Influence of low-temperature plasma on green algae culture

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Abstract. Influence of low-temperature plasma, which was formed in a high-resource arc-discharge plasma jet in an argon-air mixture, was studied for a community of green algae as *Chlorella vulgaris* Beyerinck [Beijerinck] and *Stichococcus bacillaris* Nägeli. It was found that the plasma treatment for 10 minutes led to partial cell death. At the same time, the species of *C. vulgaris* were less sensitive to the plasma treatment than the species of *S. bacillaris.* After plasma exposure, the predominant growth of the latter culture was observed in comparison with the control samples. This effect can be explained by an activation of biochemical processes in the algae due to the interaction with radicals in the low-temperature plasma. The results obtained indicate the selectivity of the low-temperature plasma effect on green algae community.

1 Introduction

Environmental studies are aimed at identifying the resilience of organisms and communities under various impacts. In particular, the use of various methods of processing biosystems to remove pathogens, to destroy cells or to stimulate the growth of organisms should be accompanied by an analysis of both instantaneous and delayed and prolonged effects. Low-temperature plasma is known to be a promising method to control living cells and tissues, as well as viruses [1-3]. The effectiveness of the plasma impact depends not only on the duration and power, but also on the type of plasma source used, which creates a technological environment characterized by many parameters [4].

The most important active factors of low-temperature plasma include the effects of charged particles, excited neutral atoms and molecules, electromagnetic fields and radiation, as well as chemical reactions. These are ultraviolet and infrared radiation, fluxes of free electrons, ions, neutral atoms and molecules, which are evaluated in a complex [5-7]. Such effects can activate the mechanisms of oxidative metabolism in cells, and at low intensity free radical oxidation is one of the types of normal metabolic processes [8], while

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their intensification leads to significant disturbances, primarily in the structure and functions of membranes [6, 8]. The effects caused by exposure to reactive oxygen and nitrogen species (as well as some others) are considered [7, 9, 10]. In our previous studies the low temperature plasma treatment was used to kill microorganisms as infusoria, fungi and pathogenic bacteria [11, 12].

The aim of our present work is to evaluate possible effects of the low-temperature plasma on the green algae community in aquatic environment.

2 Material and methods

The source of low-temperature plasma at atmospheric pressure was an arc discharge plasma jet (plasmatron) with an extended operation life and it allowed us to generate low-temperature plasma jets, which were practically free of metallic contaminants [7]. The main structural elements of the plasma source are a water-cooled rod, a cathode and a nozzle anode. The arc discharge plasma was created in a mixture of argon (Ar) gas with flow rate of 2 l/min, which was injected in the cathode-anode space, and atmospheric air (flow rate 10 l/min) injected behind the anode [7].

We studied communities of algal fouling from the photic zone of Vorontsovskaya Cave, Russia, the western Caucasus, obtained by cultivating communities selected from natural conditions. Communities were cultivated for 8 months on Bristol's medium, modified by Gollerbach, medium composition [3]. The resulting mixed cultures were diluted 1: 50 ml with culture medium the day before the experiment. The initial community consisted of two types of cells, i.e. *Chlorella vulgaris* Beyerinck [Beijerinck]and *Stichococcus bacillaris* Nägeli in a ratio of 3:2. The total number of cells was 10^6 ml^{-1}.

The influence of low-temperature plasma on the culture was carried out in a liquid medium. In a sterile Petri dish 4 cm in diameter, 4 ml of a suspension of the culture medium with algae was placed. The dish was placed at a distance of 24 cm from the plasma emitter, which operated at a current of 50 A. The exposure time was 0.5 min, 1 min, 3 min, and 10 min; morphological changes in cells and the number of cells of different types were assessed using optical microscopy. From the suspension, 200 μL were taken, placed on a glass slide, covered with a cover glass, and the number of cells in the fields of view under a microscope was counted, at least 50 fields of view, the total number of counted cells was 2000 at least.

