Physiological Responses to Oxidative Stress of Different *Chlorella vulgaris* Isolates

Dilyana Doneva[1], Juliana Ivanova[1], Lyudmila Kabaivanova[2*]

[1]*Institute of Plant Physiology and Genetics*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
E-mails: did4e_7788@mail.bg, juivanova@yahoo.com

[2]*The Stephan Angeloff Institute of Microbiology*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 26, 1113 Sofia, Bulgaria
E-mail: lkabaivanova@yahoo.com

[*]*Corresponding author*

Received: June 6, 2018 **Accepted: October 10, 2018**

Published: December 31, 2019

Abstract: *Oxidizing agents, notably hydrogen peroxide (H_2O_2), are increasingly used in a number of applications. Their manner of action on algae was investigated in this work. Different concentrations of H_2O_2 and silver ions were applied to the algal cultures to follow the effect of their action in antarctic and mesophilic isolates of the green alga Chlorella vulgaris. Oxidative stress-induced physiological responses manifested by changes in the photosynthetic apparatus and antioxidant enzymes were estimated. The algae from the antarctic region appeared to be less resistant to oxidative stress than their mesophilic counterparts, concerning growth after treatment with higher concentrations of the reagent. The increased concentration (0.05%) lead to raise of activity and isoenzyme number of catalase (CAT) in both C. vulgaris strains. The isoenzyme activity of peroxidase (POX) decreased and disappeared in the antarctic isolate. At the same time the highest concentration (0.08%) provoked restoration of POX activity in the mesophylic one. Decrease in superoxide dismutase (SOD) activity was registered and this effect was more intensive in the moderate and fast mobile isoenzymes. Isoenzyme profile at 0.08% reagent concentration for the mesophylic isolate was similar with this at 0.05% for the antarctic isolate and a fourth isoenzyme appeared.*

Keywords: *Chlorella vulgaris, Antarctic, Oxidative stress, Photosynthesis, Enzyme profile.*

Introduction

The presence of algae and fungi in water reservoirs causes economic problems because they may plug water pipes, tanks, cooling towers. To destroy them, it is necessary to find a disinfectant that is harmless for the environment. Such a substance with active ingredients may contain H_2O_2 (50%) and 0.05% silver. The active substance used is hydrogen peroxide, an environment friendly substance. The traces of silver remaining on the treated surfaces are nontoxic. It is well known that silver has a wide range of antibacterial effects, and in a combination with H_2O_2 imposes its effect on different microorganisms. There are few studies about its antiseptic effects [15, 17]. Hydrogen peroxide is extensively used as a biocide, particularly in applications where its decomposition into non-toxic by-products is important. Although increasing information on the biocidal efficacy of hydrogen peroxide is available, there is still little understanding of its biocidal mechanisms of action. The toxicity of biocides can be associated with the formation of Reactive Oxygen Species (ROS) and subsequent oxidative damage, interfering with the normal function of photosynthetic organisms [2]. The oxygen split off by the hydrogen peroxide attacks the cell walls of the microorganisms

upon direct contact [18]. The chemical reaction of the oxygen with molecules in the cell walls destroys them. This effect is boosted by silver ions that bind to the disulfide bonds of certain proteins, both of the reproduction complex as well as of the metabolic system of the microorganisms, and deactivate or precipitate them.

Oxidative stress is a natural cellular phenomenon in which an organism is subject to a change in the balance between oxidants and antioxidants in favor of the oxidants [10]. These oxidants are generally termed reactive O_2 species, and include superoxide anion radical (O_2^-), H_2O_2, and the hydroxyl radical (–OH), amongst others [6]. ROS can be extremely detrimental to cellular viability as they are damaging to DNA, proteins, lipids, and cell membranes [24]. Nevertheless, ROS generation is an unavoidable consequence of aerobic life.

In the study of algae exposed to stress situations, a group of authors summarized that major property of all formed ROS types was to induce oxidative damage to proteins, lipids, and photosynthetic structures [4, 13, 21]. At the same time, other researchers shared the opinion that the traditionally considered toxic ROS were also signaling molecules by which plant organisms regulated the course of various physiological processes by inducing protective mechanisms [9, 19, 25].

The aim of our study was to follow the physiological and biochemical changes in algal cultures after treatment with different concentrations of the mixrure of H_2O_2 and silver as well as its oxidative stress effects by estimating the activity and isoenzyme profiles of antioxidant enzymes in *Chlorella vulgaris*.

Materials and methods

Strains and cultivation conditions

Objects of the present study are isolates of the unicellular green algae *C. vulgaris* Beijerinck (Chlorophyta). The green alga is obtained from the Collection of Autotrophic Organisms (CCALA) at the Trebon Institute of Botany, Czech Republic. The antarctic strain was isolated from moss samples, collected on Livingston Island, South Archipelago. The experiments were conducted with axenic cultures of these algal strains stored in the collection of the Department of Experimental Algology of the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences.

