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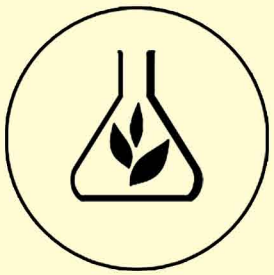

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FUNCTIONAL AND BIOCHEMICAL STATE OF *CHLORELLA VULGARIS* CELLS DURING THE CULTURE GROWTH IN LIQUID MEDIUM WITH $MnCl_2$ ADDITION AND THE BACKGROUND OF VARIOUS ORTHOPHOSPHATE CONCENTRATIONS

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In recent decades, single-celled algae of the genus *Chlorella* has been used extensively in biotechnology as a source of protein and a number of other valuable biogenic compounds. The deeper studies of these algae *in vitro* biology, including optimization of the nutrient media composition, are needed for further intensification of *Chlorella* biotechnology with the purpose to obtain target algal metabolites. We have previously shown that in some cases, the addition of $MnCl_2$ to the nutrient medium was accompanied by an increase in the level of total protein per unit of culture volume by 34% and 116% compared to the control (Ilyuchyk, Nikandrov, 2018a).

The aim of the present work is to identify the peculiarities of biomass yield, protein yield and photosynthetic pigment dynamics during *Chlorella vulgaris* growth on Tamiya medium with relatively low $MnCl_2$ concentration (0.025 or 0.05 mg/L) with the background of different orthophosphate concentration.

Materials and methods. *Chlorella vulgaris* strain IBCE C-19 from the algal collection of the Institute of Biophysics and Cell Engineering of the National Academy of Sciences of Belarus was used in the experiments.

The microalga was grown on Tamiya medium without EDTA. In the control, no KH_2PO_4 and $MnCl_2$ were added. The experimental variants of nutrient medium contained $MnCl_2$+KH_2PO_4: 0.025 mg/L+1.784 g/L; 0.025+2.007; 0.025+2.230; 0.05+1.784; 0.05+2.007; 0.05+2.230.

Chlorella was cultured at 25±1°C, stirred twice a day, supplied with illumination on the surface of the vessel of 5,000 lx, 12 h/12 h duration of light and dark phases. The inoculation dose was 3.26±0.05 million cells/ml.

The number of cells was determined visually by Goryaev chamber. After 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 days of cultivation, aliquots of culture containing 10±0.03 million cells were selected. The concentrations of total protein and pigments: chlorophylls *a* and *b*, carotenoids, in cell homogenates were determined colorimetrically. The methods and materials used are described in detail in our papers (Ilyuchyk, Nikandrov, 2018a; 2018b; 2020).

Experiments were done in nine replicates. The results were processed statistically using Statistica 6.0. The validity of differences between variants was determined by taking into account Student's coefficient (*t*) for the accepted significance level ($p \leq 0.05$).

Results and Discussion. The exclusion of $MnCl_2$ and orthophosphate (control, *K*) from the nutrient medium nevertheless caused a 54% increase in biomass after 17 days, and after 21 days its level was 49% higher than the initial one. The concentration of intracellular protein changed little, and after 5 days it fell by 17%, but by the end of cultivation it was 28% more than the initial one. The levels of chlorophylls *a* and *b* in comparison to the beginning of the experiment after 5 days were increased by 4.0 and 7.7 times, respectively. They fell down later, but by the end of cultivation they were 3.4 and 4.8 times higher than the starting point, respectively. The carotenoid content in chlorella cells fell sharply after 5 days of culture growth – by 95%, then it rose, and after 21 days it was 2 times higher than the original. Therefore, despite the absence of phosphates and manganese in the nutrient medium, the chlorella culture remained viable.

Addition of 0.025 mg/L of $MnCl_2$ and 1.784 g/L orthophosphate to the medium (variant *A*) caused after 6–7 days a growth of biomass and intracellular protein by 74% and 23%, respectively, and by the end of culture cycle – by 2.4 and 1.8 times, respectively. The concentrations of chlorophylls *a* and *b* were increased after 5 days and by the end of cultivation exceeded the initial level by 4.6 and 7.8 times. Carotenoid content in chlorella cells increased by only 66%.

In presence of orthophosphate 2.007 and 2,230 g/L in the medium (variants *B1* and *B2*) after 24 h, the biomass level was lower than the previous variant *A* by 31–32%, and in comparison with the control – by 23–24%. The maximum increase in biomass and intracellular protein in these variants was observed after 19 days – 3.0–3.4 and 1.7–1.9 times in the comparison with the beginning of culture growth, respectively. The content of chlorophyll *a* in variants *B1* and *B2* after 11-13 days increased by 46-109%. In the subsequent period, a decrease in its level was noted with reaching a maximum after 21 days. However, the value of this maximum was lower to that in variant *A* and even the control variant. The content of chlorophyll *b* had the maximum after 21 days: 6.4 and 7.4 times from the initial value. However, its level was also lower compared to variant *A*, but exceeded that of the control variant. As for the level of carotenoids, when growing in *B1* variant, it increased in the period of 15–21 days compared with the beginning of cultivation by 1.8–2.3 times. In the case of the *B2* combination, the carotenoid content dropped by 95% by the end of the culture cycle.

In case of increase of $MnCl_2$ to 0.05 mg/L at an orthophosphate concentration of 1.784 g/L in medium (variant *C*), biomass and intracellular protein levels reached a maximum after 19 days: the increase was 4.9 and 2.2 times compared to the beginning of cultivation, respectively. Moreover, these values exceeded those of variants *A*, *B1* and *B2* by 1.8–2.2 and 1.3–1.5 times, respectively. The content of chlorophylls *a* and *b* increased (as in variant *A*) after 5 days (2.5 and 3.1 times, respectively, by the beginning of culture growth) and by the end of the experiment exceeded the initial level by 5.4 and 3.4 times, respectively. However, these values were 37% and 2.8 times lower than those of variant *A* and even the value of the control variant by 24% and 47%. The dynamics of the level of carotenoids was complex and of oscillatory manner. It was noteworthy that after one day of growth, the concentration of carotenoids in chlorella cells on medium of variant *C* was significantly lower than in variants *K*, *A* and *B* – by 3.6–5.0 times. However, after 21 days, the value increased by more than an order of magnitude and exceeded that at the beginning of algal growth and was noticeably higher than in variants *K*, *A* and *B*.

The addition of orthophosphate to the variant *C* of nutrient medium at concentrations of 2.007 and 2.230 g/L (variants *D1* and *D2)* was accompanied by a decrease in the yield of chlorella biomass and intracellular protein compared to variant *C* by 42–48% and 17–28%, respectively. Although it exceeded the other variants of the biomass yield, but had no fundamentally differences from them in terms of intracellular protein content. The maximum content of chlorophylls *a* and *b* in chlorella cells during cultivation on media of variants D1 and *D2* was lower not only compared to the other variants of the medium, but even in comparison to the control variant. Only carotenoids concentration differed significantly in comparison to these variants or exceeded those of *B2* variant.

The above materials showed quite clearly the possibility of a significant change in the physiological and biochemical state of the chlorella cell with changes in the composition of the culture medium. This particular study focused on biomass yield and intracellular protein concentration. The best results were achieved at a nutrient medium with 0.05 mg/L $MnCl_2$ and 1.784 g/L orthophosphate (variant *C*). However, chlorella produces many biologically active compounds. Here it is enough to mention the so-called chlorellin, the chemical nature of which, despite its strong biological effect, remains unclear. Certainly, to obtain other target products from chlorella, the optimal nutrient composition will differ from the described above.

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