JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Development And Symbiosis of a Chlorella Strain in Natural and Extreme Conditions of The Aquatic Environment

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ABSTRACT

The article studies the influence of natural and extreme conditions of the aquatic environment on the development and symbiosis of a strain of the unicellular planktonic green microalgae Chlorella vulgaris BIN, 6-10 microns in size. The studies were carried out in 2019, 2020 on the basis of laboratory analysis of samples taken at a depth of 10 cm from 15 control points of the Nizhny Fermsky pond of the Federal State Budgetary Educational Institution of Higher Education RSAU-Moscow Agricultural Academy named after K.A. Timiryazev, and in an open aquarium (bioreactor) with a volume of 50 liters in which, using the Arduino hardware and software complex, the temperature and lighting optimal for the growth of microalgae of the Chlorella vulgaris BIN strain were automatically maintained, as well as biochemical and physical conditions for the growth and development of microalgae were simulated strain Chlorella vulgaris BIN. In a separate study, completed in January 2020, the development and symbiosis of chlorella with unicellular and multicellular aquatic organisms was studied in an open aquarium with artificial lighting and temperature maintenance, in the presence of a critically large amount of macro and microelements used by the microalga of the Chlorella vulgaris BIN strain at the growth and reproduction. To calculate the number of chlorella cells in the samples, a Goryaev two-grid camera was used, in which the identification and quantitative accounting of chlorella cells was carried out according to the results of photographs and video filming, with the resolution settings of the Bresser LCD MICRO 5MP digital microscope at 125, 500 times, and at 1000 times electronic ZOOM magnification. As a result of the work carried out, a number of limiting factors have been identified that affect the growth and development of microalgae of the Chlorella vulgaris BIN strain and associations of microorganisms that coexist in symbiosis with microalgae.

KEYWORDS:
Chlorella vulgaris BIN, microorganisms, symbiosis, aquatic environment, heterotrophic organisms, hydrobitis

ARTICLE HISTORY:
Received April 19, 2021
Accepted April 16, 2021
Published May 02, 2021

DOI:
10.5455/jcmr.2021.12.02.03

VOLUME: 12
ISSUE: 2
ISSN: 2146-8397

INTRODUCTION

The genus Chlorella includes unicellular green algae with spherical or ellipsoidal cells, 2 to 10 microns in diameter, with one or two nuclei and one cup-shaped chloroplast. The microorganism itself is covered with a durable cellulose shell. Chlorella does not have any devices for movement, such as flagella or cilia, therefore it moves in the direction of water flow, gradually rising or falling in the water column when the density inside the chlorella cell changes, which depends on gaseous and liquid waste products of chlorella of different densities. Chlorella is an active oxygen producer during

photosynthesis. It includes all the necessary substances for its development, while chlorella, unlike a number of algae, does not produce toxic compounds. Reproduction of chlorella is carried out by autospores, which are formed in even quantities as a result of the division of the mother cell [1]. The considered strain of microalgae Chlorella vulgaris BIN was obtained by selection of the strain Chlorella vulgaris IGF № C-111, during its cultivation in industrial and municipal wastewater. In turn, the strain Chlorella vulgaris IGF № C-111 was isolated in 1974-1977 in the Nurek reservoir of Tajikistan [2]. The advantages of the Chlorella vulgaris BIN strain are: uniform distribution of cells in the culture medium, the possibility of being in a state of prolonged anabiosis, the absence of adhesion to higher vegetation and the precipitation of cells from a homogeneous suspension, resistance to damage by algophages, rickettsiae and bacteria, the possibility of cultivation without balloon carbon dioxide, the ability to quickly change the type of food , from autotrophic to heterotrophic, depending on environmental conditions. Upon entering the water body, the Chlorella vulgaris BIN strain does not settle to the bottom, floats freely in the upper water layer, intensively divides and becomes the dominant microalgae in the upper, meter-long water layer.

