THE RESISTANCE OF A NEW STRAIN *CHLORELLA* SP. R-06/2, ISOLATED FROM AN EXTREME HABITAT TO ENVIRONMENTAL STRESS FACTORS

G. Gacheva[1] and P. Pilarski[1]*

[1]Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 21, 1113 Sofia, Bulgaria

Summary. The resistance of a new strain – *Chlorella* sp. R-06/2, isolated from a geothermal well (42 °C) in Rupite, to stress caused by temperature, light intensity and concentration of mineral elements in the nutritive medium was studied. The results obtained showed that *Chlorella* sp. R-06/2 was not sensitive to high light intensity and had a high photosynthetic productivity preserved over a broad temperature range 26-39 °C. Furthermore, *Chlorella* sp. R-06/2 did not die when cultivated continuously at 44 °C and survived the stress temperature (51 °C) and light influence (2x8 kLx) up to 4 h. *Chlorella* sp. R-06/2 did not have special requirements concerning the concentration of nutrients and there was no lag phase, i.e. no adaptation was needed. After 20 days starvation, the normal growth of the culture was restored at intensive conditions. The high light, temperature and salt resistance of the studied strain as well as the relative stability of its chemical composition (proteins 39-53 %, carbohydrates 20-26 %, lipids 15-30 %, and pigments1,5-4 %) are of particular interest for further experimental work and the mass outdoor cultivation.

Key words**:** *Chlorella*, microalgae, resistance, stress influence.

***Corresponding author, e-mail: psp@bio.bas.bg

INTRODUCTION

Microalgae are producers of valuable biomass and raw materials for pharmaceuticals, cosmetics and the food industry and are, thus, important subjects of ecological, physiological and biochemical studies. Exploration for and research on new algal strains that are suitable for intense cultivation for biomass in outdoor installations are a traditional area of research in Bulgaria. Strains under consideration for biomass production must be highly productive in managed culture and resistant to variable and extreme environmental conditions.

Many strains which are kept in museum collections have been isolated from the nature. However, only these ones which possess physiological, biochemical and technological characteristics corresponding fully to the available climatic conditions, cultivation equipment and accepted technology should be selected for cultivation. According to many authors (Vladimirova et al. 1962; Ruzicka and Ramon, 1966; Richmond, 1986) the organisms with high optimal growth temperature have higher productivity since they possess wider possibilities for adaption. Nowadays special attention is paid to the isolation and characterization of new algal strains from thermal springs.

The green chlorococcal algae of genus *Chlorella* are very suitable not only for different laboratory studies, but also for mass intensive cultivation. *Chlorella* spp. reproduces mainly by autospores (four to eight in one cell) which is a very important technological advantage. These algae do not have special requirements to the environmental conditions, multiply intensively and are widely distributed.

The aim of this study was to determinate the tolerance range of a new strain *Chlorella* sp. R-06/2 isolated from a thermal spring in Rupite (42 ^{o}C) to temperature stress, light influences and changes in the concentration of mineral elements in the nutrition medium.

MATERIALS AND METHODS

The studies were conducted with the strain *Chlorella* sp. R-06/2, isolated from a geothermal flood (42 °C) in Rupite. The strain is kept in

the collection of Department "Experimental algology" of Institute of Plant Physiology "Acad. M. Popov", Bulgarian Academy of Sciences.

The cultivation of the experimental strain was conducted on an equipment constructed at the Institute of Plant Physiology – 1) a block for intensive cultivation of algae at set temperature and two light intensities 8 and 16 kLx and ventilation with air enriched with 2-3 % of carbon dioxide, 2) a block with temperature gradient (15-50 °C, illumination 8 and 16 kLx and ventilation with air enriched with 2-3 % of carbon dioxide).

For intense cultivation in laboratory conditions the mineral nutritive medium of Setlik (1967), modified by Georgiev at al. (1978) was used.

The concentration of the dried algal biomass was analyzed based on weight mass. The protein content was measured following the method of Lowry (1951). The carbohydrate content was determined following the antrone method of Jaaska (1964). Total lipid content was measured as described by Petkov (1990): 20-30 mg of biomass were extracted 2 times with a mixture of chloroform and methanol (2:1). Pigment content was determined spectrophotometrically after an extraction with boiling methanol. The pigment quantity was calculated using Mackiney coefficients (1941).

