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POSSIBILITY OF *DESMODESMUS ARMATUS* (CHOD.) HEGEW. CULTIVATION IN MIXOTROPHIC CONDITIONS

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The study is devoted to investigation of the possibility of green microalga Desmodesmus armatus cultivation in mixotrophic conditions. Microalgae were cultivated on the waste water from RAS. To create mixotrophic conditions in the nutrient medium, glucose was added in the amount of 0.5, 2 and 4 g/l. Cultivation was carried out in a climatic room with a 16-hour photoperiod for 21 days. The rate of biomass growth was analyzed in the cultivation dynamics with presence of glucose in a nutrient medium. In the obtained biomass, the indicators of crop productivity were analyzed – the amount of protein, lipids and photosynthetic pigments. It is noted that the introduction of glucose in an amount of 0.5 g/l allows to increase in biomass yield by six times. It is established that in all variants of the experiment the amount of protein remains unchanged, however, the amount of lipids, chlorophyll a and carotenoids increases. The optimum concentration of glucose for the growth of Desmodesmus armatus in mixotrophic conditions is the amount of 0.5 g/l of nutrient medium.

Keywords: Desmodesmus armatus (Chod.) Hegew., waste water from RAS, mixotrophic conditions.

Introduction. Photoautotrophic way of feeding in terms of scale and significance has become one of the main ways of feeding algae and other green plants. However, many algae have the ability to switch easily under certain conditions from a photoautotrophic feeding method to assimilation of various organic compounds and to carry out a heterotrophic, photoheterotrophic or mixotrophic type of diet. Mixotrophs are organisms that have a mixed type of nutrition - autotrophic nutrition with inorganic substances (due to photosynthesis) and heterotrophic (exogenous organic compounds) (Zolotareva et al., 2008).

The ability to grow on organic substrates in the dark or in the light in the absence of CO_2 is shown for a large number of species belonging to green, yellow-green, diatom algae and cyanobacteria (Afiukwa et al., 2007; Gribovskaia et al., 2011). For example, *Chlorella vulgaris* is an optional autotrophic microorganism that can use different types of nutrition. It can grow under the condition of lighting, using carbon dioxide, and in the dark under heterotrophic conditions. Chlorella is capable of mixotrophy, or a mixed type of nutrition, that is, simultaneous use in the processes of biosynthesis of CO_2, which is assimilated in the process of photosynthesis, and organic compounds coming from outside (Heredia-Arroyo et al., 2011). However, for the green microalga *Desmodesmus armatus*, there is no data on the possibility of culturing it in mixotrophic conditions. The use of mixotrophic cultivation conditions would provide a sufficient amount of *D. armatus* biomass for a short time for the needs of aquaculture.

Representatives of different algae groups can act as a source of useful products, in particular: lipids, proteins, carbohydrates, dyes, biologically active compounds, etc. (Becker, 2007; Brown et al., 2002). To simulate mixotrophic conditions and increase the yield of useful metabolites in the cultivation medium, it is possible to add organic substances that are supposed to stimulate growth and the development of algae and at the same time the accumulation of valuable metabolic products. The yield of these products can be increased when certain organic carbon sources are added to the cultivation medium. Organic substrates can be monosaccharides, organic acids, including amino acids (glycine, alanine, etc.). The assimilation of sugars by algae is significantly affected by their concentration, as well as illumination. The introduction of glucose into the nutrient medium affects the biosynthesis and the ratio of photosynthetic pigments (Sivash et al., 2001) carbohydrate metabolism and the fractional composition of proteins (Mushak, 2007). Glucose as an organic substrate can fully meet the need for algoculture in both carbon and energy (Sivash et al., 2001).

The purpose of this study was to assess the possibility of culturing *Desmodesmus armatus* in mixotrophic conditions when introducing glucose into the nutrient medium.

Materials and Methods. The research was conducted on an algological pure culture of green alga, *Desmodesmus armatus* (Chod.) Hegew.

(IBASH-A), obtained from the collection at the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine.

Microalgae *D. armatus* cells have an oval-cylindrical shape, often with widely cone-narrowed and rounded poles. Their dimensions are 10-20 µm in length; 3.5 - 8 µm in width. All the operations connected with the culture planting were carried out under sterile conditions (www.algaebase.org).

As a nutrient medium for the cultivation of *D. armatus*, waste water was used from a recirculating aquaculture system, standardized for pH values (U-160 MU ion meter) and total mineralization (COM-100 Water Quality Tester Conductometer) (Cheban et al., 2015). Glucose in the amount of 0.5, 2 and 4 g / l was added to the nutrient medium.

