EXPERIMENTAL ARTICLES

UDC 582.263.1:[604.2:547.979.8] https://doi.org/10.15407/biotech12.02.046

INDUCTION OF CAROTENOGENESIS IN *Desmodesmus armatus* (Chod.) Hegew CULTIVATED ON THE CLOSED WATERSIDE FROM RECIRCULATING AQUACULTURE SYSTEM

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Received 25.02.2019
Revised 29.03.2019
Accepted 10.05.2019

The aim of the study was to develop the biotechnology approach for obtaining secondary carotenoids of green microalgae *Desmodesmus armatus* (Chod.) Hegew. under conditions of two-stage cultivation on waste water from recirculating aquaculture system in response to the action of inducers of different origin. By chemical nature, secondary carotenoids are C40-ketocarotinoids — intermediates of enzymatic oxidation of β-carotene to astaxanthin.

The study presents the conditions for cultivation of *D. armatus* on the waste water from the recirculating aquaculture system by a two-stage accumulation process, where conditions for rapid growth of biomass were created at the first stage, and the biosynthesis of the target product was induced by the introduction of carotenoid biosynthesis precursors ($C_6H_{12}O_6$, CH_3COONa), promoters of free radical oxidation ($FeSO_4$ / H_2O_2) or osmotic stress (NaCl) into the nutrient medium.

It was shown that the first phase of cultivation was characterized by high growth and productive indices: the amount of biomass is up to 13 g/l, the content of total proteins was 37.9 %, lipids — 26 % and total carotenoids — 7.5 % per gram of dry biomass. Among carotenoids, the presence of zeaxanthin, lutein, β-carotene, insignificant amounts of astaxanthin, canthaxanthin, esters of adonixanthin and astaxanthin were detected.

The features of the adaptive response of *D. armatus* to the influence of factors that induce secondary carotenogenesis are established. Among them is retention of the number of cells or doubling of their number during the use of chemical activators. Decrease in the activity of cytochrome oxidase as an indicator of the metabolic activity of the culture.

Thus, the possibility of increasing the content of β-carotene and astaxanthin in *D. armatus* biomass, essential for fish and crustaceans, by introducing promoters of free radical oxidation and osmotic stress NaCl (200 mM) or Fe^{2+} (200 mM) and H_2O_2 (10^{-4} mM) into the waste water from RAS in the second phase of cultivation was established. Metabolic disbalance in *D. armatus* cells, which were observed under the influence of chemical factors, led to a redistribution of the main nutrients profile. Biosynthesis and accumulation of lipids were activated against the background of intensive carotenogenesis.

Key words: *Desmodesmus armatus*, two-stage accumulative cultivation, recirculating aquaculture system RAS, secondary carotenoids.

To date, most of the technologies of microalgae cultivation are aimed at correcting their nutrient composition due to saturation with various essential compounds, including carotenoids [1]. The latter are used as food supplements, preventive agents, they are also irreplaceable components of feed and premixes in fish and crustaceans aquaculture [2]. So, the color of the skin, muscles and caviar is associated with the content of carotenoids and their composition, and they are also an important factor in the formation of the reproductive products of fish and the survival of their larvae [3]. As in breeding of fish in aquaculture, the composition of feed is formed artificially, the introduction of

carotenoids in the diet is very important [4, 5]. Astaxanthin, zeaxanthin, β-carotene also play an important role in providing antioxidant protection, reproductive functions, promoting growth and immunity. Microalgae are the qualitative natural source of carotenoids in the composition of aquaculture feeds and premixes [6, 7]. Only some representatives belong to those capable of synthesizing β-carotene, astaxanthin, essential for aquaculture. Thus, the commercial producer of β-carotene is halophilic green algae *Dunaliella salina*, and the source of natural astaxanthin is *Haematococcus pluvialis*. The first microalgae requires high salinity of the medium, while cultivating of another is accompanied by difficulties in avoiding of culture contamination. Their cultivation on the waste water from the recirculating aquaculture system (RAS) can be quite labor-consuming [8, 9]. Therefore, the search for new producers of carotenoids among the representatives of algae and the development of schemes for their obtaining is relevant.