Green algal lines were intensively cultivated in laboratory cultures before being exposed to the stress effects. For the *C. vulgaris* isolate, the nutrient medium of Setlik Simer, modified by Georgiev et al. [11] was used. *C. vulgaris* is cultivated at 26 °C. The growth and development of algae was carried out in 200 ml glass vessels under continuous illumination with luminescent lamps with intensity of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and an aeration of 100 $l \cdot m^{-3} \cdot h^{-1}$. Air was enriched with 2% CO_2.

The agent used represents a solution with two basic components – H_2O_2, as an oxidizing factor, and silver with its biocidic effect. It is expected to find a wide application in the waste water treatment, preventing the development of algae in pools, which is a current problem and to favor the reduction of unwanted sludge through biocatalytic effect, accelerating the oxidation process. An advantage is its rapid biodegradability, making it environmentally friendly. Hydrogen peroxide decomposes into water and oxygen. Oxygen destroys biofilms and thus allows silver ions to destroy unwanted microorganisms underneath them.

Algal suspensions were treated with different concentrations of this agent (0.03%, 0.05%, 0.08%).

Algal growth

Growth of algae was measured gravimetrically as absolute dry weight. 10 ml aliquots of the algal suspension were placed in centrifuge tubes and centrifuged after 10 minutes of treatment with 1 ml of 11.5% CH_3COOH. After the supernatant was discarded, the precipitated cells were dried in a desiccator at 105 °C for 20 hours. The absolute dry weight (DW) was measured.

Functional activity of the photosynthetic apparatus

The changes in photosynthetic activity were determined by measuring the chlorophyll fluorescence kinetics using a modulated pulsed amplitude fluorimetry (PAM 101-103, H. Walz, Germany) by the method of Krause et al. [16]. Induced kinetics was recorded and analyzed using the FIP 4.3 program [26]. Stress-induced changes in photosystem II and its characteristic oxygen release were performed by measuring chlorophyll fluorescence and oxygen release. Pulse-modulated (PAM) chlorophyll fluorescence was recorded on a PAM fluorometer (model PAM 101-103, H. Walz, Effeltrich, Germany). Cells adapt in dark 20 minutes before the measurement. The F_0 level is measured at a frequency of 1.6 kHz and a light intensity of 0.060 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD. The maximum fluorescence level (*Fm*) was recorded at saturation impulses at an intensity of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD with a duration of 0.8 s.

Oxygen release by lightning and continuous lighting of cells of *C. vulgaris* was determined using the polarographic electrode described by Zeinalov [30]. Measurements were performed as described by Apostolova et al. [3], Ivanova et al. [14] and Dobrikova et al. [8]. During measurements, cells with an optical density (OD) of 1.7 (at 760 nm) form a 2 mm layer on the electrode. The samples were pre-illuminated with 25 flashes and then adapted in the dark for 5 minutes before the measurements. Oxygen lightning is induced by continuous (4 J) and short ($t_{1/2}$ = 10 μs) periodic flashes with 650 ms of dark intervals between the individual flashes. The initial oxygen burst was recorded after illumination with continuous white light (450 $\mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$). The following parameters are used to assess oxygen release: Y_4 – amplitude of oxygen release after the fourth flash; *A* – amplitude of initial oxygen release under continuous illumination.

Native electrophoresis in polyacrylamide gel

Native polyacrylamide gel electrophoresis (PAGE) was performed according to Davis method [7] on 7.5% gels-plates with dimensions 10/10 cm. Electrophoresis was performed at a current of 2 mA on a sample applied for about 90 minutes. Gels were sampled with equal amounts according to the quantity of total protein.

Visualization of isozyme profiles after native PAGE

The anode peroxidase isoenzymes (POX) were expressed by the Ornstein method [20] at room temperature by incubating the gels for 10 minutes in an incubation medium containing 0.5 mM benzidine hydrochloride (as H-donor) and 4 mM H_2O_2 dissolved in 0.05 M acetate buffer. Peroxidase isoenzymes appear as brown streaks on a light background. The superoxide dismutase isoenzymes (SOD) were expressed on the gels by the method of Greneche et al. [12]. The plates were incubated for 30 minutes in the dark, at a shaker, at room temperature in 100 ml of Tris-HCl buffer, pH 8.2, containing 10 mg of NBT, 75 mg of Na2-EDTA and 3 mg of riboflavin. The gels were then placed for 5 min on a fluorescent lamp

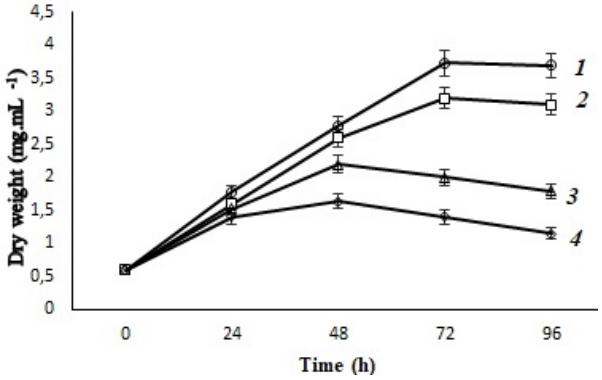
PIL1 LF18W/54 1000lm, Poland. Superoxide dismutase isozymes appeared as colorless strips on a dark blue background. The catalase isoenzymes were stained by the method of Woodbury et al. [29]. The plates were incubated for 20 minutes in the dark at room temperature in 10 mM H_2O_2 and then in a 1% $K_3Fe(CN)_6$ solution and $FeCl_3$ for another 10 min on a shaker. Catalase isozymes appeared as colorless strips on a dark green background. Quantitative differences in isoenzymes between treatments for the POX, CAT and SOD enzymes were measured based on the area and intensity of plaque isoenzyme staining by the "Image J" program after scanning.