Cultivation in the light was carried out in a climatic room under a 16-hour photoperiod, when illuminated with fluorescent lamps 2500-4000 lux and 28 ± 2 °C (Cheban et al., 2015). Cultivation lasted for 21 days, every 7 days the growth activity of monoculture was analyzed. The density of the culture was determined spectrophotometrically by an indirect optical index at 750 nm at SF-46. The transition from the units of optical density (D_{750}) to the value of absolutely dry biomass (ADB) was carried out through the empirical coefficient k (Hevorhyz et al., 2008).

On day 21, the entire biomass was removed and a complex determination of the compounds (proteins, lipids and pigments) was performed therein. Isolation of algal cells from the culture medium was carried out by centrifugation at 5,000 rpm within 15 minutes on ‘Herauses’ Biofuga stratos. After centrifugation, the biomass of the microalgae was disintegrated by ultrasound on USDN-2T.

The pigments were extracted with a chloroform : ethanol mixture (2:1), centrifuged at 3000 rpm before discoloration of the extract. The pigment concentration was calculated according to the generally accepted formulas from the optical density values at wavelengths corresponding to absorption maxima of chlorophyll a and b and total carotenoids (Sanchez et al., 2008).

The content of main nutrients was defined in the studied samples. Total lipids extracted with Folch method (Folch et al., 1957) were determined after acid hydrolysis of the samples with the subsequent reaction between decomposition products and the phospho-vanillin reagent (Knight et al., 1972). Total protein content was defined by Lowry method (Lowry et al., 1951). All calculations were performed on dry weights. To determine humidity and dry weights, previously weighed samples were dried at 60°C for 24 hours to constant mass.

Mean values were considered significantly different at $P \leq 0.05$ according to Student’s criterion. The results were analysed statistically with Microsoft Excel software according to generally accepted methods.

Results. To study the effect of glucose on the growth of the culture and on its physiological and metabolic parameters, we applied sterile glucose into the medium to a final concentration of 0.5 g/l, 2 g/l, 4 g/l. At this stage it was noted that during the first seven days of the study the amount of biomass sharply increased by 2.5 times. On the 14th day of cultivation, there is a significant deterioration in the growth of *D. armatus* culture when glucose is introduced. However, for 21 days the culture is adapted and the amount of biomass is doubled. We can also observe that a culture grows better in an environment with a glucose concentration of 0.5 g/l. In comparison with the initial indices, the amount of *D. armatus* biomass under such conditions increased almost 6-fold at the final stage of cultivation (Fig. 1).

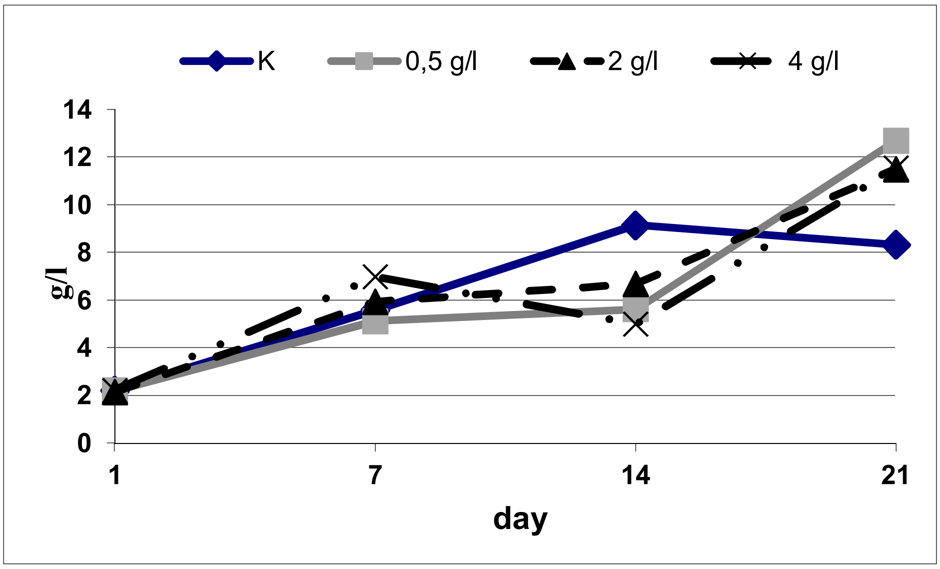


Fig.1. The biomass of D. armatus, provided that glucose is present in the nutrient medium

Thus, we have demonstrated the possibility of culturing *D. armatus* in myxotrophic conditions, using glucose as a carbon source.