Nowadays potential producers of ketocarotenoids are considered to be representatives of green algae of the genera *Chlorella*, *Chlamydomonas*, *Muriellopsis* and *Scenedesmus* [10, 11]. We have shown the possibility of using green microalgae *Desmodesmus armatus*, which is one of the potential producers of secondary carotenoids grown on the waste water from RAS, as sufficient for the nutritional composition of the feed substrate for *Daphnia magna* [12]. The ability of this microalgae to rapid biomass accumulation, as well as the simple technology of industrial cultivation, makes it one of the most promising objects in aquaculture. The great advantage of *D. armatus* in comparison with other is not only the simplicity of cultivation, but also the lability of the chemical composition, which allows conducting biosynthesis of valuable chemical compounds, among which carotenoids occupy a dominant position. However, taking into account our previous results, in order to increase aquatic feed's value of such biomass, the quantitative content of valuable ketocarotenoids should be increased. This can be achieved by introducing inducers of carotenogenesis into the nutrient medium, namely waste water from RAS. For other representatives of microalgae it has been shown that reactive oxygen species (ROS) are involved in the induction of secondary carotenoids synthesis, as the introduction of generators (sodium chloride, hydrogen peroxide, ferrous sulphate) into the medium triggers the hyperinsynthesis of secondary carotenoids. Most likely, the ROS act as secondary messengers, activating ways of biosynthesis of secondary carotenoids, in particular astaxanthin and its esters, by activation of the corresponding enzymes or inducing the expression of the genes that encode them. There is also an increase in the concentration of ROS under conditions of osmotic stress or with the reduction of the efficiency of carbon dioxide fixation. Mechanisms of induction of the secondary carotenogenesis with sodium acetate and glucose are also known. Perhaps these compounds are included in the energy and structural metabolism of algae cells via acetyl-CoA [13, 14].

In order to obtain the target product from the algaculture, it is expedient to use two-phase accumulating cultivation with the addition of activating compounds, mentioned above [15]. Using such a scheme at the first stage makes possible to create conditions for the rapid growth of biomass, and on the second, to induce biosynthesis of carotenoids.

Therefore, the aim of the study was to develop schemes for obtaining secondary carotenoids of green microalga *D. armatus* (Chod.) Hegew. under conditions of two-stage cultivation on the waste water from RAS in response to the action of precursors of biosynthesis, inducers or carotenogenesis stimulants.

Materials and Methods

The studies were carried out using a unialgal culture of green algae *Desmodesmus armatus* (Chod.) Hegew (IBASH-A), obtained from the collection of the Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, for which we express our gratitude to it.

Conditions of cultivation of the initial culture. The original unialgal culture of *D. armatus* was grown on the waste water from RAS. The water was taken from a mechanical filter, poured into aliquots, autoclaved for 30 min at 121 °C, standardized for pH and total mineralization. The inoculation was carried out in a ratio of the inoculum: the nutrient medium that is 1:10. All the manipulations related to the culture sowing were performed in a laminar-box. During the cultivation, physicochemical parameters were monitored: pH (7.5–8) (U-160 MU ionometer) and total mineralization of the medium (495±5 ppm) (Water Quality Tester COM-100 conductometer) [15, 16].

Climatic conditions of cultivation. The initial culture of *D. armatus* was grown in a climatic room at a temperature of 21±2 °C, illuminated with fluorescent lamps of 2500–4000 lux and a 16-hour photoperiod in 500 ml Erlenmeyer flasks [15, 16]. The distance to the light source does not exceed 0.5 m.

Conditions of two-stage cultivation. The first phase of accumulative culture of *D. armatus* lasted 16 days until the optimal density of culture was achieved (5×10^6 cells/l). Biomass of the first phase served as a source of inoculum, that was introduced into the medium for the second phase in a ratio of 1:10. A corresponding carotenogenesis inducer was introduced into each of the media: $FeSO_4$ (0.11, 0.22, 0.45 mM) with H_2O_2 (10^{-4} mM), NaCl (50, 100, 200 mM), $C_6H_{12}O_6$ or CH_3COONa (10, 25, 50 mM). Growth and productive indices of culture were analyzed in the dynamics of cultivation.

Evaluation of growth activity. The amount of biomass was determined from the culture density using an optical index at 750 nm at CaryWin UV 60 (Agilent, USA). 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:

$$ADB = k \times D_{750}.$$

The coefficient k (k = g unit density per liter) for the culture of *D. armatus* was determined experimentally in three independent repeats [17]. Morphological parameters were analyzed microscopically using the Goriaev camera, the MicroMed-3300 trinocular microscope (×1000) (Ukraine), and the computer program Micam 2.0. (http://science4all.nl/?Microscopy_and_Photography). The physiological state of the cells was assessed by the cytochrome oxidase test [18].

Evaluation of algal culture productivity. The microalgae suspension was centrifuged at 8000 rpm for 15 min on the Biofuge Stratos "Heraeus" (Germany) centrifuge. The hydrated cells were disintegrated on USDN-2T, 60 μA sonicator for 10 min [16]. The amount of total protein was determined by the method of Lowry [19], the total lipids by reaction with the phosphorus-vanillin reagent [20], the content of chlorophyll a, b and total carotenoids in acetone extracts by spectral photometry with CaryWin UV 60 (Agilent, USA) in range of wavelengths from 400 to 800 nm [21, 22]. The obtained values were converted to absolute dry biomass.

Thin layer chromatography (TLC). The fractional composition of carotenoids was analyzed by chromatography in a thin layer of sorbent (TLC) on "Silufol-UV-254" plates (Czech Republic) in a hexane: acetone (9:1) solvent system in an ascending manner in accordance with the requirements of the State Pharmacopoeia [23]. The plates with applied samples were placed in a darkened chromatographic chamber preliminarily saturated with a mixture of solvents. Carotenoid fractions were identified by the specific color of the spots and compared with typical values of *Rf* for primary and secondary carotenoids [24].