Results and discussion

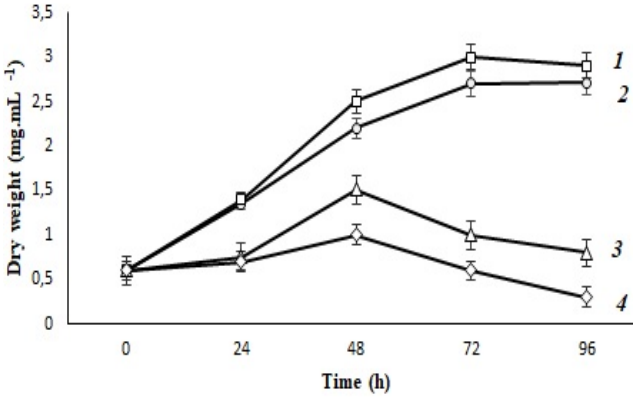
Following the aim of the present study the physiological and biochemical changes in the green microalga *C. vulgaris* were followed and the differences between the antarctic and mesophilic isolates after application of stress factors were estimated.

Production of biomass

The results showed regular inhibition of growth of mesophylic *C. vulgaris* isolates, which increased with increasing the concentration of the solution of H_2O_2 and silver. At the lowest concentration tested, growth inhibition was minimal. As the concentration of the agent increased, there was a significant inhibition of biomass accumulation. However, for the antarctic isolate, slight growth stimulation was observed after treatment with the lowest concentration of the preparation (0.03%), whereas at a concentration of 0.08%, growth was significantly suppressed (Fig. 1A, B).



A) Mesophilic: 1 – control; 2 – 0.03% oxidizing agent;
3 – 0.05% oxidizing agent; 4 – 0.08% oxidizing agent.



B) Antarctic, 1 – 0.03% oxidizing agent; 2 – control;
3 – 0.05% oxidizing agent; 4 – 0.08% oxidizing agent.

Fig. 1 Influence of different oxidizing agent concentrations on growth of *C. vulgaris*

Influence of the algicide agent on the functional activity of the photosynthetic apparatus

The effect of the preparation on photosynthetic oxygen release and primary photochemistry of the mesophilic and antarctic isolates of *C. vulgaris* were investigated. Green algae oxygen release curves were analyzed at different concentrations of the H_2O_2 and silver solution and indicated inhibition of photosynthetic oxygen release in both cases – continuous illumination and flashes. The degree of inhibition was dependent on the concentration and the characteristics of the particular isolate. Inhibition of oxygen release was associated with a decrease in the number of functionally active centers of PSII (parameter A, Table 1).

Table 1. Influence of stress agent on oxygen release at continuous illumination (parameter A) of *C. vulgaris*. Data were presented as % of untreated cells.

Agent concentration, %	**Oxygen release, % *C. vulgaris***	
	Mesophilic	**Antarctic**
0.03	68	83
0.05	41	56
0.08	37	38
Control	100	100

Oxygen release in flashes was more strongly influenced compared to oxygen release in continuous illumination. After treatment oxygen-releasing centers involved in both mechanisms (non-cooperative and cooperative mechanisms), i.e., fast and slow PSII centers were affected. It was also established that the degree of inhibition of oxygen release in flashes exposure in the mesophilic *C. vulgaris* was greater at concentration of 0.08% than in the antarctic line (Table 2). This result was in correlation with the estimated growth at the same concentration.

Table 2. Influence of the agent on oxygen release at exposure to flashes (parameter Y_4) of *C. vulgaris*. Data were presented as % of untreated cells.

Agent concentration, %	**Oxygen release, % *C. vulgaris***	
	Mesophilic	**Antarctic**
0.03	76	89
0.05	31	34
0.08	28	28
Control	100	100

The influence of biocide on primary photochemistry of PSII in mesophilic and antarctic strains of *C. vulgaris* was investigated. For this purpose, the fluorescence ratio *Fv/Fm* was determined (Table 3). Changes in this respect and in both investigated forms were smaller than the effect on oxygen release, lead us to the assumption that the changes were greater in the oxygen-releasing system.

Table 3. Influence of biocide agent on the fluorescence ratio *Fv/Fm* (maximum quantum yield) of *C. vulgaris*. Data were presented as % of untreated cells.

Agent concentration, %	**Fluorescence ratio, *Fv/Fm* *C. vulgaris***	
	Mesophilic	**Antarctic**
0.03	98	97
0.05	85	82
0.08	76	75
Control	100	100

Isoenzyme profiles

Changes in the isoenzyme profiles of the antioxidant enzymes SOD, CAT and POX in the green alga *C. vulgaris* were registered under the influence of oxidative stress.

