

Cultivating *Desmodesmus armatus* (Chod.) Hegew. in recirculating aquaculture systems (RAS) waste water

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Abstract. The possibility of using RAS waste water as a cultivation medium for *Desmodesmus armatus* (Chod.) Hegew. was investigated. Algal biomass increased continuously reaching its maximum on the day 40 of cultivation. *D. armatus* culture was characterized by a maximum content of total proteins of 23% on day 40 of cultivation. At the same time, 17 proteinogenic amino acids, including 9 replaceable and 8 essential acids, were detected and identified in *D. armatus* biomass. Among these amino acids, maximum contents of asparagine and glutamine were noted. A gradual increase in the number of total photosynthetic pigments until day 40 of cultivation was also observed. On day 40, the content of chlorophyll *a*, *b*, and carotenoids in *D. armatus* cells was maximal at 11.17, 7.07, and 12.05 mg g⁻¹ dry weight, respectively. Therefore, cultivating microalgae *D. armatus* in RAS waste water permits obtaining an active growing culture that is characterized by a constant increase of biomass and high nutrient contents.

Keywords: biomass, chlorophyll *a*, *b*, carotenoids, recirculating water system.

Introduction

The advantage of biotechnology for the controlled cultivation of photosynthetic organism is the low loss of valuable biomass, which is used both in natural and processed conditions (Zolotareva et al. 2008). The high protein content, including essential amino acids, and carbohydrates, lipids, and pigments in numerous microalga species and their lability under various cultivation conditions render it possible to use them as sources of biologically active substances and to manage their biosynthesis in the required directions (El-shimy and Ismail 2007). Knowledge about the mechanisms of microalgal adaptation to various cultivation conditions and the chemical components of culture media is not only of theoretical interest for producing highly-productive strains, but also of great practical importance, particularly for the development of modern technologies for the manufacture of productive feeds and premixes (Ladygina 2007). Simultaneously, is important that the algal mass obtained is non-toxic. Thus, possibilities for the comprehensive use of microalgae requires actively screening highly productive strains and optimizing culture conditions. This is because changes in the functional activity and nutrient composition of microalgae during dynamic cultivation are possible during the formation of population structures with

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the requisite biosynthetic properties (Zolotareva et al. 2008).

For most algal representatives, the general guidelines were developed for cultivation under laboratory conditions depending on the purpose of the experiment; these included the choice of medium, the inoculation of producer's culture into the culture medium, and accumulative cultivation over a certain period. Thus, the nutritive elements and different sources of carbon nutrition (various forms of nitrogen $[\text{NH}_4^{4+}, \text{NO}_3^{-}, \text{NO}_2^{-}]$, phosphorus and others) were necessary for increasing the algal biomass (Nunez et al. 2001, Mallick 2002, Denisov and Zhuikov 2007). Common approaches in biotechnology include using mixtures of macro- and microelements that contain all the necessary components as culture media (Zolotareva et al. 2008). However, artificial media are quite expensive. On the other hand, the yield of algae biomass with improved productive characteristics can be achieved only by changing the composition of the culture medium (Chubchikova et al. 2013). So, it is necessary to find alternative culture media that, on the one hand, meet the mineral component needs of algal cultures and that, on the other, help to reduce the technological costs of producing algal biomass (Golub 2013, Mamedova et al. 2013). Therefore, the physiological characteristics of algae, such as active photosynthesis, the ability to directly utilize nitrogen and phosphorus ions and other biogenic elements, and to withstand the high concentrations of toxic substances and metabolites permits using waste water of various origin as culture media (Makarova et al. 2009, Woertz et al. 2009). This approach allows, on the one hand, to reduce the expense of cultivation systems and, on the other, to clean waste water of nutrients (Gorbunova and Zubko 2009). The waste water from recirculating aquaculture systems (RAS) is rich in nutrients, particularly in various forms of nitrogen, and can be considered as a culture medium.

The possibility of cultivating the blue-green algae *Anabaena hassalii* in RAS waste water has been demonstrated previously (Cheban et al. 2014). However, protococcus algae are one of the most active agents used to clean waste waters (Samorí et al.

2013). The phototrophic ability of the *D. armatus* algal culture can enrich the aquatic environment with oxygen that accelerates oxidation processes and the mineralization of organic compounds. In addition, *D. armatus* biomass can be used as a source of proteins, amino acids, vitamins, polyunsaturated fatty acids, and pigments, including carotenoids (Sharapova et al. 2012). This permits using the *D. armatus* biomass as a source of complex nutrients and for the needs of aquaculture (Cetin and Mert 2006). Consequently, *D. armatus* culture productivity was estimated using RAS waste water as the culture 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. RAS waste water was used as the culture medium. The water was taken from a mechanical filter, poured onto aliquots, autoclaved at 121°C for 30 min, and standardized at a pH index of 7.5-8 with total mineralization of 495 ± 5 ppm (Cheban et al. 2014). Fitzgerald's medium No 11 with Zehnder's and Gorham's modifications was used as the comparative medium (Zolotareva et al. 2008). The cultivation was carried out in 500 mL Erlenmeyer's flasks at a temperature of $21 \pm 2^\circ\text{C}$, illuminated with 2500 lux fluorescent lamps for a 16-hour photoperiod (Gaysina et al. 2008). The inoculation was conducted at a ratio of inoculum:culture medium of 1:10. The physicochemical parameters of the culture medium were monitored throughout cultivation (pH, Ionometry U-160 MU; total mineralization, Water Quality Tester COM-100 conductivity meter). Biomass was determined by culture density using the optical index at 750 nm on SF-46. The transition from units of optical density (D750) to the value of absolutely dry biomass (ADB) was done with empirical coefficient k :