The microscope control was conducted with a microscope Amplival – Carl Zeiss – Jenna with magnification 1250X. The biological state of the culture – development phases, degree of synchronization, algological and bacterial pollution etc, was controlled daily.

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

RESULTS AND DISCUSSION

The growth rate and the productivity of *Chlorella* sp. R-06/2 were evaluated for different initial algal cell concentrations resp. different illumination. Cultivation was conducted at a normal concentration of the nutritive medium, temperature of 36 °C, and illumination of 8 Klx. The results obtained are shown in Fig. 1. It was found that the growth rate [μ] was highest for low initial algal concentrations using maximal illumination of the algal cells. Growth rate was lower with higher initial concentrations,

and the photosynthetic productivity of the cells was also lower. The absolute quantity of the synthesized biomass (the productivity) remained relatively constant and high at the investigated densities from 1.20 g/l at lowest initial density to 1.45 g/l at the highest density.

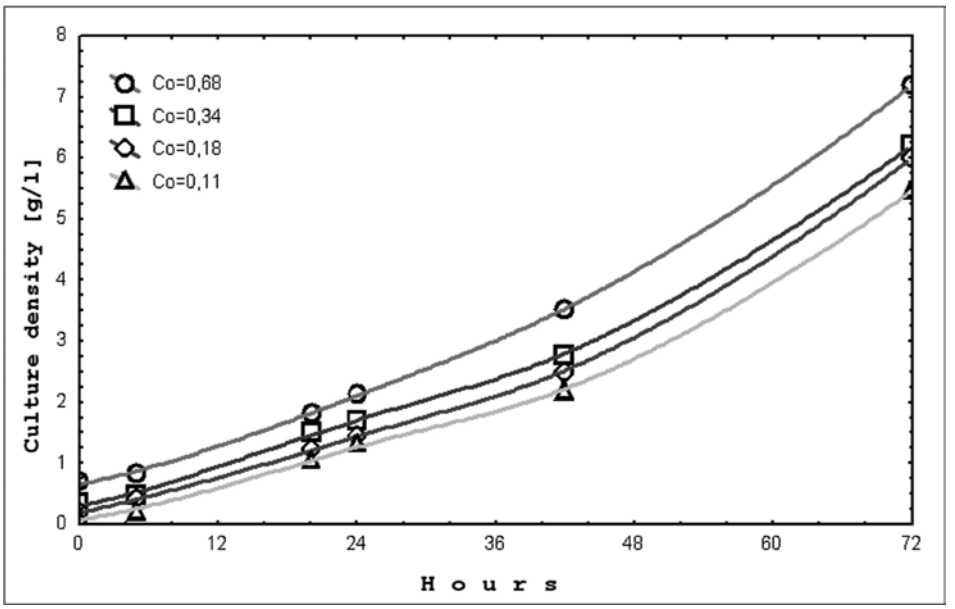

Fig. 1. Growth curves of algal culture depending on the start density of the culture.

The characterization of the temperature range of *Chlorella* sp. R-06/2 growth was conducted on a block with temperature gradient (26-44 °C) at two light intensities – 8 kLx и 16 kLx. The results obtained are shown in Fig. 2. The cultures became lighter in color and showed minimal growth at 44 °C. The cells retained their vitality and did not die at both studied light intensities – 8 kLx и 16 kLx. At low light intensity in the temperature range 26-39 °C, the difference in the productivity of *Chlorella* sp. R-06/2 was 14 % and at high light intensity – 18 %; the strain maintained high productivity in a wide temperature range.