Isolation of individual carotenoids was carried out by preparative TLC. The zones corresponding to the individual compounds were removed from the support and eluted with petroleum ether (carotene) and ethanol (xanthophylls). The purity of the obtained fractions and their amount were checked spectrophotometrically by Cary 60 (Agilent, USA) using the software CaryWin UV.

Statistical processing of results. Statistical processing of the obtained results was carried out using Microsoft Excel software. Differences in the results are significant at the level $P \le 0.05$ by the Student's criterion.

Results and Discussion

When developing schemes for the induction of secondary carotenogenesis on the waste water from RAS, the classical scheme of two-phase accumulation culture was chosen as the basis and adapted to increase the yield of secondary carotenoids of *D. armatus* (Fig. 1).

Thus, it is known from literature sources that the most effective method for obtaining biomass of microalgae enriched with carotenoids is a two-stage accumulative culture. Under such conditions, optimal conditions for rapid growth of biomass are created at the first phase, and biosynthesis of target metabolites is induced at the second phase [25]. This method avoids the biosynthesis of the target metabolite in a culture whose cells are actively dividing, since a combination of both processes can be inefficient. In the first phase of cultivation, the inoculum of the original culture was introduced into a fresh nutrient medium, which was the waste water from RAS. This made it possible to obtain an actively growing culture, with high productive indicators: the amount of total protein — 37.9%, lipids — 26% and total carotenoids — 7.5%. In addition, up to the end of the first phase of cultivation, a rapid increase in the biomass to 13 g/l was observed (Table 1).

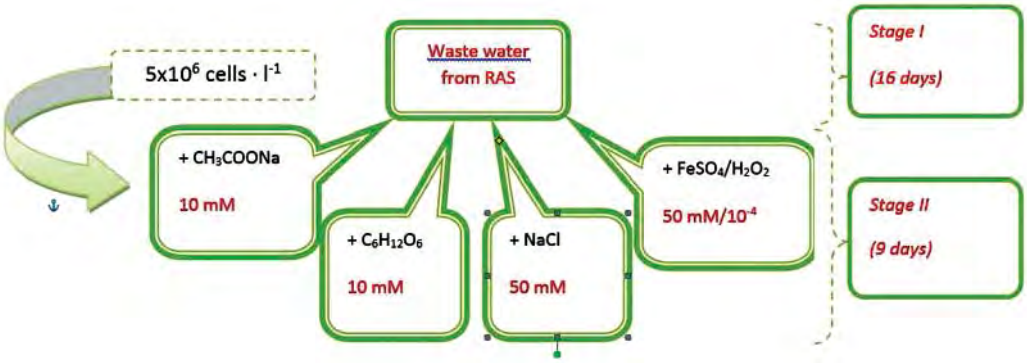

Fig. 1. **Scheme of two-stage accumulative cultivation of *D. armatus***

Table 1. **Dynamics of biomass and number of cells, content of total protein, lipids and carotenoids at the first stage of *D. armatus* cultivation**

Day of cultivation	Biomass, $g \cdot l^{-1}$	Proteins, %	Lipids, %	Carotenoids, %
1	8.8±0.57	33.6±2.17	23.4±1.69	4.6±0.11
5	9.1±0.81	35.3±2.87	24.0±1.92	5.8±0.34
10	10.7±0.85	37.0±3.11	25.7±2.41	6.3±0.54
15	13.0±0.97	37.9±3.67	26.0±2.89	7.5±0.87

This is probably due to the availability of components of mineral nutrition in the waste water. At the same time, 8 fractions of carotenoids were detected in *D. armatus* biomass. Among these compounds, the basis is formed by primary carotenoids, in particular zeaxanthin, lutein, β-carotene. Also, a small amount of astaxanthin, canthaxanthin, as well as esters adonixanthin and astaxanthin, is present (Fig. 2).

So, stage I of cultivation allows to obtain a culture characterized by a constant increase in biomass, a high content of proteins, lipids, carotenoids. Such a culture can be used in the future to receive target metabolites, in particular for the accumulation of secondary carotenoids.

The transition of the culture to the second phase was caused by the introduction of carotenoid biosynthesis precursors