Superoxide dismutase activity

The hydrogen peroxide is known to produce a burst of reactive ROS, i.e. a state of oxidative stress in plants (algae) by a mechanism of redox cycling and generates a bulk of them. Aerobic organisms had developed complex antioxidant defense systems to combat the deleterious effects of oxidative stress [22]. The antioxidant systems include enzymes eliminating ROS such as SOD, CAT and POX. Our results revealed that 4 isoenzymes appeared in the profile of SOD isolated from *C. vulgaris*, depending on the concentration of the solution causing oxidative stress. Under the action of 0.05% and 0.08%, isoenzyme 1 (IE1) activity in the mesophilic *C. vulgaris* decreased by 24% and 39%, respectively. Isoenzyme 2 (IE2), at 0.05% was decreased by 47% relative to the control, and at 0.08% (H_2O_2 and silver solution) – by 33%. Isoenzyme 3 (IE3), at 0.05% solution decreased its activity by 23%. At 0.08%, the activity of isoenzyme 3 decreased by 44% but was accompanied by the appearance of a new isoenzyme 4 (IE4) (Fig. 2A and Table 4).

After treatment of the cells of antarctic *C. vulgaris* with 0.03% solution, IE3 increased its activity by 50%. Upon addition of 0.5% of the agent, SOD reacted with a decrease in the activity of all isoenzymes compared to those in the control. A new fourth isoenzyme, analogous to that shown under No. 4 was administered when 0.08% was applied to the mesophilic isolate.

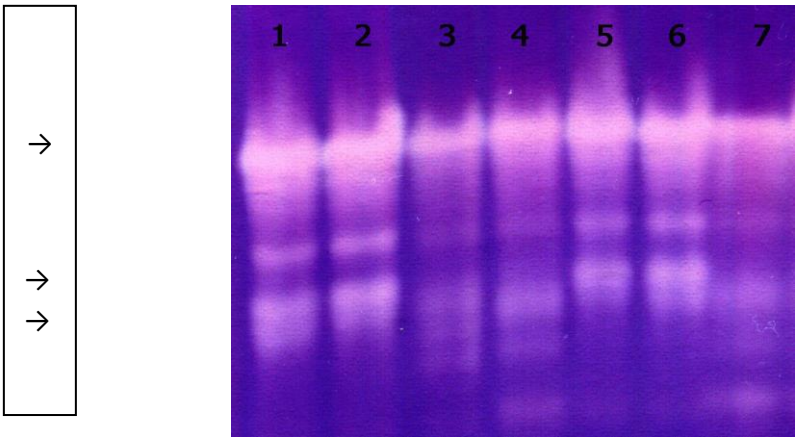
The lower concentrations (0.03) of the oxidizing agent stimulalate the total SOD activity of the antarctic strain, while at the highest concentration it was not registered. In the mesophilic strain this activity gradually decreased (Fig. 2B).

Catalase activity

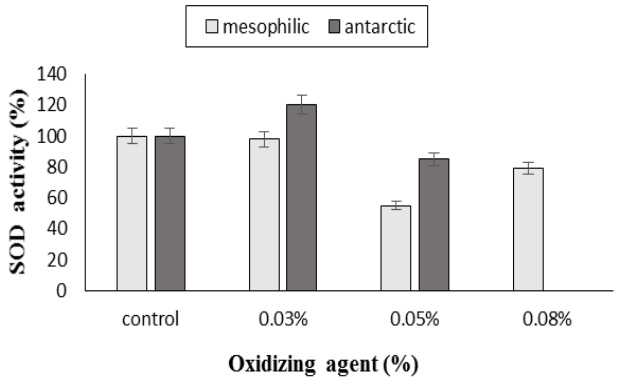
Under the influence of higher concentrations of the agent in the cells of mesophilic *C. vulgaris*, new CAT isoenzymes were activated. Upon exposure to 0.05% solution, isoenzymes IE3 and IE5 were induced and at 0.08% – isoenzymes IE3, IE4 and IE5 (Fig. 3A).

As a result of the effect of 0.03% solution, a decrease in CAT activity (for IE1 – by 31% and for IE2 by 15%) was established. The effect with 0.05% resulted in activation of isoenzymes IE1 and IE2, respectively by 113.7% and 13.7%. Upon exposure to 0.08% agent solution, the activity of isoenzymes IE1 and IE2 was close to that of the control. An activity of IE3 was

increased by 292%, compared to its activity after treatment with 0.05%. Only IE4 appeared at 0.08% treatment. The activity of IE5 was similar to that after treatment with 0.05%.



A) Effect of the biocide agent on the SOD izoenzyme profile of *C. vulgaris*;
1 – control (mesophile); 2 – 0.03% biocide (mesophile);
3 – 0.05% biocide (mesophile); 4 – 0.08% biocide (mesophile);
5 – control (antarctic); 6 – 0.03% biocide (antarctic);
7 – 0.05% biocide (antarctic).

