei fotonice. Efectul combinat al iluminării slabe şi al adăugării glucozei în mediul de cultură readuce conţinutul în pigmenţi fotosintetici la un nivel apropiat de cel al culturilor martor. Dacă condiţiile de mai sus se combină cu hipoxia, raportul molar dintre clorofilele de tip a şi b creşte semnificativ, datorită acumulării clorofilelor b din regiunile periferice ale complexelor antenare supradezvoltate. Sinergismul dintre surplusul de carbon anorganic şi hipoxie şi, pe de altă parte, dintre condiţiile de mixo/heterotrofie şi fluxul fotonic scăzut, se reflectă şi în producţia netă de oxigen fotosintetic a culturilor algale.