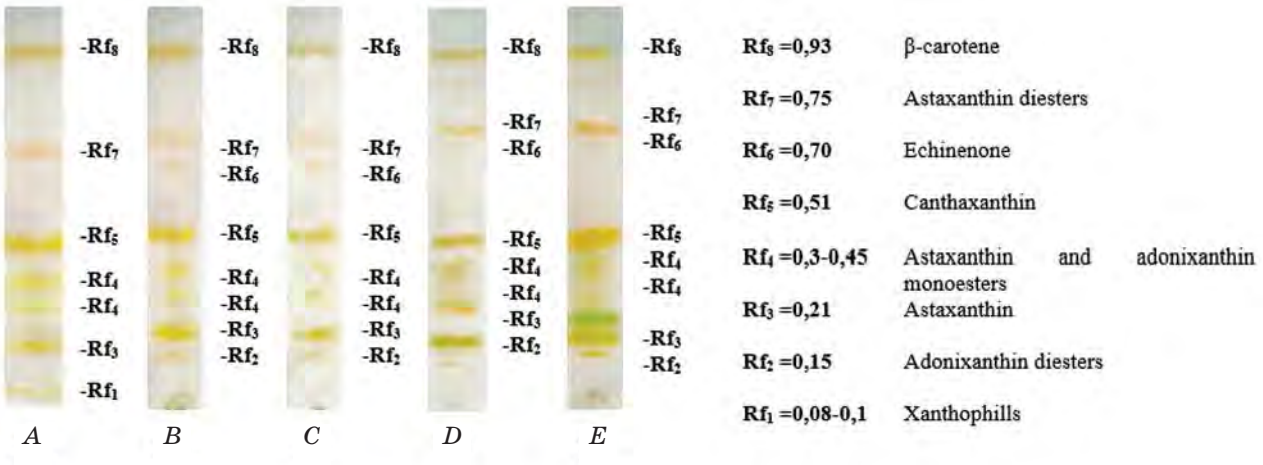

Fig. 2. **Fractional content of *D. armatus* carotenoids at the stage I (*A*) and II of cultivation after introduction of CH_3COONa (*B*), $C_6H_{12}O_6$ (*C*), NaCl (*D*), Fe^{2+} /H_2O_2 (*E*)**

($C_6H_{12}O_6$, CH_3COONa), promoters of free-radical oxidation ($FeSO_4/H_2O_2$) or osmotic stress (NaCl) into the nutrient medium. It is important to control the physiological state and growth activity of the culture. In all cases, mass death of cells was not observed while introducing inducers (Fig. 3). The addition of $FeSO_4/H_2O_2$ and NaCl led to inhibition of growth activity. Under these conditions, the number of cells remained at the level of the first day of the second stage of cultivation for all days of cultivation and was about 4×10^6 cells/l at day 9.

Such suppression of the growth activity of the culture may be due to changes in osmotic balance or the accumulation of reactive oxygen substances [26]. At the same time, when CH_3COONa was applied at the terminal stage, the number of cells did not exceed 8×10^6 cells/l, and when $C_6H_{12}O_6$ was applied, this value was within 9×10^6 cells/l.

In addition, changes in the number of cells were also accompanied by changes characteristic of the carotenoids accumulation, in particular, it is shown for other species of genera *Desmodesmus* and *Acutodesmus*, provided that carotenogenesis is introduced into the medium of inducers [26, 27].

Against the background of morphological changes, the inhibition of metabolic activity of the culture was observed, which is proved by the cytochrome oxidase test (Fig. 4).

The effect of $FeSO_4$ with H_2O_2 and NaCl led to a decrease in the activity of cytochrome oxidase by a factor of two as early as the 3rd day of the second phase of cultivation. Most likely, osmotic balance disturbances have the same effect on biosynthetic processes as the accumulation of ROS. At the same time, the precursors of the biosynthesis of carotenoids, sodium acetate and glucose, negatively suppress biosynthetic processes in cells, as against the background of a slight decrease in growth indices, the activity of cytochrome oxidase at terminal stages of cultivation decreases by only 23% in both cases. At the same time, the important question is what metabolites are accumulated under such conditions. The introduction of inductors into the medium primarily affects the efficiency of the accumulation of pigments. Thus, the use of promoters of free-radical oxidation ($Fe_2^+ + H_2O_2$) and osmoregulator (NaCl) leads to an increase in the number of carotenoids from 14% to 27% (Fig. 5).