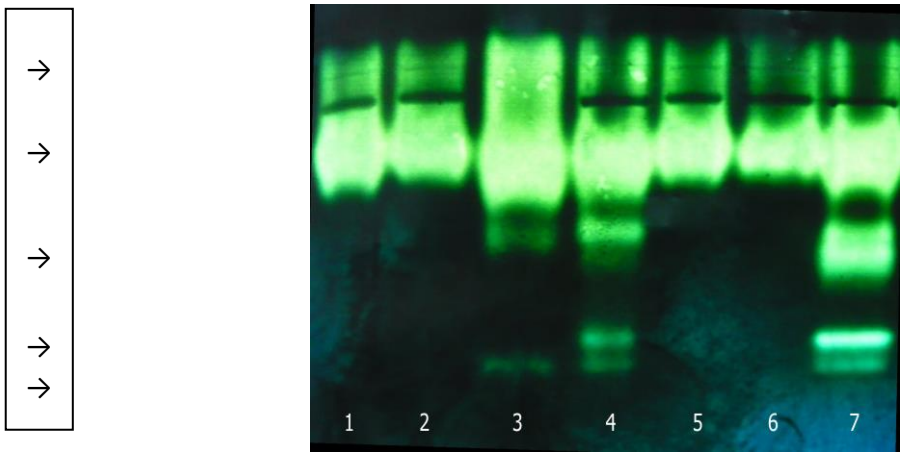


B) Effect on total SOD activity

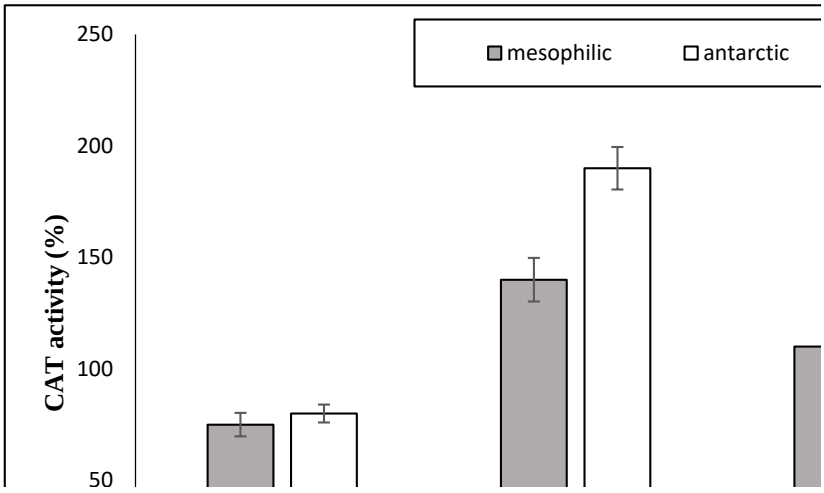
Fig. 2 Effect of biocide agent on SOD

Table 4. Changes in the isoenzyme profiles in *C. vulgaris* (SOD)

Agent concentration, %		**Mesophilic**			**Antarctic**	
		0.03	**0.05**	**0.08**	**0.03**	**0.05**
	Isoenzymes, %					
IE1	100	100	76	61	100	74
IE2	100	100	67	53	100	65
IE3	100	100	77	56	150	49
IE4	-	-	-	+ (new)	-	+ (new)



A) Effect of the agent on the isoenzyme profile of *C. vulgaris* CAT;
1 – control (mesophile); 2 – 0.03% biocide (mesophile);
3 – 0.05% biocide (mesophile); 4 – 0.08% biocide (mesophile);
5 – control (antarctic); 6 – 0.03% biocide (antarctic);
7 – 0.05% biocide (antarctic).



B) Effect on total CAT activity

Fig. 3. Effect of biocide agent on CAT

The antarctic *C. vulgaris* treated with 0.03% agent solution also responded with a slight decrease in the activity of isoenzymes IE1 and IE2 by 23.3% and 17%, respectively. Upon exposure with 0.05%, the IE2 was activated with 13% and new isoenzymes with numbers 3, 4 and 5 were revealed (Fig. 3A and Table 5).

After treatment of the cells of the antarctic isolate with 0.05% solution, the activity of the moderate – mobile isoenzymes (IE3, IE4, IE5) increased compared to the mesophilic one, the latter lacking IE4. On the other hand, the slow-mobile isoenzymes (IE1 and IE2) were with a higher activity in the mesophilic form. The isoenzyme profile of the antarctic isolate at 0.05% was similar to that of the mesophilic one at 0.08% of the agent. IE3 in the antarctic isolate increased its activity by 293% towards the activity of IE3 in the mesophilic isolate.

Table 5. Isoenzyme activity (%) after treatment with the oxidizing agent (CAT)

Agent concentration, %		Mesophilic			Antarctic	
		0.03	0.05	0.08	0.03	0.05
	Isoenzymes, %					
IE1	100	70	213	100	77	100
IE2	100	83	115	100	89	100
IE3	-	-	+(new)	+(new)	-	++(new)
IE4	-	-	-	+(new)	-	+(new)
IE5	-	-	+(new)	+(new)	-	+(new)

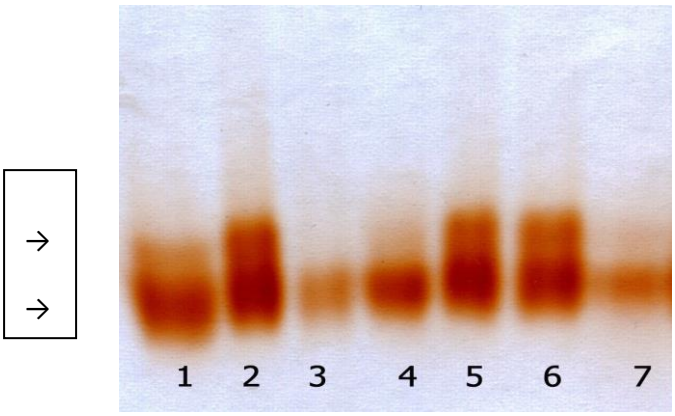
The results for the total CAT activity measured showed enhancement at concentration 0.05% of the agent, higher for the antarctic strain (Fig. 3B).