MATERIALS AND METHODS

Starting from January 2019, a set of works on algolization of the Nizhny Fermsky pond, located on the territory of the Russian State Agrarian University-Moscow Agricultural Academy named after K.A. Timiryazev (Figure 1).

Fig.1: Nizhny Fermsky Pond, 2019

The complex of works includes a seasonal pouring of 150 liters of a suspension of microalgae of the Chlorella vulgaris BIN strain, with a concentration of 50 million cells per milliliter (hereinafter: cells / ml), provided by LLC “Algotek”. In the period from June 15 to August 15, 2019, water samples were taken from Nizhny Fermsky pond after the development and distribution of the planktonic strain Chlorella vulgaris BIN as the dominant microalgae species on the surface of the reservoir. Sampling was carried out with a 1 liter sampler, at a depth of 10 cm, at 15 control points located around the perimeter and in the center of the pond. In the problem laboratory of the RSAU-Moscow Agricultural Academy named after K.A. Timiryazev, the samples were then taken and mixed in a test tube using a mechanical pipette, and then analyzed using a Bresser LCD MICRO 5MP digital microscope. To calculate the number of chlorella cells in the samples, a Goryaev two-grid chamber was used, in which the identification and quantitative registration of chlorella cells took place at microscope resolution settings of 125, 500 times, and at 1000 times electronic ZOOM magnification. For the calculation was used the formula [3]:

$$A = H \cdot M \cdot 12499 \qquad (1)$$

where: A – number of cells in 1 ml; H – number of suspension dilutions before calculation; M – the number of cells of the strain Chlorella vulgaris BIN obtained by counting in twenty large squares of the Goryaev chamber.

Confidence limits, the random error in estimating the measured value when counting cells in the Goryaev chamber were calculated according to GOST R 8.736-2011, for a confidence probability P = 0.95 according to the formula [4]:

$$\varepsilon = t \cdot S_{\bar{x}} = t \cdot \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n(n-1)}} \qquad (2)$$

where: n – the number of large Squares in the Goryaev chamber in which the cells were counted, taking into account the count in twenty large squares in the Goryaev chamber (n =20); x_i – the number of cells in each of the large squares of Goryaev's chamber where i has the values i =1, 2, 3, 4,…., 20; t – Student's coefficient, for the confidence probability P=0,95 and n =20 equal to 2,093; $\bar{x}$ – the arithmetic mean of the measurement result.

The arithmetic mean of the measurement result is determined by the formula [4]:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

(3)

As a result of the analysis of samples from the test tube, after taking into account and subtracting micron background organic and inorganic contaminants, the total content of cells of the strain Chlorella vulgaris BIN was determined at the surface of the Nizhny Fermsky pond, it ranged from 198±15 thousand cells/ml to 355±18 thousand cells/ml. A photograph taken from a Bresser LCD MICRO 5MP digital microscope when analyzing a sample in a Goryaev camera at a resolution of 500 times is shown in Figure 2.

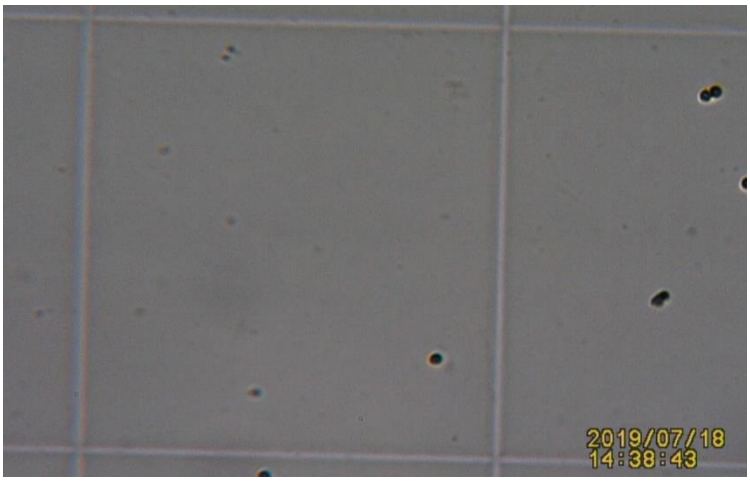


Fig.2: Analysis of a sample from Nizhny Fermsky pond in the Goryaev chamber at a resolution of 500 times on a Bresser LCD MICRO 5MP digital microscope