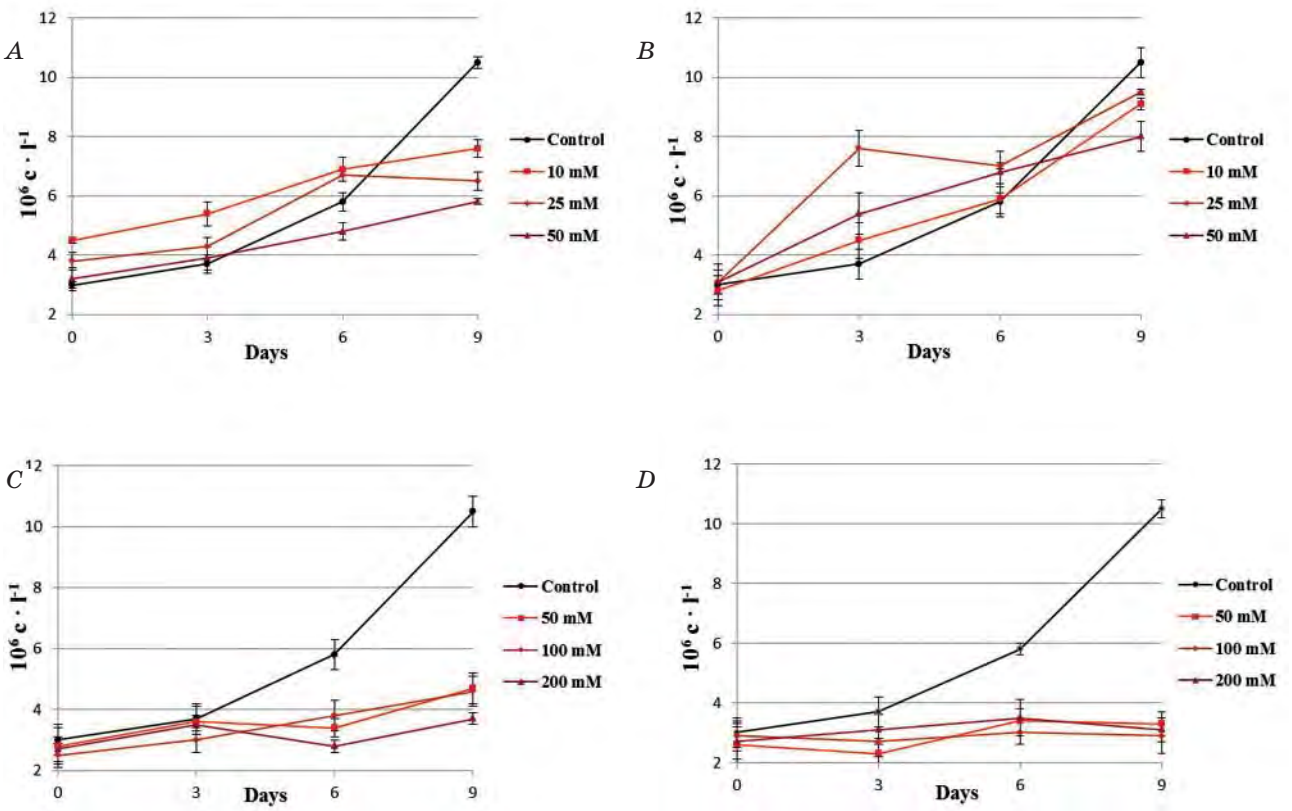

Fig. 3. ***D. armatus*** **cell number dynamics at cultivation stage II after introduction of CH_3COONa (*A*), $C_6H_{12}O_6$ (*B*) NaCl (*C*), Fe^{2+}/H_2O_2 (*D*)**