Intense stress, such as overheating and/or overillumination of algal

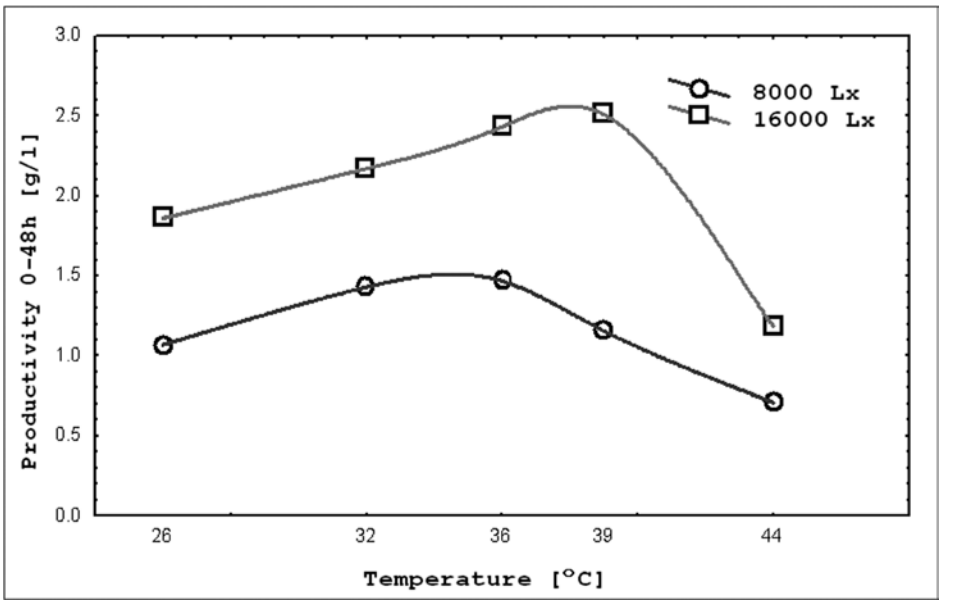

Fig. 2. Productivity [ΔC] of Chlorella sp. R-06/2 for 48 h at variable temperatures and light intensities.

cultivars in outdoor production units are likely and expected. We have attempted to study such potential events in the laboratory. Algae were initially cultivated at 8 kLx light intensity and 36 °C. Twenty-four hours later, the cultivars were transferred to the temperature gradient block and held at 51 °C for 4 h at two light intensities – 8 and 16 kLx. The cultivars were then returned to conditions of 36 °C and 8 kLx light intensity. The results are shown in Fig. 3.

A decrease in growth rate was observed under the combined influence of high temperature and high light intensity. However, the culture did not die and did not stop growing.

To determine the optimal concentration of the background nutritive medium for algal growth as well as the concentrations at which stress conditions occur for *Chlorella* sp. R-06/2, zero, ¼, ½, 1 and 2x doses of the mineral elements in regime of increasing density were tested. Cultivation was conducted under conditions of 36 °C and 8 kLx illumination (Fig. 4, Fig. 5, Fig. 6).

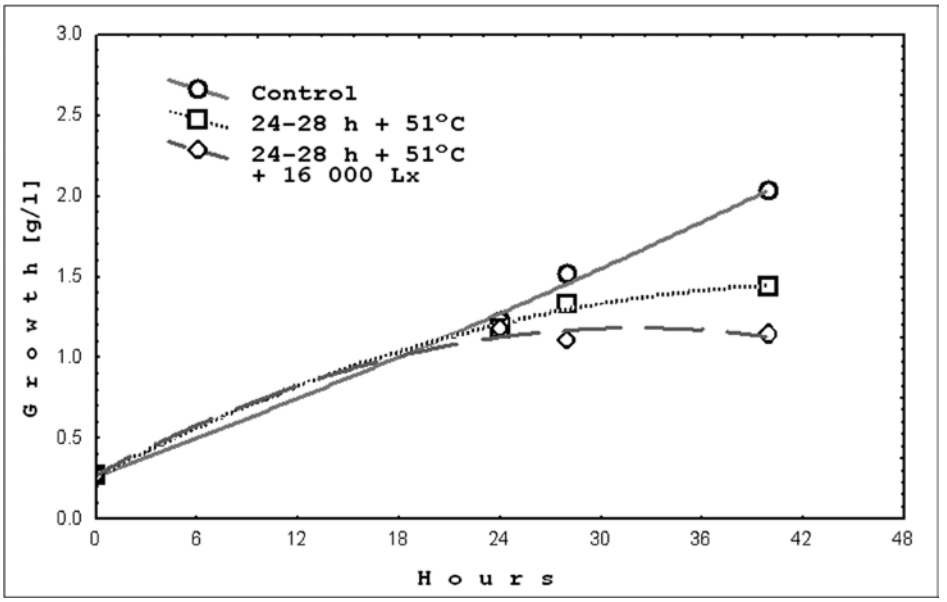

Fig. 3. Growth of *Chlorella* sp. R-06/2 under temperature stress and variable illumination regimes.