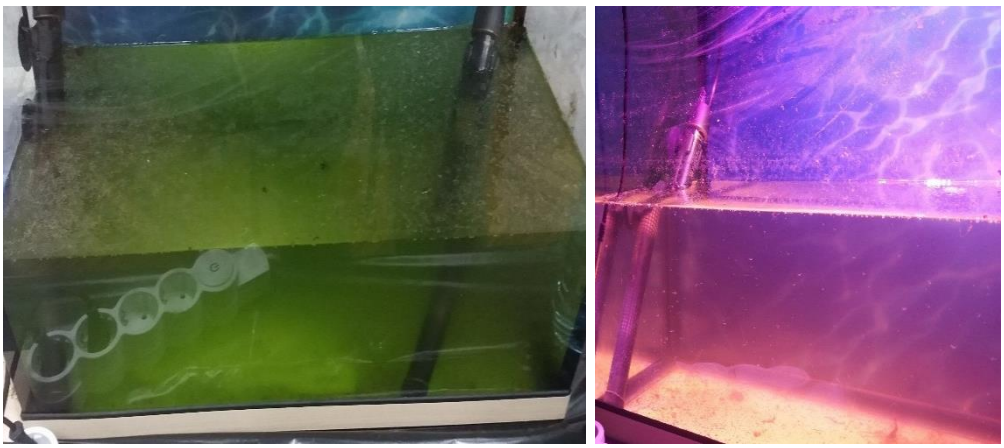
It can be noted that, despite the influence of external biological communities (including the eating of chlorella by fry, rotifers, ciliates, amoebas and other aquatic organisms), the Chlorella vulgaris BIN strain introduced into the reservoir retained its viability for six months.

To assess the possibility of self-regulation of the Chlorella population inside the pond under conditions favorable for the growth and reproduction of microalgae, the Chlorella vulgaris BIN strain, 25 liters of water from the Nizhny Fermsky pond were placed in an aquarium, in which in automatic mode, on the basis of the Arduino hardware and software complex, the optimal temperature for the growth of microalgae of the Chlorella vulgaris BIN strain was maintained in the range from 27 °C to 30 °C, and with the help of a Smart Led Grow Light 160W phytolamp, bright artificial lighting simulating bright summer sunlight (with at intervals of 16 hours light on and 8 hours light off), Figure 3.

Fig.3: Cultivation of microalgae strain Chlorella vulgaris BIN under artificial light, in water from Nizhny Fermsky pond

For accelerated growth of microalgae in the aquarium three times, with an interval of four days, microelement fertilizer was supplied 4 ± 0.01 grams of potassium nitrate (KNO3) manufactured by the Buisk Chemical Plant according to TU 2384-064-32496445-2003, which was weighed on an OEM MH-200 electronic balance. During the first 5 days, stable growth of microalgae of the Chlorella vulgaris BIN strain was observed under optimal conditions, from a concentration of 310±16 thousand cells/ml to a concentration of 6895±122

thousand cells/ml. At the same time, an accelerated growth of the microcommunity of heterotrophs (rotifers, ciliates, amoebas and other microorganisms) eating the population of algae was also observed. During the next two days, there was a sharp drop in the number of microalgae of the Chlorella vulgaris BIN strain to a concentration of 293±15 thousand cells/ml, at which the change in the number stabilized and did not change much further (Figure 4).