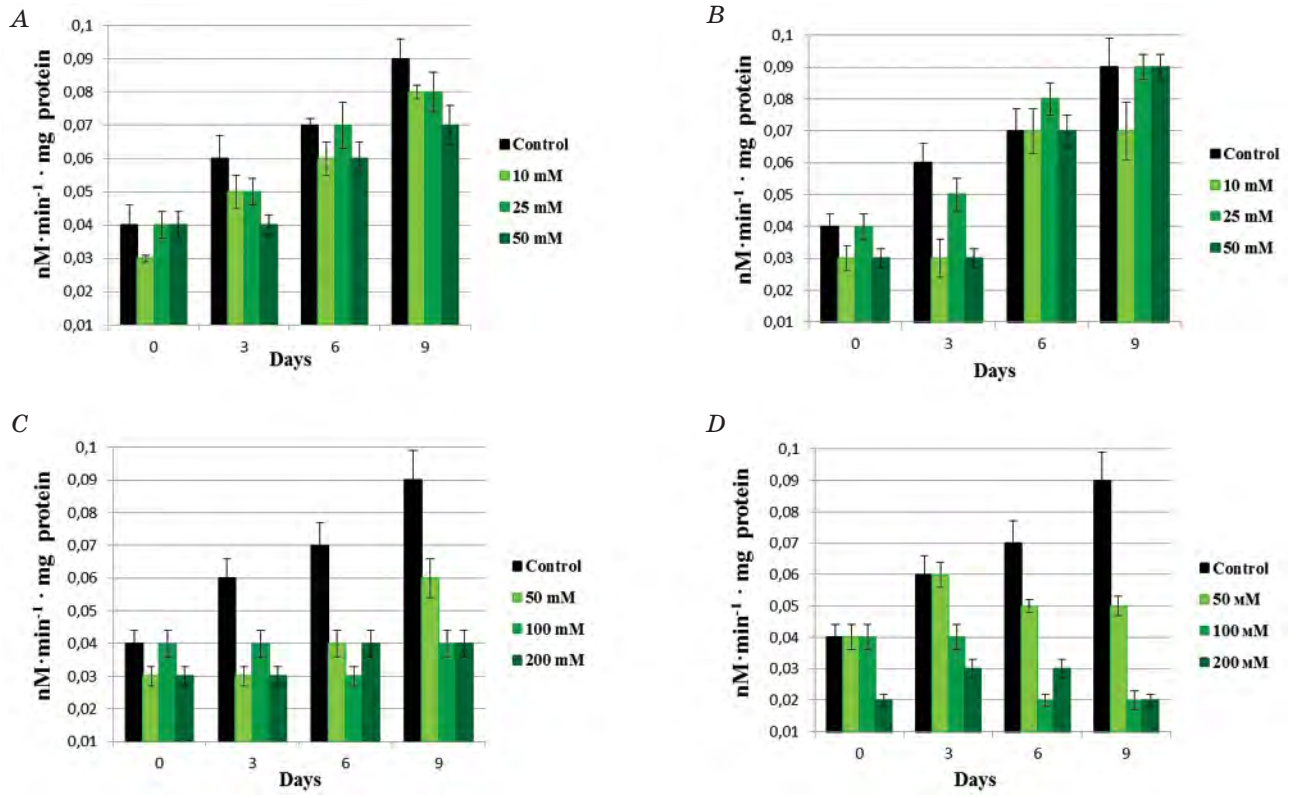

Fig. 4. ***D. armatus*** **cytochrome oxidase activity dynamics at the cultivation stage II after introduction of CH_3COONa (*A*), $C_6H_{12}O_6$ (*B*) NaCl (*C*), Fe^{2+}/H_2O_2 (*D*)**

Note. Here and after * — differences among groups are statistically significant at $P \leq 0.05$

Table 2. **Fractional content of *D. armatus* carotenoids**

Fraction	CH_3COONa	$C_6H_{12}O_6$	NaCl	Fe^{2+}/H_2O_2	Control
β-Carotene	17.87±0.16*	23.70±1.24*	32.45±2.02	37.04±2.86*	29.1±2.16
Astaxanthin monoesters	10.17±0.16*	12.76±0.54*	13.19±1.89*	12.32±1.03*	5.11±0.93
Astaxanthin diesters	9.30±0.53*	13.08±0.54*	11.76±0.97*	11.54±0.68*	3.04±0.18
Astaxanthin	36.52±3.55*	21.49±2.13*	20.23±1.65*	23.73±2.67*	13.6±2.17

At the same time the use of glucose and sodium acetate leads to increase in the content of carotenoids to only 18%. However, changes in the composition of the nutrient medium affect not only the qualitative composition of carotenoids, but also their quantitative characteristics. Thus, during the second stage of cultivation, the quantitative composition of individual fractions of carotenoids varies significantly (Table 2). A redistribution of the carotenoid profile was observed when precursors of biosynthesis were introduced. Thus, a decrease in the content of primary carotenoids and an increase in the fraction of secondary carotenoids were noted.

With the use of free radical oxidation promoters (Fe^{2+} + H_2O_2) and osmotic stress (NaCl), the share of β-carotene increases to 37%, astaxanthin — to 24%. When NaCl and $FeSO_4/H_2O_2$ were used, the content of β-carotene increased in parallel with the content of secondary carotenoids. In this case, it is obvious that β-carotene is formed *de novo* in chloroplasts and is transported to the cytoplasm, where it is oxidized through a series of intermediates to astaxanthin by the