At 48h and zero salt concentration in the nutritive medium, growth was limited, but after adding mineral elements corresponding to the full dose of the nutritive medium at the 480th h the normal culture growth recovered completely.

A decrease in the growth rate within the first days was not observed when ¼, ½, full and 2x dose of the nutritive medium was used. In addition, no adaptation of the algal culture to the respective concentration was needed or it occured very quickly in the first hours after the inoculation.

At ¼ and double concentration the linear growth was retained up to the 72th h, at ½ concentration to the 144th h and at full dosage up to the 240th h. Moreover, the maximal density reached was different: 5,50 g/l at the 144th h, 9, 32 g/l and 11, 92 g/l at the 408th h and 10,57 g/l at the 384th h. At a double concentration of the medium a growth delay was registered from the 144th to the 216th h followed by a new linear growth till the end of the experiment (480th h).

The persistence and solid growth of the strain at 2x concentration of

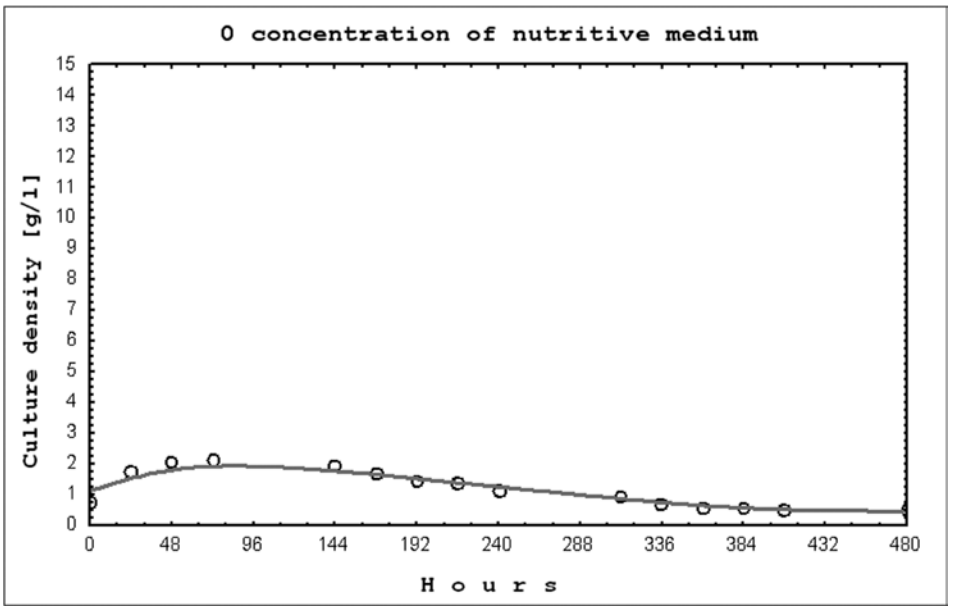

Fig. 4. Growth of the algal culture at 0 concentration of the nutritive medium.