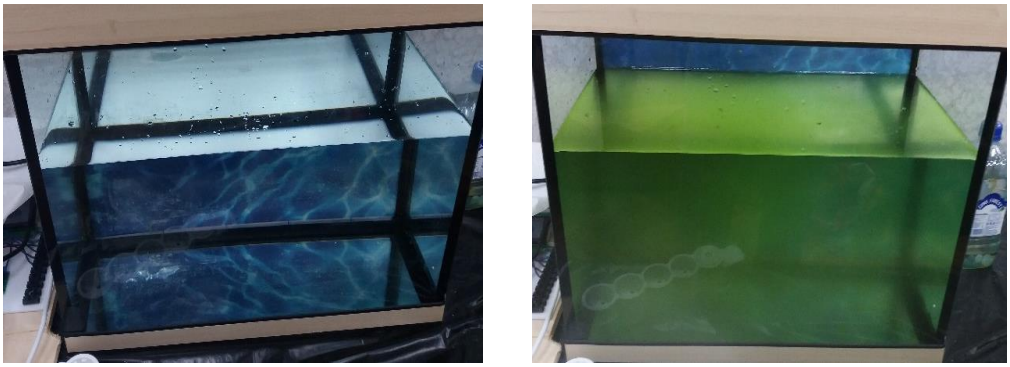
a) *b)*

Fig.4: Gradual change in the number of microalgae of the Chlorella vulgaris BIN strain from 6895 thousand cells/ml (a) to 293 thousand cells/ml (b)

Moreover, the composition of the microbiological community in the samples after 8 days of research was very similar to the composition of the microbiological community in the samples from the pond. In general, the study confirmed the possibility of self-regulation of the number of microalgae of the Chlorella vulgaris BIN strain using first-order consumers (rotifers, ciliates, amoebas and other microorganisms).

The most interesting results associated with the creation of a symbiotic community of microalgae of the Chlorella vulgaris BIN strain with unicellular and multicellular microorganisms were obtained during the following experimental study. The study was based on earlier work on accelerated cultivation of microalgae in various nutrient media, including organic and microelement feeding in the form of refined sunflower oil and potassium nitrate [5, 6]. The originally cleaned and sterilized aquarium was filled with 30 liters of tap water with a pH of 7, measured with pH meters, pH/ORP WP (Martini) and HI99161 (Foodcare). Then, 500 ml was poured into the aquarium in the form of a suspension of microalgae Chlorella vulgaris BIN, with a cell concentration of 8000±140 thousand cells/ml. Together with the suspension, an extremely large amount of nutrients was added to the aquarium and then mixed in it in the form of 100±1 ml of refined, deodorized sunflower oil made according to GOST 1129-2013 and 30.7±0.01 grams of potassium nitrate made according to TU 2384-064-32496445-2003 [7]. It should be noted that these concentrations are critical for most aquatic organisms, for example, the concentration of potassium nitrate in 1 g/l in an aquarium exceeds the maximum permissible concentration of nitrates for NO3 according to GN 2.1.5.2280-07 by 13.6 times, the content of organic acids contained in sunflower oil is also many times exceeds the MPC specified in GN 2.1.5.2280-07 [8]. Upon stirring, a clearly visible oil film immediately appears on the surface of the water, which also does not meet the hygienic requirements for the protection of surface waters specified in SanPiN 2.1.5.980-00 [9]. The purpose of the experiment was to study the development and symbiosis of chlorella in an open aquarium in the presence of a

critically large amount of macro and micronutrients. In the first 6 days of the experiment, every 48 hours an additional portion of potassium nitrate was added to the aquarium in the amount of 30.7±0.01 g. During the entire experiment, artificial lighting was used, created using a 160W Smart Led Grow Light phytolamp, with an artificial light on for 16 hours and darkness for 8 hours. In the first 3 days of the experiment, a steady increase in the number of cells of the strain Chlorella vulgaris BIN was observed from the initial 130 thousand cells/ml diluted in water to 1 million cells/ml. During the next 3 days, the process of rapid cell death of the Chlorella vulgaris BIN strain began to concentrations of 18±3 thousand cells/ml, which was apparently associated with an extremely high concentration of nitrates and waste products of chlorella, which is destructive for this microalga strain. The development and death of the microalgae strain Chlorella vulgaris BIN is shown in Figures 5, 6 and 7.