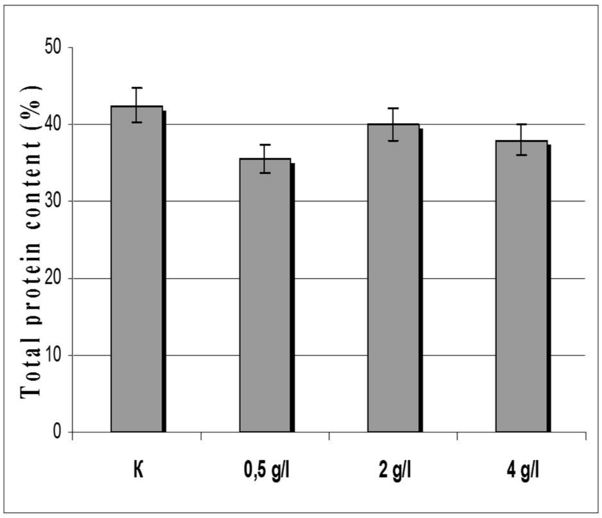
Microalgae, which are involved in aquaculture as a food object, should be characterized by high protein content (Ishaq et al., 2016). It is known that the biomass of green algae can contain about 30-50% protein, and the amount of this protein can vary depending on the conditions of cultivation and nutrient composition (Becker, 2007, Cetin et al., 2015). The changed cultivation conditions affect not only the quantitative indices of the protein in biomass, but its qualitative characteristics can also change. That is why we decided to determine the effectiveness of our nutrient medium saturated with glucose on the accumulation of protein by algal cells. We noted that the introduction of glucose did not lead to an increase in the amount of proteins in algal cells (Fig. 2).

Such results are somewhat contradictory in the literature (Mushak, 2007) on the stimulating effect of monosaccharides on protein biosynthesis.

A completely different picture was noted when studying the amount of lipids and pigments in *D. armatus* biomass. When the alga *D. armatus* was cultivated at a glucose concentration of 0.5 g/l, a decrease in lipids was observed with respect to the control values. However, even with an increase in glucose concentration to 2 g/l, the lipid content increases to 16.8%, and at a concentration of 4 g/l, the amount of lipids is 14.1%. It is with the increase in lipids in the biomass of algae that most authors attribute the stability of their cells to the changed conditions of cultivation, as it is known that the stress resistance of cell membranes is largely determined by protein-lipid interactions (Vasilenko et al., 2014).

It is known that with changing over to mixotrophic growth conditions, algae do not lose the ability for intensive photosynthesis, but an increase in the concentration of osmotically active substances in cells can lead to a slowdown of all biochemical reactions. The intensity of photosynthesis in the mixotrophic cultivation conditions will be indicated by the accumulation of pigments in the biomass of the alga under investigation. The introduction of glucose leads to an increase in the amount of chlorophyll a and carotenoids (Fig. 3). The amount of chlorophyll a in biomass *D. armatus* increases by 1.5 - 2 times in all variants of the experiment. The optimal concentration for the accumulation of chlorophyll a is 0.5 g/l.

The amount of carotenoids increases proportionally to the increase in the amount of glucose in the nutrient medium and reaches the maximum values provided that 4 g/l of glucose is applied. Most likely, this result is evidence of the formation of a stressful situation for *D. armatus* cells, provided that glucose is introduced into the nutrient medium in significant amounts.



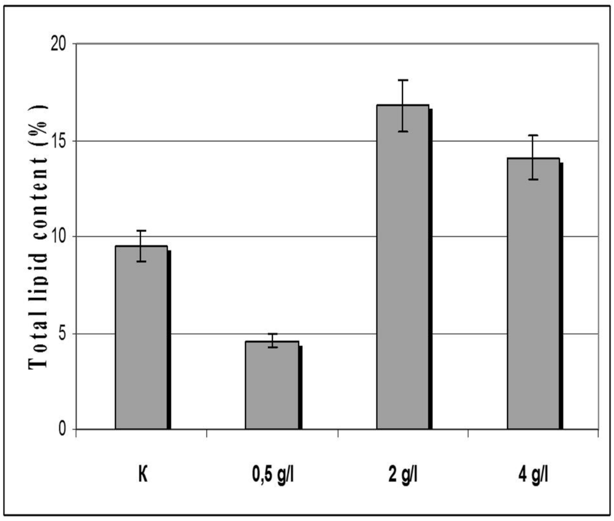


Fig.2. The content of total protein and total lipids of D. armatus, provided that glucose is present in the nutrient medium