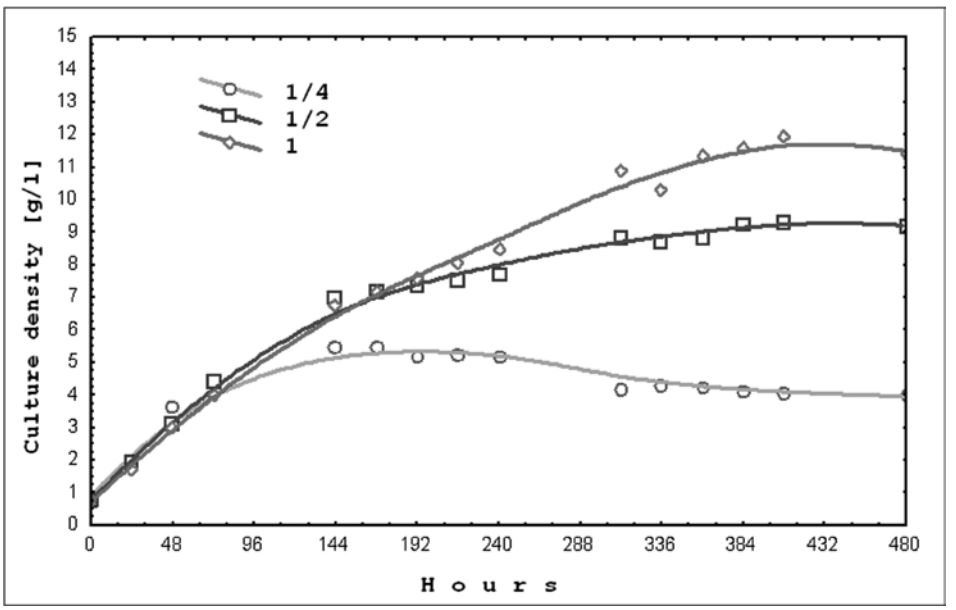

Fig. 5. Growth of the algal culture at ¼,½ and 1 x concentration of the nutritive medium.

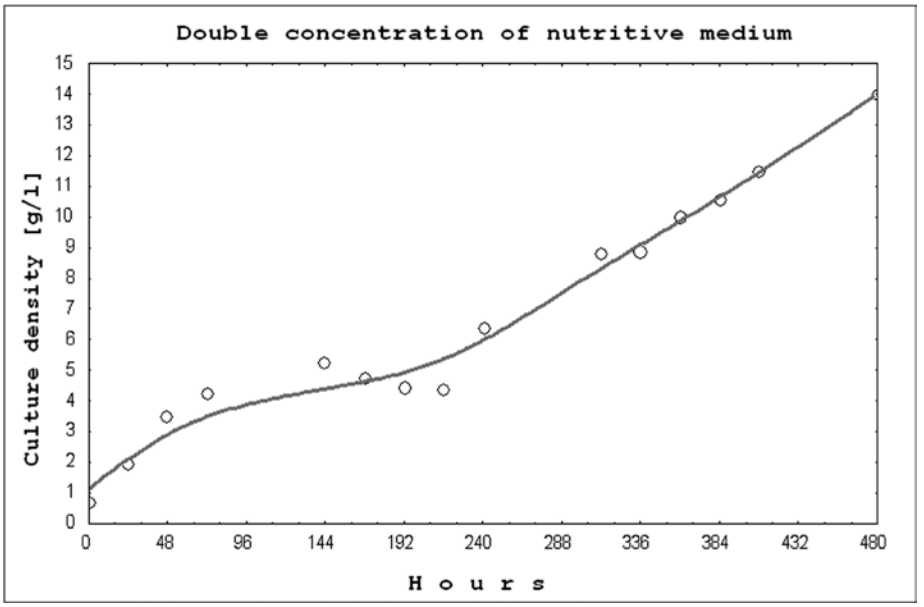

Fig. 6. Growth of the algal culture at 2x concentration of the nutritive medium.

mineral elements in the nutritive medium are of interest for outdoor mass cultivation, especially when using technology for thin layer cultivation (5-10 mm) and increased density (8-12 g/l) of the algal suspension (Livansky, Pilarski, 1993; Livansky et al., 1993, 1995).

The microalgae possess exceptional metabolism plasticity. The cultivation parameters depending on natural and climatic conditions vary in wide limits during mass cultivation and the qualitative content of the biomass is changed. This causes difficulties when a standard product is produced. For that reason, studies of the effects of temperature and light, the two most important factors for algal growth, on the biomass qualitative content (proteins, carbohydrates, lipids and pigments) of *Chlorella* sp. R-06/2 were conducted.

Protein content ranged between 39 – 53 % and increased with the rise of the temperature and illumination intensity (Fig. 7). The average content at 8 kLx was 44 % and at 16 kLx was 10 % higher – 49 %.