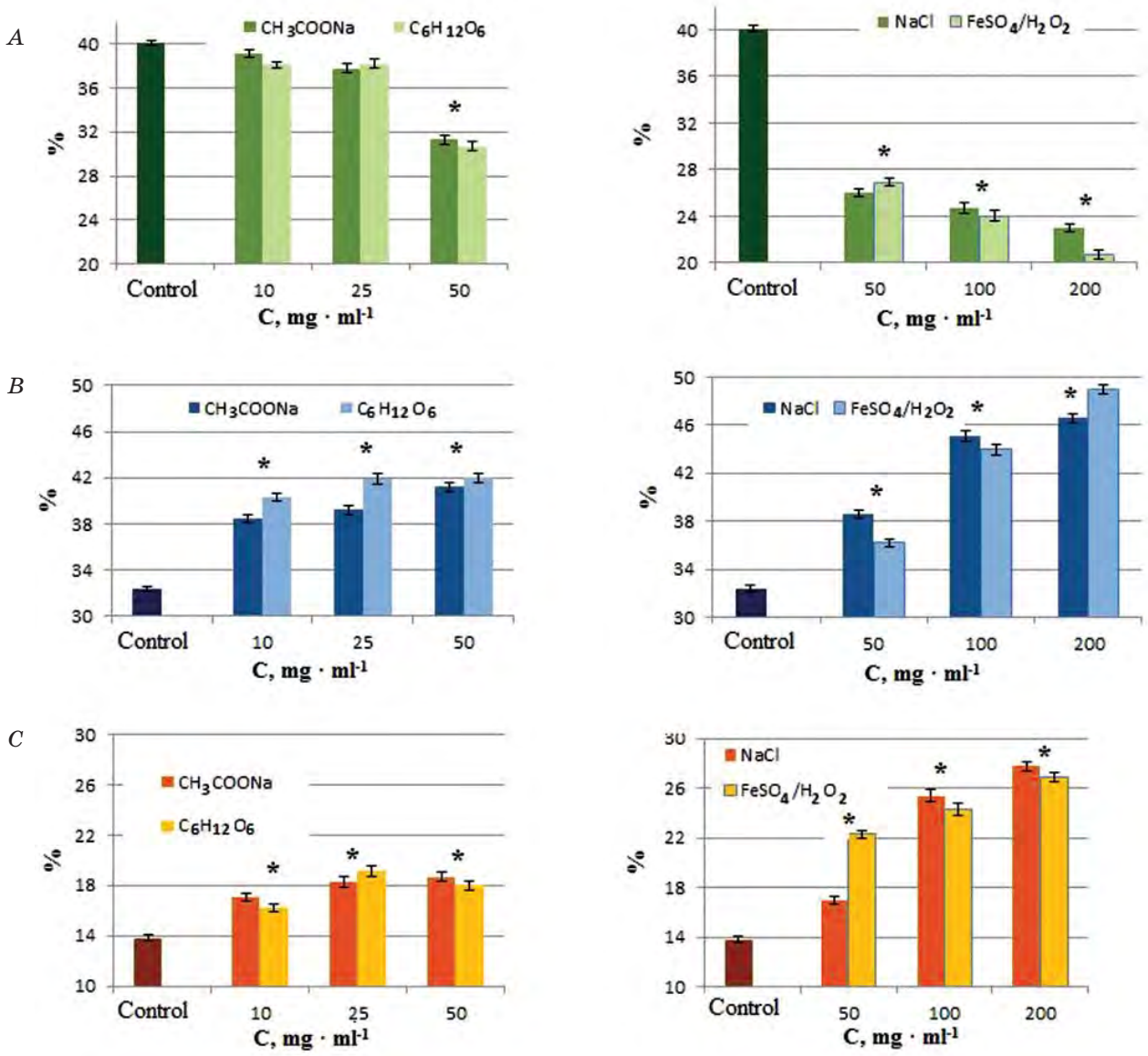

Fig. 5. **Content of total proteins (*A*), lipids (*B*), carotenoids (*C*) of *D. armatus* at the end of cultivation stage II during the induction of carotenogenesis**

action of ketolases and hydroxylases, that are accumulated in *D. armatus* cells due to abiotic stress. This phenomenon may indicate the functioning of two main pathways of secondary carotenoid biosynthesis or their branching at key points of bifurcation [28].

Intensification of carotenogenesis can also lead to changes in the metabolism of other nutrients. Thus, during the induction of biosynthesis of secondary carotenoids, the profile of the main nutrients is redistributed [26]. The basic role in stressful conditions is played by adaptive metabolic systems, in particular quantitative and qualitative changes in biosynthesis of lipids and proteins. Considering the factors that activate carotenogenesis and the physiological effects of their accumulation, special attention is paid to the connection of the biosynthesis of secondary carotenoids with the activation of biosynthesis and the accumulation of lipids, which are an energy reserve [29]. Thus, the accumulation of biomass in *D. armatus* culture was mainly due to an increase in the lipid content. When introduced into the medium CH_3COONa in *D. armatus* biomass, the content of total lipids increased to 41% against the background of a decrease in the total proteins content. Similar trends were also observed using $C_6H_{12}O_6$ at the same concentrations. An increase in the content of carotenoids is always accompanied by an increase in the content of lipids, since fatty acids

are necessary for the esterification of free OH groups in ketocarotenoid molecules, and neutral lipids are provided for their dissolution and functioning in the cytoplasm [29]. On the issue of patterns of protein accumulation in microalgae under conditions of enhanced carotenogenesis, there is no consensus in the literature. One of the opinions is that the enhanced carotenogenesis activates the synthesis of proteins, in particular those that stabilize the lipid structures of accumulation of secondary carotenoids [30]. In other cases, and in ours in particular, the induction of carotenogenesis proceeds without the intensification of protein synthesis [31, 32]. So, with the use of stress factors ($FeSO_4$/H_2O_2, NaCl), the protein content decreased almost 4-fold to the end of the second phase of cultivation and was at the level of 20%. At the same time, mass accumulation of carotenoids (25%) occurred against a background of intensive lipogenesis (46%).

Thus, the study shows the possibility of increasing the content of β-carotene and astaxanthin in *D. armatus* biomass, essential for fish and crustaceans, by introducing into the waste water from RAS promoters of free radical oxidation and osmotic stress NaCl (200 mM) or Fe^{2+} (200 mM) and H_2O_2 (10^{-4} mM) at the second phase of cultivation. Moreover, along with the processes of carotenogenesis activation, a redistribution of the main nutrient profile and accumulation of lipids occurs, which does not affect the feed value of this biomass.

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ІНДУКЦІЯ КАРОТИНОГЕНЕЗУ *Desmodesmus armatus* (Chod.) Hegew, КУЛЬТИВОВАНОЇ НА СКИДНІЙ ВОДІ ІЗ РИБОВОДНОЇ УСТАНОВКИ ЗАМКНУТОГО ВОДОПОСТАЧАННЯ

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Метою роботи було розробити схему отримання вторинних каротиноїдів зеленої мікроводорості *Desmodesmus armatus* (Chod.) Hegew. за умов двостадійного культивування на скидній воді з рибоводної установки замкнутого водопостачання — УЗВ у відповідь на вплив індукторів різної природи. За хімічною природою

ИНДУКЦИЯ КАРОТИНОГЕНЕЗА *Desmodesmus armatus* (Chod.) Hegew, КУЛЬТИВИРУЕМОЙ НА СБРОСНОЙ ВОДЕ С РЫБОВОДНОЙ УСТАНОВКИ ЗАМКНУТОГО ВОДОСНАБЖЕНИЯ

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Целью работы была разработка схем получения вторичных каротиноидов зеленой микроводоросли *Desmodesmus armatus* (Chod.) Hegew. в условиях двухстадийного культивирования на сбросной воде с рыбоводной установки замкнутого водоснабжения — УЗВ в ответ на воздействие индукторов различной природы.