After exposure, the suspension was placed in a fresh medium at a concentration of 1 ml of suspension in 50 ml of medium and cultivation was continued under conditions of 25° C and illumination intensity of about 2×10^{19} $m^{-2}s^{-1}$. The state of the cultures was monitored on the 10th and 30th days of cultivation by calculating the number of cells of different types in the culture with accuracy 2%. A control experiment was carried out at similar dilution procedures from the original culture. The numbers of cells in the control culture and those in the plasma-exposed cultures were compared. The experiments were carried out in duplicate, in two series. The temperature and pH of the medium were monitored during the experiment. The obtained experimental data were processed statistically using an Excel software.

3 Results and discussion

It was found that the cold-plasma treatment resulted in weak changes in the temperature and acidity level of the culture medium. The temperature increased during 20 minutes of constant irradiation by no more than 3° C (Fig. 1), which is within the range of permissible changes for the cultivation of the studied organisms. The acidity of the medium for 10

minutes of constant irradiation decreased from 7.2 to 6.8 (Fig. 2). The detected changes of pH-level are obviously into the zone of optimum culture development.

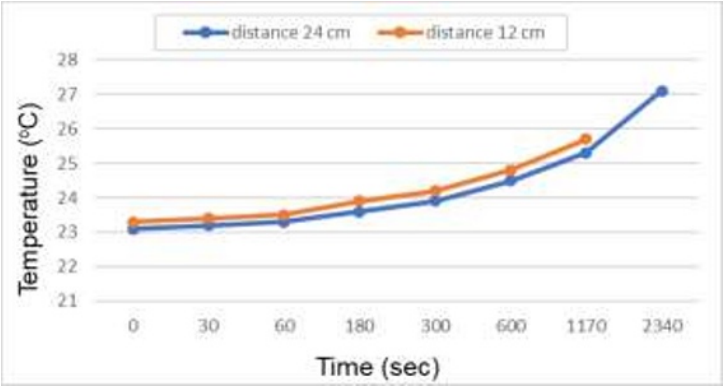

Fig. 1. Dependences of the liquid environment temperature on duration of the plasma treatment at distances of 12 and 24 cm from the plasma emitter.

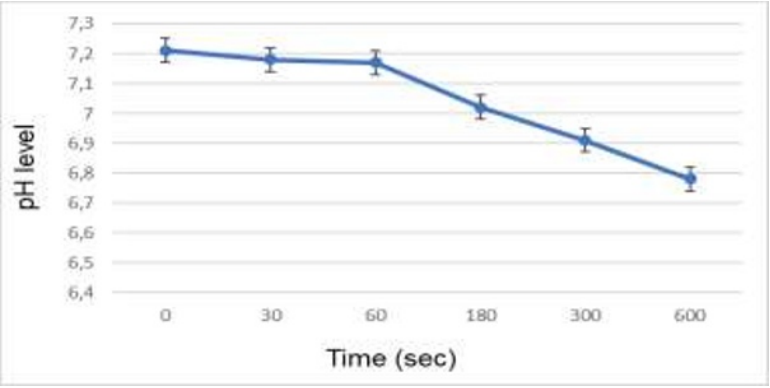

Fig. 2. Dependence of the pH level in liquid environment on duration of the plasma treatment at a distance of 24 cm from the plasma emitter.

The experiments showed that the action of low-temperature plasma for 1-5 minutes did not cause statistically significant changes in the structure of communities. When exposed to the community for 10 minutes, it was revealed that the initial ratio of the number of species changed; immediately after the impact, the share of *S. bacillaris* decreased to 5% (Fig. 3).