The carbohydrate content varied from 20 % to 26 % of dried algal biomass (23 % difference) (Fig. 8). It was higher at lower temperatures

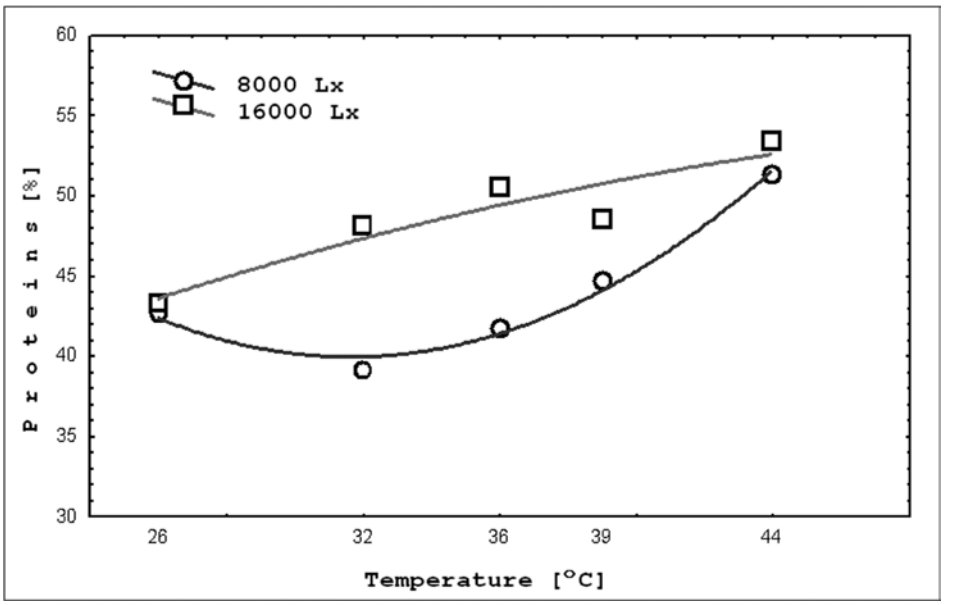

Fig. 7. Protein content (% of dried algal biomass) in *Chlorella* sp. R-06/2 depending on the temperature and illumination conditions.

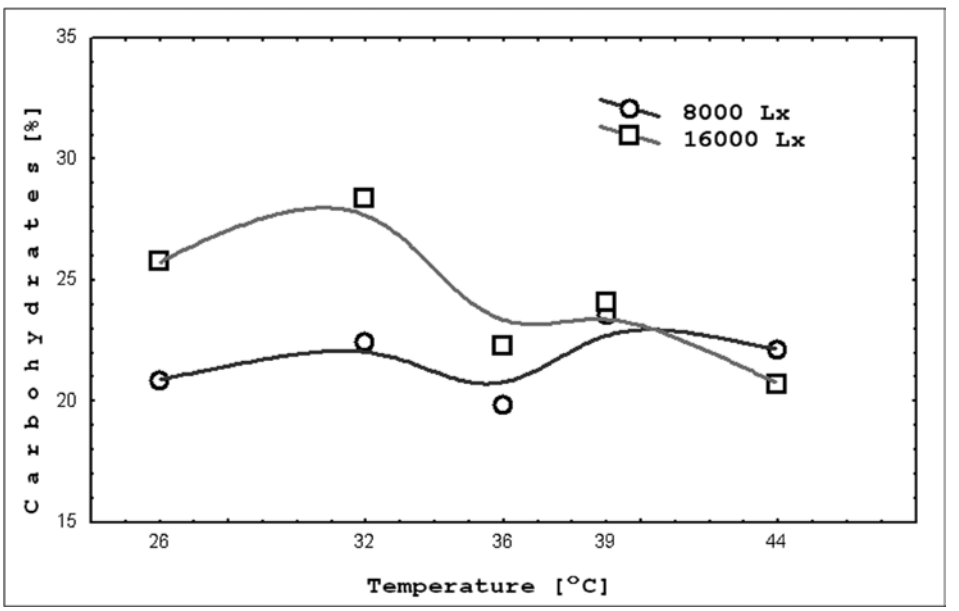

Fig. 8. Carbohydrate content (% of dried algal biomass) in *Chlorella* sp. R-06/2 depending on the temperature and illumination conditions.

and higher light intensity. When the temperature was optimal or higher no considerable differences and dependence on light were observed.

Lipids were the only metabolites that showed large variation depending on the light and temperature (Fig. 9). The lipid content varied from 15 to 29 % of dried algal biomass. The mean lipid content in the biomass was 22 % at 8 kLx and with 23 % higher in comparison with 16 kLx – 17 %.