вторинні каротиноїди є С40-кетокаротиноїдами — інтермедіатами ферментативного окиснення β-каротину до астаксантину.

Розроблено умови культивування *D. armatus* на скидній воді з УЗВ шляхом двостадійного накопичувального культивування, де на першому етапі було створено умови для швидкого нарощування біомаси, а на другому — індуковано біосинтез цільового продукту шляхом внесення у живильне середовище попередників біосинтезу каротиноїдів ($C_6H_{12}O_6$, CH_3COONa), промоторів вільнорадикального окиснення ($FeSO_4/H_2O_2$) чи осмотичного стресу (NaCl).

Показано, що культура I фази культивування характеризується високими ростовими та продуктивними показниками: кількістю біомаси до 13 г/л, загального протеїну — 37,9%, ліпідів — 26% та сумарних каротиноїдів — 7,5%. Серед каротиноїдів встановлено наявність зеаксантину, лютеїну, β-каротину, незначної кількості астаксантину, кантаксантину, ефірів адоніксантину та астаксантину.

Встановлено особливості адаптивної відповіді *D. armatus* на вплив факторів, що індукують вторинний каротиногенез, які включають: збереження чисельності клітин або збільшення її вдвічі в період використання хімічних активаторів; зниження активності цитохромоксидази, як показника метаболічної активності культури.

Таким чином, показано можливість збільшення у біомасі *D. armatus* вмісту цінних для аквакультури риб та ракоподібних β-каротину та астаксантину шляхом внесення у скидну воду з УЗВ промоторів вільнорадикального окиснення та осмотичного стресу NaCl (200 мМ) чи Fe^{2+} (200 мМ) з H_2O_2 (10^{-4} мМ) на другій фазі культивування. Порушення обміну речовин у клітинах *D. armatus*, які спостерігалися за дії хімічних факторів, призводять до перерозподілу профілю основних нутрієнтів. На фоні інтенсивного каротиногенезу активувалися процеси біосинтезу та накопичення ліпідів.

Ключові слова: *Desmodesmus armatus*, двостадійне накопичувальне культивування, рибоводна установка замкнутого водопостачання — УЗВ, вторинні каротиноїди.

По химической природе вторичные каротиноиды это С40-кетокаротиноиды – интермедиаты энзимного окисления β-каротина до астаксантина.

Разработаны условия культивирования *D. armatus* на сбросной воде с УЗВ путем двухстадийного накопительного культивирования, где на первом этапе были созданы условия для быстрого наращивания биомассы, а на втором — индуцирован биосинтез целевого продукта путем внесения в питательную среду предшественников биосинтеза каротиноидов ($C_6H_{12}O_6$, CH_3COONa), индукторов свободнорадикального окисления ($FeSO_4/H_2O_2$) или осмотического стресса (NaCl).

Показано, что культура I фазы культивирования характеризуется высокими ростовыми и продуктивными показателями: количеством биомассы до 13 г/л, общего протеина — 37,9%, липидов — 26% и суммарных каротиноидов — 7,5%. Среди каротиноидов установлено наличие зеаксантина, лютеина, β-каротина, незначительного количества астаксантина, кантаксантина, эфиров адониксантина и астаксантина.

Установлены особенности адаптивного ответа *D. armatus* на воздействие факторов, индуцирующих вторичный каротиногенез, который включает: сохранение численности клеток, или увеличение ее вдвое в период использования химических активаторов; снижение активности цитохромоксидазы как показателя метаболической активности культуры.

Таким образом, показана возможность увеличения в биомассе *D. armatus* содержания ценных для аквакультуры рыб и ракообразных β-каротина и астаксантина путем внесения в сбросную воду с УЗВ промоторов свободнорадикального окисления и осмотического стресса NaCl (200 мМ) или Fe^{2+} (200 мм) с H_2O_2 (10^{-4} мМ) на второй фазе культивирования. Нарушение обмена веществ в клетках *D. armatus*, которые наблюдались при воздействии химических факторов, приводят к перераспределению профиля основных нутриентов. На фоне интенсивного каротиногенеза активировались процессы биосинтеза и накопление липидов.

Ключевые слова: *Desmodesmus armatus*, двухстадийное накопительное культивирование, рыбоводная установка замкнутого водоснабжения — УЗВ, вторичные каротиноиды.

The work was carried out within the State Program of Yuriy Fedkovych Chernivtsi National University, Ukraine.