*Note: * - a significant difference from the reference values*

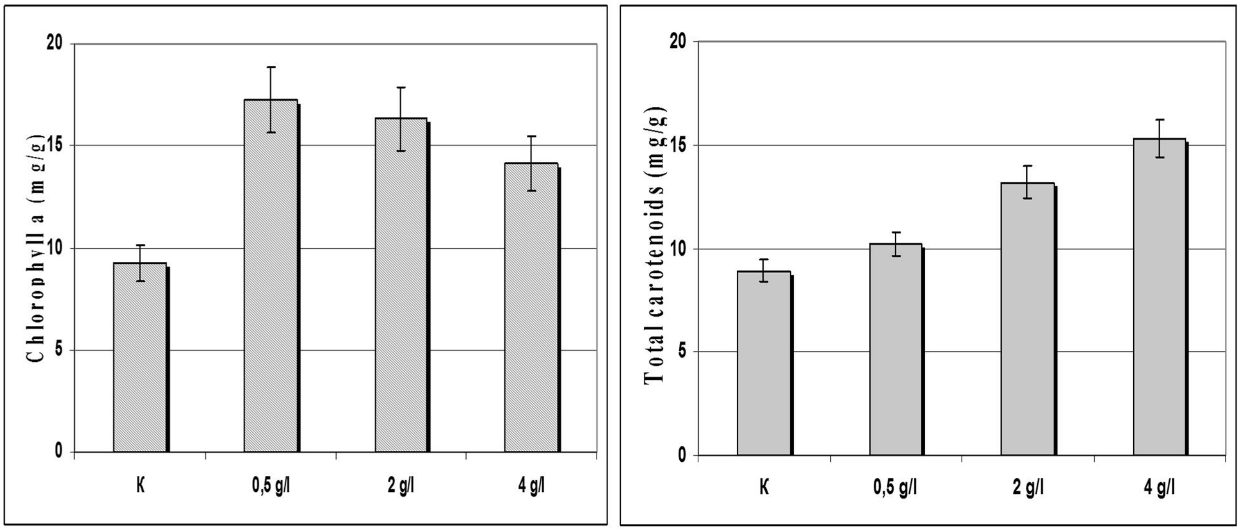


Fig.3. The content of chlorophyll a and total carotenoids D. armatus, provided that glucose is present in the nutrient medium

*Note: * - a significant difference from the reference values*

The amount of carotenoids increases proportionally to the increase in the amount of glucose in the nutrient medium and reaches the maximum values provided that 4 g/l of glucose is applied. Most likely, this result is evidence of the formation of a stressful situation for *D. armatus* cells, provided that glucose is introduced into the nutrient medium in significant amounts.

Thus, the results of our studies have shown the possibility of mixotrophic growth for *D. armatus* culture. The introduction of glucose into the culture medium leads to a significant accumulation of biomass and pigments. However, under such conditions, an increase in the amount of protein is not observed. At the same time, the high content of chlorophyll a indicates the glucose support of the pigment system of this culture.

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МОЖЛИВІСТЬ КУЛЬТИВУВАННЯ *DESMODESMUS ARMATUS* (CHOD.) HEGEW. У МІКСОТРОФНИХ УМОВАХ

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Робота присвячена вивченню можливості культивування зеленої мікроводорості Desmodesmus armatus у мікотрофних умовах. Мікроводорість культивували на скидній воді із УЗВ. Для створення мікотрофних умов у живильне середовища вносила глюкозу у кількості 0.5, 2 та 4 г/л. Культивування проводили у кліматичній кімнаті при 16-годинному фотоперіоді протягом 21 доби. В динаміці культивування аналізували швидкість нарощування біомаси за умов присутності глюкози у живильному середовищі. У отриманій біомасі аналізували показники продуктивності культури – кількість білка, ліпідів та фотосинтезуючих пігментів. Відмічено, що внесення глюкози у кількості 0,5 г/л дозволяє збільшити вихід біомаси у шість разів. Встановлено, що у всіх варіантах досліду кількість білка залишається незмінною, проте збільшується кількість ліпідів, хлорофілу а та каротиноїдів. Оптимальною концентрацією глюкози для вирощування Desmodesmus armatus у мікотрофних умовах є кількість 0,5 г/л живильного середовища.

Ключові слова: Desmodesmus armatus (Chod.) Hegew., стічні води з РАН, міксотрофні умови

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