Peroxidase activity

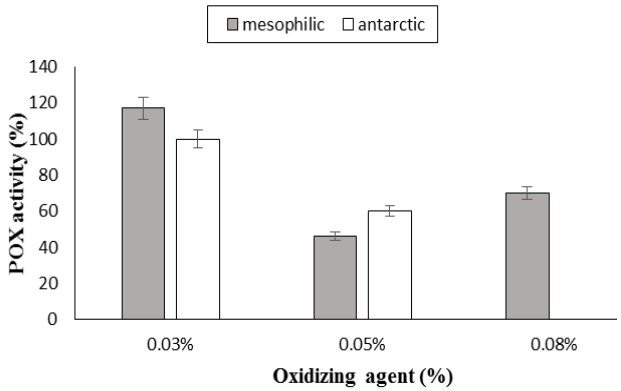
In the biomass of the *C. vulgaris* mesophilic isolate, and treated with the lowest solution concentration (0.03%), the occurrence of two POX isoenzymes was recorded. Upon treatment with 0.03%, the isoenzymes were activated. The activity of IE1 was increased by 79% relative to the control. When 0.05% and 0.08% were applied, only IE2 appeared, as upon treatment with 0.05% it reacted with a 40% decrease. At 0.08% a similar activity to the control was registered.

Antarctic *C. vulgaris* reacted with the appearance of two isoenzymes after exposure to all the concentrations tested. Upon exposure to 0.05% of the reagent solution, the activity of both isoenzymes decreased, for IE1 – by 66% and for IE2 – by 30% relative to the control (Fig. 4A and Table 6).

The algicidic action of a number of substances causes oxidative stress. The hydrogen peroxide produces a burst of ROS, i.e., a state of oxidative stress. This is the reason to suppose a shift of isoenzyme composition of the ROS-scavenging enzymes. Oxidative stress lead to a significant increase in antioxidant enzyme activity, which was reduced after prolonged treatment [23]. A significant increase in the activity of the antioxidant enzymes CAT, POX and SOD was registered. The activity of CAT and POX was reduced after 6 days of treatment and SOD after 4 days, which the authors explained with damage to cells of *Aphanizomenon flos-aque* from the prolonged oxidative stress. The same authors also found a significant suppression of photosynthetic activity. Data from our study after exposure to the biocidic solution confirmed this trend for enhancing enzyme activity at low reagent concentrations and declining activity at the highest applied concentrations. The results also confirmed the data for the photosynthetic activity decline. There were data for short-term (24 h) experiments to study the effect of phenanthrene and anthracene on the growth of *Scenedesmus armatus*. Anthracene had been found to stimulate the total activity of SOD in cells, and this effect was usually more pronounced when increasing the concentration [1]. However, we detected suppression of SOD activity from higher concentrations of the applied solution of H_2O_2 and silver. In a study of tryptamine for selective algicidic activity against various cyanobacteria and eukaryotic microalgae, Churro et al. [5] found that when algicide treatment with tryptamine of *Ankistrodesmus falcatus* (green algae) and cyanobacteria *Aphanisomenon gracile* the increase in catalase activity was negligible.



A) Effect of the agent on the isoenzyme profile of *C. vulgaris* POX-
1 – control (mesophile); 2 – 0.03% biocide (mesophile);
3 – 0.05% biocide (mesophile); 4 – 0.08% biocide (mesophile);
5 – control (antarctic); 6 – 0.03% biocide (antarctic);
7 – 0.05% biocide (antarctic).



B) Effect on total activity of *C. vulgaris* POX in mesophilic and antarctic isolates

Fig. 4 Effect of biocide agent on POX

Unlike the values for the CAT total activity, the total activity of POX decreased with the agent concentration of 0.05%. At 0.08% a slight increase was visualized due to the presence of IE2 (Fig. 4B).