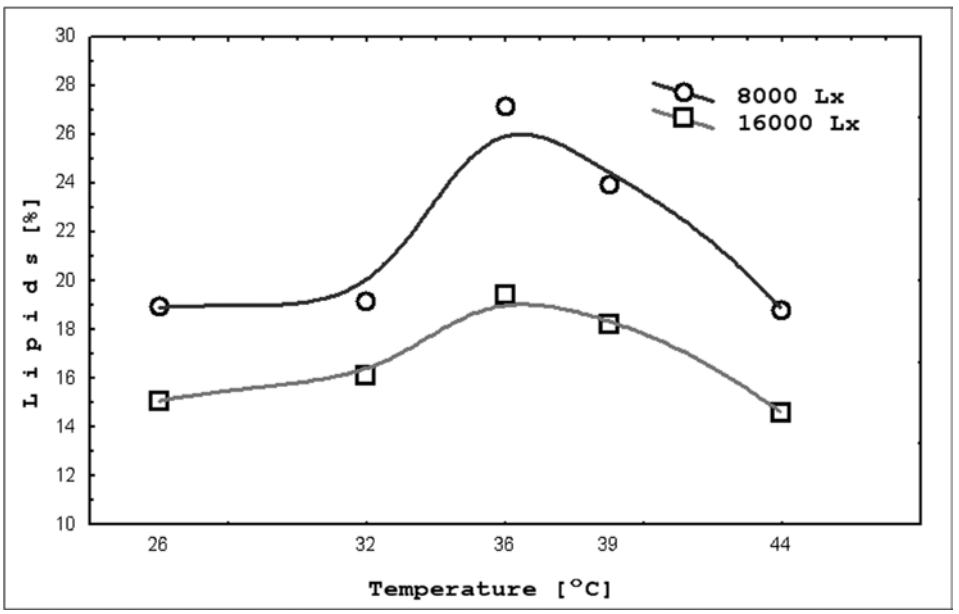

Fig. 9. Lipid content (% of dried algal biomass) in *Chlorella* sp. R-06/2 depending on the temperature and illumination conditions.

Generally, we registered an increase of lipid content at both light intensities used. The same tendency was observed for the pigment content (Fig. 10).

It is normal that the pigment quantity decreased with the increase of the light intensity and conversely increased with decreasing the light intensity. At high extreme temperatures and light intensities the pigment amount decreases because of the destructive changes (Richmond, 2004).

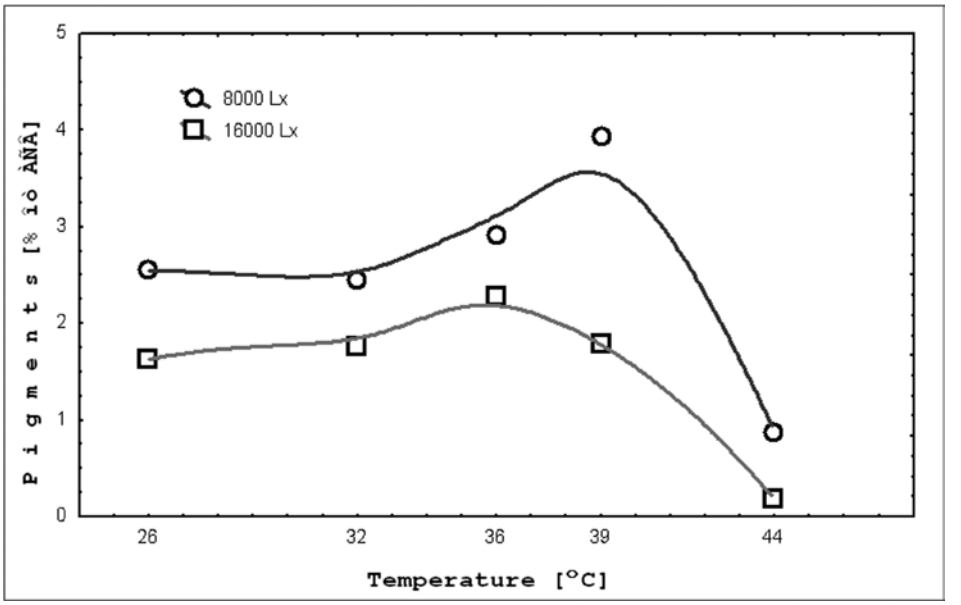

Fig. 10. Pigment content (% of dried algal on the biomass) in *Chlorella* sp. R-06/2 depending on the temperature and illumination conditions.