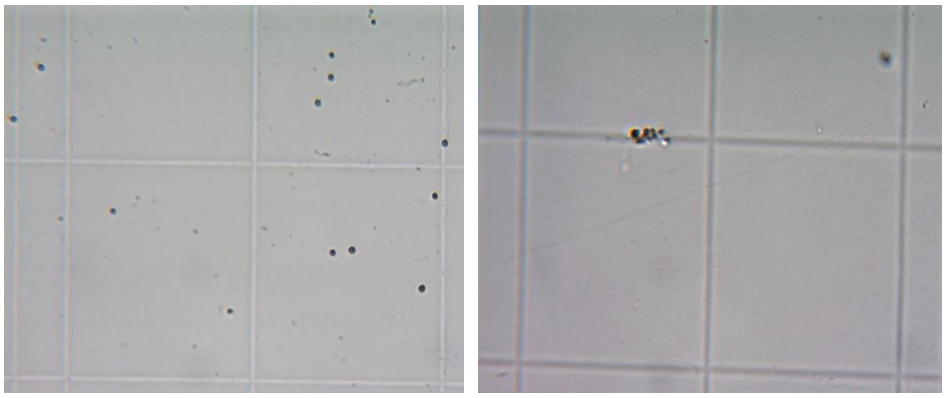


a) b)

Fig.5: Filling with water (a), aquarium after adding a suspension, cell concentration 130 thousand cells/ml (b)

a) b)

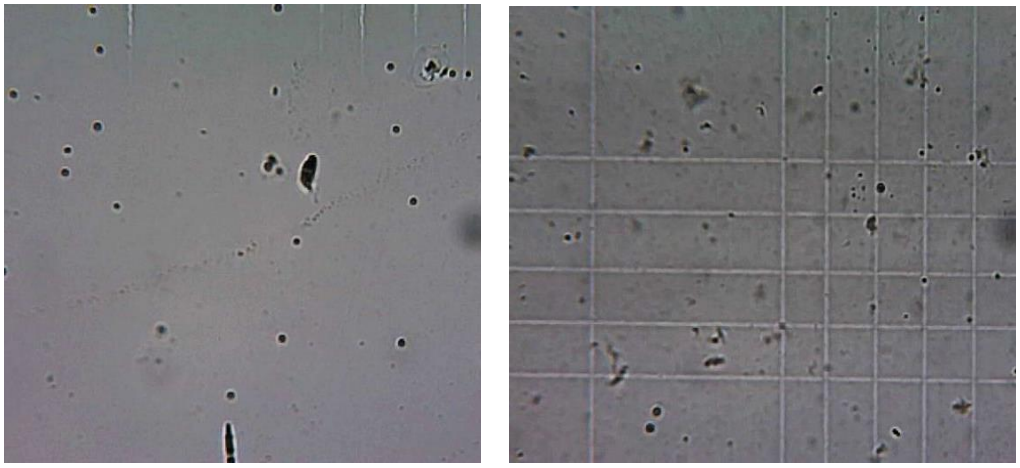
Fig.6: Aquarium after three days of experience, cell concentration 1 million cells/ml. (a), fatty film on the surface of the suspension (b)



a) b)

Fig.7: Cell counting after 3 days of the experiment, 1 million cells/ml. (a), the concentration of cells after 6 days of the experiment, less than 20 thousand cells/ml (b)

After 6 days of research, the experiment was not completed, artificial lighting worked in the same mode, 16 hours of lighting and 8 hours of darkness, but microelement and organic additives were no longer supplied to the aquarium, which led to the creation of new symbiotic communities in the aquatic environment. Over the next two days, mobile forms of microorganisms appeared, and a white viscous film that does not allow oxygen to pass through began to form on the surface of the aquarium (Figure 8).