Table 6. Isoenzyme activity (%) after treatment with the oxidizing agent (POX)

Agent concentration, %		**Mesophilic**			**Antarctic**	
		0.03	**0.05**	**0.08**	**0.03**	**0.05**
	Isoenzymes, %					
IE1	100	179	-	-	77	34
IE2	100	85	60	100	87	70

Wang et al. [28] investigating the differential expression of SOD isoenzymes under the influence of various types of abiotic stress, found that the expression of MnSOD was constitutively high in the vegetative cells of the green alga *Haematococcus pluvialis* and progressively decreased after the onset of stress. Under stress conditions, the expression of the Cu/ZnSOD isoform increased 8-fold [27]. These authors found that the MnSOD and FeSOD

isoenzymes underwent various changes at stress induction. They suggested that under stress conditions, additional MnSOD-isoenzymes were expressed or activated. The observed decrease in the level of transcripts and the activity of MnSO4 isoenzymes after several days under stress conditions were explained by the assumption that SOD represented a short-term (rapid) strategy to protect against oxidative stress. When stress continued, cells developed a long-term strategy of protection and survival, including other biosynthetic pathways and also production of low molecular weight antioxidants. The solution of H_2O_2 and silver possessed an algicidic effect with concentration increase up to 0.1%. Both *C. vulgaris* isolates reacted with decrease of biomass production with the increase of algicide concentration. This was the reason to recommend its use in the control of undesirable development of algae, which can cause the plugging of tube systems in purification stations and variety productions. Its fast biodegradation makes it suitable for ecological expedient control of undesired algal flora. The increase of reagent concentration also led to the raise of activity and isoenzyme number of CAT in both *C. vulgaris* strains. POX activity was retained comparatively stable in the control and at low concentrations in both *C. vulgaris* strains. The treatment with higher concentrations decreased strongly the isoenzyme activity and in the antarctic isolate was not registered. The highest concentration provoked restoration of POX activity in the mesophilic isolate. The increased concentration for both *C. vulgaris* strains lead to decrease in the SOD activity and this effect was more intensive in the moderate and fast mobile isoenzymes. Isoenzyme profile at 0.08% for the mesophylic isolate was similar with this at 0.05% for the antarctic isolate.

Conclusion

The simultaneous effect of the two algicidic constituents of the agent, used in combating unwanted algal contamination showed they affected the photosynthetic apparatus, as well as the enzyme profile of the mesophilic and antarctic *C. vulgaris* strains by provoking isoenzyme synthesis. Both strains were compared to establish the agent imposed concentration-dependent oxidative stress. At the lower concentration growth and enzyme activity of antarctic strain were slightly stimulated while at the highest concentrations of the agent growth retardation and decreased activity was proven. Due to the specific habitat the antarctic strain appeared to be more susceptible to the effect of this oxidizing agent.

References

1. Aksman A., Z. Tukaj (2004). The Effect of Anthracene and Phenanthrene on the Growth, Photosynthesis, and SOD Activity of the Green Alga *Scenedesmus armatus* Depends on the PAR Irradiance and CO_2 Level, Arch of Environ Contam and Toxic, 47(2), 177-184.
2. Almeida A., T. Gomes, K. Langford, K. Thomas, K. Tollefsen (2017). Oxidative Stress in the Algae *Chlamydomonas reinhardtii* Exposed to Biocides, Aquatic Toxicology, 189, 50-59.
3. Apostolova E. L., A. G. Dobrikova, P. I. Ivanova, I. B. Petkanchin, S. Taneva (2006). Relationship between the Organization of the PSII Supercomplex and the Functions of the Photosynthetic Apparatus, Journal of Photochemistry and Photobiology B: Biology, 83, 114-122.
4. Breusegem F., J. Dat (2006). Reactive Oxygen Species in Plant Cell Death, Plant Physiol, 141, 384-390.
5. Churro C., E. Alverca, F. Sam-Bento, S. Paulino, V. Figueira et al. (2009). Effects of Bacillamide and Newly Synthesized Derivatives on the Growth of Cyanobacteria and Microalgae Cultures, Hydrobiologia, 649, 195-206.