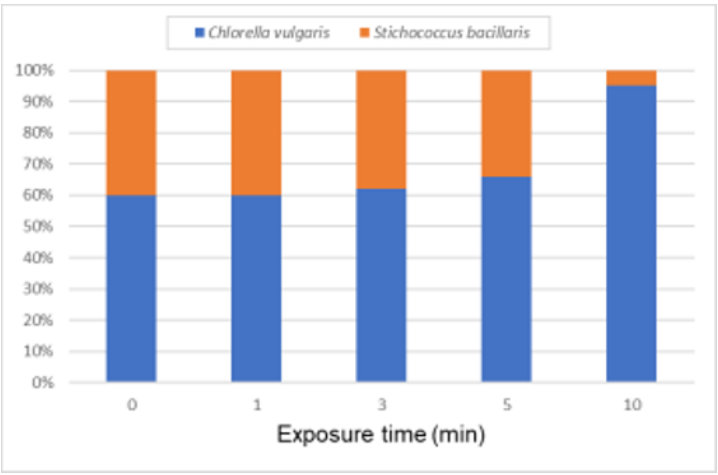

Fig. 3. Changes in the ratio of species in culture at different times of exposure to the low-temperature plasma.

After 10 days of cultivation, the proportion of *S. bacillaris* in the culture was 15%, and on day 30 it reached 20% (Fig. 4). The observed partial elimination of *S. bacillaris* community can be explained by the larger cell size compared to the algae of *C. vulgaris* species.

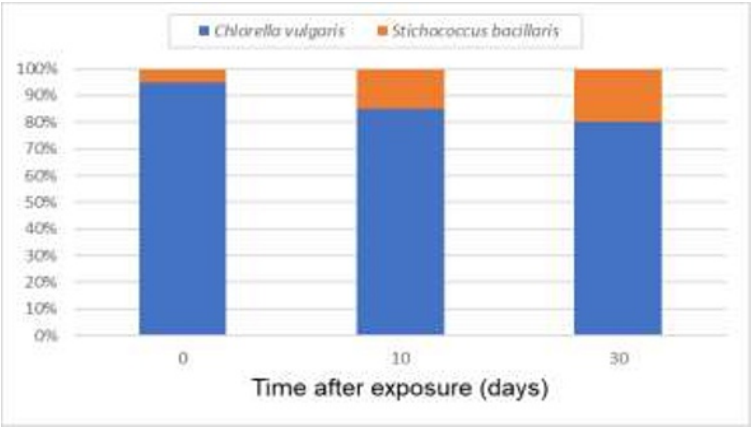

Fig. 4. Changes in the ratio of species in the community after plasma-exposure for 10 min.

The analysis of the growth rate of the culture after exposure to the cold plasma revealed a difference between the increase in the number of cells in the control community and in the experiment. After 1 and 3 minutes of exposure, by the 30th day of cultivation, a significant decrease in the number of cells was observed in comparison with the control by 8 and 12%, respectively. In an experiment with a 5-minute exposure, an increase in the number compared to the control was revealed by 4% by the 10th day of cultivation and a decrease by 24% by the 30th day of cultivation, while the ratio of species in the community changed, the cells of *C. vulgaris* and *S. bacillaris* were 70: 30. After 10 minutes of exposure, the decrease in the number in the culture reached 30% by the 30th day of cultivation.

The relevance of this study consists in assessing the impact of low-temperature plasma on the communities of green algae in the aquatic environment, identifying the resistance of species to the effects of low-temperature plasma and the dynamics of community recovery.

The effect on the algal community consisting of the species *C. vulgaris* and *S. bacillaris* is considered, with the greatest changes observed in *S. bacillaris*, which can be associated with the larger size of the algal cells compared to the cells of *C. vulgaris*. Further research is needed to better understand the effect of low-temperature plasma on cells of different sizes.

4 Conclusions

It was revealed that under the studied conditions, the effect of low-temperature plasma on the suspension of green algae for 0.5-5 minutes did not cause changes in the community. The plasma effect on a suspension of green algae for 10 minutes caused partial cell death, the species *C. vulgaris* were less sensitive to the effect than the species *S. bacillaris*. The algae cultivation of after exposure revealed an increase in the growth rate of the culture in comparison with the control samples, especially for the species *S. bacillaris*. This effect can be explained by activation of biochemical processes in algae because of the interaction with plasma-related radicals. The obtained results demonstrate possibilities to control selectively the algae community by low-temperature plasma treatment.

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