Conclusions

- A new, highly temperature strain of genus *Chlorella* - *Chlorella* sp. R-06/2 was isolated.
- *Chlorella* sp. R-06/2 was not sensitive to the high light intensities and had high photosynthetic productivity.
- *Chlorella* sp. R-06/2 preserved high productivity in a broad temperature range – from 26 to 39 °C and did not die when cultivated continually at 44 °C. It persisted 4 h at the stress temperature (51 °C) and light (16 kLx).
- The *Chlorella* sp. R-06/2 culture and its normal growth were fully restored after 20 days of starvation and removal of the stress influence. The fast restoration of the culture growth after elimination of the stress influence represents valuable biotechnological quality of the strain.
- The qualitative content of the biomass of *Chlorella* sp. R-06/2

(proteins – 39-53 %, carbohydrates – 20-26 %, lipids- 15-30 % and pigments – 1.5-4.0 %) is in the requirement limits for outdoor cultivation.

- The complex evaluation of the qualitative characteristic of the isolated strain (rate and temperature range of growth, productivity, qualitative content of biomass) is a reason to recommend *Chlorella* sp. R-06/2 as a particularly perspective strain for mass cultivation and further investigations.

Acknowledgements: The authors gratefully acknowledge the support by the project "Progress in plant investigations for improvement of the sustainability of agriculture" (PISA – INI-14/ 05.09.2005).

References

Georgiev, D., H. Dilov, S. Avramova, 1978. Millieu nutritif tamponne et méthode de culture intensive des microalgues vertes. Hydrobiology (Bulgaria) 7, 14-23 (In Bulgarian).

Jaaska, R., 1964. Influence of the nitrogen starvation regime on the chemical compostiton of some strains of green algae. Izv. AN Est. SSR. 13, 33-39 (In Russian)

Livansky, K., P.S. Pilarski, 1993. Carbon dioxide supply to algal cultures. II. Efficiency of CO_2 absorption from a natural gas supplied to the recirculation pipe of a cultivation unit. Archiv fur Hydrobiologie, 97 (Algological studies 69), 113-123.

Livansky, K., M. Kajan, P. Pilarski, 1993. pCO_2 and pO_2 profiles along the flow of algal suspension in open solar culture units: Verification of a matematical model. Archiv für Hydrobiologie, 98 (Algological studies 70), 97-119.

Livansky, K., M. Kajan, P. Pilarski, 1995. Productivity, respiration and chemical composition of the green algae *Scenedesmus incrassatulus* grown in outdoor cultivation units with and without baffles. Archiv fur Hydrobiologie 106 (Algological studies 76), 111-128.

Lowry, O., N. Rosenbrough, A. Z. Farr, R. J. Randball, 1951. Protein

measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265-275.

Mackinney, G., 1941. Criteria for purity of chlorophyll preparations. J. Biol. Chem., 132, 91-96.

Petkov, G., 1990. Lipids of photoautotrophically cultivated microalgae. Ph. D. thesis, IPP, BAS, 119 pp. (In Bulgarian).

Richmond, A., 1986. Cell response to environmental factors. In: Handbook of microalgal mass culture, ed. A. Richmond. CRC Press, Inc., Boca Raton, Florida, 69-101.

Richmond, A., 2004. Biological principles of mass cultivation. In: Handbook of microalgal culture, biotechnology and applied phycology, ed. A. Richmond. Blackwell Science Ltd., 125-177.

Ruzicka, J., J. Ramon, 1966. Dependence of the production of dry weight on temperature in three strains of the genus Scenedesmus. In: Mater. IV Koord. Sobr. naucn. Symp. SEV, Inst. Zootechn., Krakow, 208, 202-216.

Setlik, I., 1967. Contamination of algal cultures by heterotrophic microorganisms and its prevention. Ann. Rep. Algol. for the Year 1966, Trebon, CSAV, Inst. Microbiol., 89-100.

Vladimirova, M. G., V. E. Semenenko, 1962. Intensive culture of unicellular algae. M., Academy of Science USSR, 44-46 (In Russian).