6. Dalton T. P., H. G. Shertzer, A. Puga (1999). Regulation of Gene Expression by Reactive Oxygen, Annual Review of Pharmacology and Toxicology, 39, 67-101.
7. Davis B. J. (1964). Disc Electrophoresis. II. Method and Application to Human Serum Proteins, Annals of the New York Academy of Sciences, 15, 404-427.
8. Dobrikova A., I. Domonkos, Ö. Sözer, H. Laczkó-Dobos, M. Kis et al. (2013). Effect of Partial or Complete Elimination of Light-harvesting Complexes on the Surface Electric Properties and the Functions of Cyanobacterial Photosynthetic Membranes, Physiologia Plantarum, 147(2), 248-60.
9. Edreva A. (2005). Generation and Scavenging of Reactive Oxygen Species in Chloroplasts: A Submolecular Approach, Agriculture, Ecosystems & Environment, 106, 119-133.
10. Halliwell B., J. M. C. Gutteridge (Eds.) (1999). Free Radicals in Biology and Medicine, Oxford University Press, Oxford.
11. Georgiev D., Ch. Dilov, S. Avramova (1978). Buffer Nutrient Medium and a Method for Intensive Cultivation of Green microalgae, Hidrobiologia, 7, 14-24.
12. Greneche M., J. Lallemand, O. Michaud (1991). Comparison of Different Enzyme Loci as a Means of Distinguishing Ryegrass Varieties by Electropkoresis, Seed Science and Technology, 19, 147-158.
13. He Y., M. Klisch, D. Hader (2002). Adaptation of Cyanobacteria to UV-B Stress Correlated with Oxidative Stress and Oxidative Damage, Photochemistry Photobiology, 76(2), 188-196.
14. Ivanova P. I., A. G. Dobrikova, S. G. Taneva, E. L. Apostolova (2008). Sensitivity of the Photosynthetic Apparatus to UV-A Radiation: A Role of Light-harvesting Complex II-Photosystem II Supercomplex Organization, Radiation and Environmental Biophysics, 47, 169-177.
15. Izadi A., F. Farnaz, S. Soufiabadi, F. Vafaee, S. Kasraei (2013). Antibacterial Effect of Sanosil 2% and 6% and Sodium Hypochlorite 0.5% on Impressions of Irreversible Hydrocolloid (Alginate) and Condensational Silicone (Speedex) Avicenna, Journal of Dental Research, 5(1), 1-4.
16. Krause G., E. Weis (1991). Chlorophyll Fluorescence and Photosynthesis: The Basics, Annual Review of Plant Biology, 42, 313-349.
17. Linley E., S. Denyer, G. Mc Donnell, C. Simons, J. Maillard (2012). Use of Hydrogen Peroxide as a Biocide: New Consideration of Its Mechanisms of Biocidal Action, J Antimicrob Chemother, 67, 1589-1596.
18. Matthijs H. C., P. M. Visser, B. Reeze, J. Meeuse, P. C. Slot, G. Wijn, R. Talens, J. Huisman (2012). Selective Suppression of Harmful Cyanobacteria in an Entire Lake with Hydrogen Peroxide, Water Res, 46(5), 1460-1472.
19. Mittler R. (2002). Oxidative Stress, Antioxidants and Stress Tolerance, Trends Plant Sci, 7(9), 405-410.
20. Ornstein L. (1964). Emzyme Bulletin, Canalco Industrial Corporation, Rockvill, Maryland, 12.
21. Pitzschke A., C. Forzani, H. Hirt (2006). Reactive Oxygen Species Signaling in Plants, Antioxidants and Redox Signaling, 8(9-10), 1757-1764.
22. Pradedova E., O. Isheeva, R. Salyaev (2011). Classification of the Antioxidant Defense System as the Ground for Reasonable Organization of Experimental Studies of the Oxidative Stress in Plants, Russian Journal of Plant Physiology, 58(2), 210-217.
23. Shi D., F. Xie, C. Zhai, Z. Stern, Y. Liu, S. Liu (2009). The Role of Cellular Oxidative Stress in Regulating Glycolysis Energy Metabolism in Hepatoma Cells, Molecular Cancer, 8, 32-39.

24. Storz G., J. A. Imlay (1999). Oxidative Stress, Current Opinion in Microbiology, 2, 188-194.
25. Suzuki N., R. Mittler (2006). Reactive Oxygen Species and Temperature Stress: A Delicate Balance between Signaling and Destruction, Physiologia Plantarum, 126, 45-51.
26. Tyystjärvi E., J. Karunen (1990). A Microcomputer Program and Fast Analog to Digital Converter Card for the Analysis of Fluorescence Induction Transients, Photosynthesis Research, 26, 127-132.
27. Wang J., F. Chen, M. Sommerfeld, Q. Hu (2004). Proteomic Analysis of Molecular Response to Oxidative Stress by the Green Alga *Haematococcus pluvialis* (*Chlorophyceae*), Planta, 220, 17-29.
28. Wang J., M. Sommerfeld, Q. Hu (2011). Cloning and Expression of Izoenzymes of Superoxide Dismutase in *Haematococcus pluvialis* (*Chlorophyceae*) under Oxidative Stress, Journal of Applied Phycology, 23, 995-1003.
29. Woodbury W., A. Spenser, M. Stahmann (1971). An Improved Procedure Using Ferricyanide for Detecting Catalase Isoenzymes, Analytical Biochemistry, 44, 301-305.
30. Zeinalov Y. (2002). Equipment for Investigations of Photosynthetic Oxygen Production Reactions, Bulgarian Journal of Plant Physiology, 28, 57-67.

Assist. Dilyana Doneva, Ph.D.
E-mail: did4e_7788@mail.bg

Dilyana Doneva works as an Assistant in Bulgarian Academy of Sciences, Institute of Plant Physiology and Genetics, Laboratory "Photosynthesis – Activity and Regulation". Her professional interests include photosynthetic gas exchange, chlorophyll fluorescence, oxidative stress, secondary metabolites, antioxidant enzymes.

Assist. Prof. Juliana Ivanova, Ph.D.
E-mail: juivanova@yahoo.com

Juliana Ivanova works as an Assistant Professor in Bulgarian Academy of Sciences, Institute of Plant Physiology and Genetics, Laboratory "Experimental Algology". Her professional interests include algae cultivation, immobilization, interactions between bacteria and algae, biologically active substances from algae, stress factors.

Assoc. Prof. Lyudmila Kabaivanova, Ph.D.
E-mail: lkabaivanova@yahoo.com

Lyudmila Kabaivanova works as an Associate Professor, Scientific secretary of The Stefan Angeloff Institute of Microbiology, BAS and is a Head of Department "Applied microbiology". Her professional interests include biologically active substances from bacteria and algae with different applications.