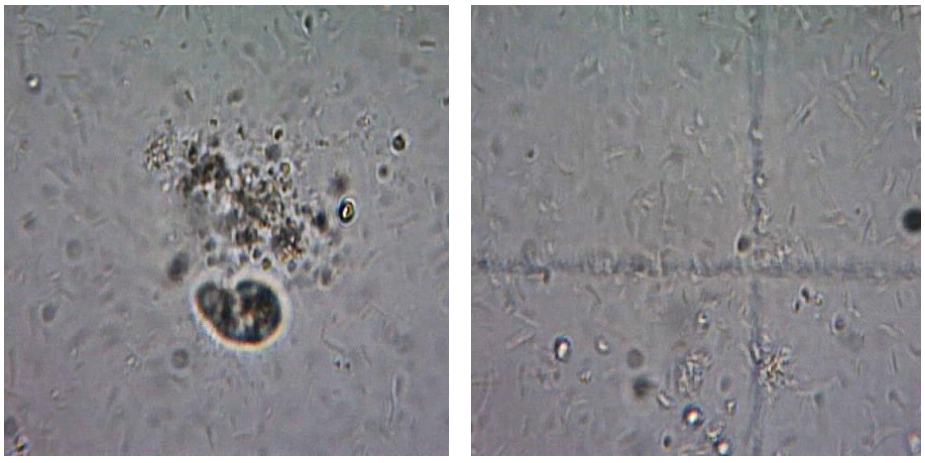


a) *b)*

Fig.8: Cells of the Chlorella vulgaris BIN strain surrounded by: multicellular organisms (a); stuck together organic compounds (b)

Then, within 7 days, bacterial communities appeared and multiplied, processing organic compounds (lipids, proteins, amino acids, carbohydrates) isolated by the Chlorella vulgaris BIN microalgae strain in the course of their life. On the 15th day after the start of the entire experimental study, the thickness of the organic viscous film reached a thickness of 2 - 3 mm, and the number of unicellular and multicellular organisms in a liquid suspension exceeded 500 million, as shown in Figures 9 and 10.

Fig.9: The surface of the suspension is covered with a thick organic film, 2-3 mm thick, a sapropel 2 mm thick has formed at the bottom of the aquarium, including dead chlorella cells



a) *b)*

Fig.11: 1000-fold ZOOM-increase: the ciliate eats microorganisms (a); microorganisms in the corners of the large squares of the Goryaev chamber, the cell concentration is more than 500 million cells/ml

RESULTS

As a result of the study, biological mechanisms of regulation of the number of microalgae Chlorella vulgaris BIN were identified, including due to heterotrophic organisms that eat this alga. It was possible to identify the mechanisms of transformation of the culture medium of the microalga strain Chlorella vulgaris BIN into a culture medium for a self-sufficient and viable symbiotic community of microorganisms and this microalga, actively producing biomass.

CONCLUSIONS

The influence of dramatically changing environmental conditions, simulated in laboratory conditions, significantly affects the number of microalgae of the Chlorella vulgaris BIN strain, but even under favorable conditions, due to the natural influence of aquatic organisms and waste products of this microalga, there is no spontaneous increase in the number of microalgae of the Chlorella vulgaris BIN strain up to values above 7 million cells/ml. At the same time, a quick return to the previous values of the number of cells of the strain Chlorella vulgaris BIN in the aquatic environment has a positive effect on the ecosystem of the aquatic environment and prevents its excessive pollution with the waste products of these microalgae. Under extreme conditions of the influence of abiotic factors on the development of the microalga Chlorella vulgaris BIN, its significant viability and the ability to form stable symbiotic communities were revealed. In the future, it is planned to continue this experimental one by including the laboratory study of the microelement and organic composition consumed and produced by the Chlorella vulgaris BIN microalga strain and the symbiotic community of microorganisms.

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