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

Coefficient k ($k = g \times \text{absorbance units/l}$) for the *D. armatus* culture was determined experimentally in three independent replicates (Gorbunova and Zhondareva 2012).

The microalgae suspension was centrifuged at 8000 g per 15 minutes with Biofuge Stratos (Heraeus Instruments). The biomass was disintegrated on UZDN-2T after centrifugation. The content of proteins (Vohnivenko et al. 2009), the qualitative and quantitative amino acid composition, the total content of chlorophyll *a*, *b* (Campbell et al. 1998), and total carotenoids (Sánchez et al. 2008) were determined in crude cells. All calculations were performed on dry weights. Amino acid hydrolysis was performed with concentrated hydrochloric acid at a temperature of 106°C for 24 hours. The complete composition of amino acids was analyzed on an automatic T-339 amino acid analyzer (Mikrotechna, Czech Republic) at the O. Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine. The spectra of pigments in the acetone extracts were measured by spectrophotometry on SF-46 in the wavelength range of 400-800 nm. The calculations of pigment concentrations were conducted according to formulas (Jeffrey and Humphrey 1975) by optical density indexes at wavelengths

corresponding to maxima absorption of chlorophyll *a*, *b*, and total carotenoids.

Statistical Analysis

Mean values were considered significantly different at $P \leq 0.01$ according to Student's criterion. The results were analyzed statistically with Microsoft Excel software according to generally accepted methods.

Results

The growth of biomass indices was observed against a background of exponential increases in total mineralization up to day 10 of cultivation in both the RAS waste water and the artificial medium. However, beginning from day 20 of cultivation, the general mineralization indexes returned to the initial levels. A slight increase in total mineralization was correlated with changes in pH. An increase in pH up to day 40 was noted, but it did not exceed pH 9.0 during cultivation in waste water (Fig. 1). Low culture growth was noted during the first days of *D. armatus* cultivation in both culture media, which was obviously caused by the microalgae adapting to the new

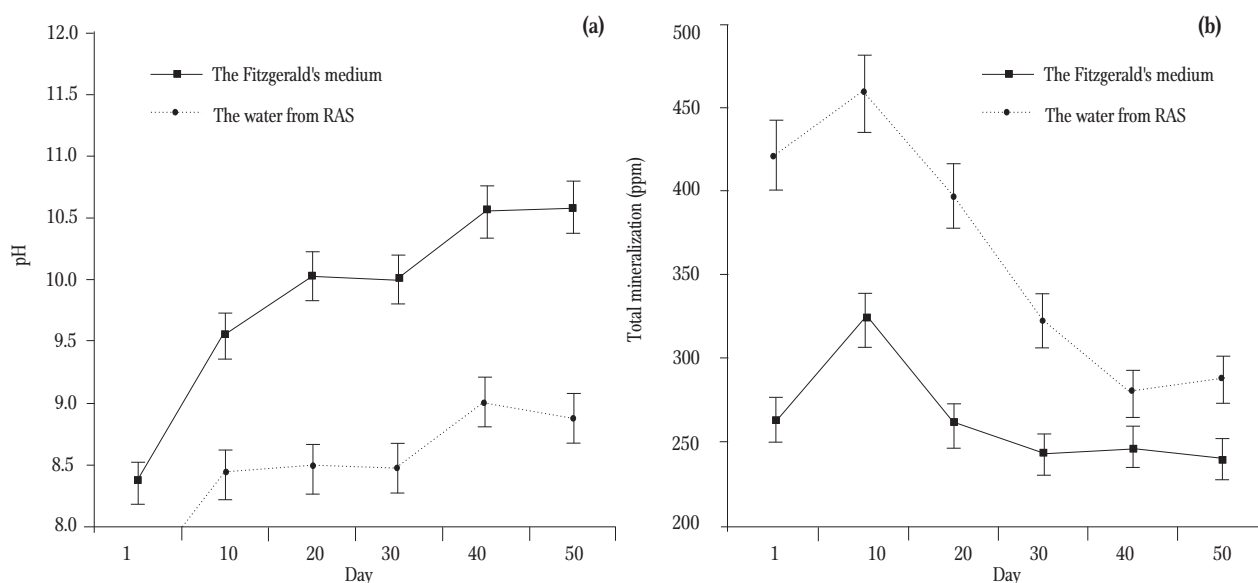


Figure 1. The changes of pH (a) and total mineralization (b) in control (Fitzgerald's medium) and cultures with water from RAS of *D. armatus*.

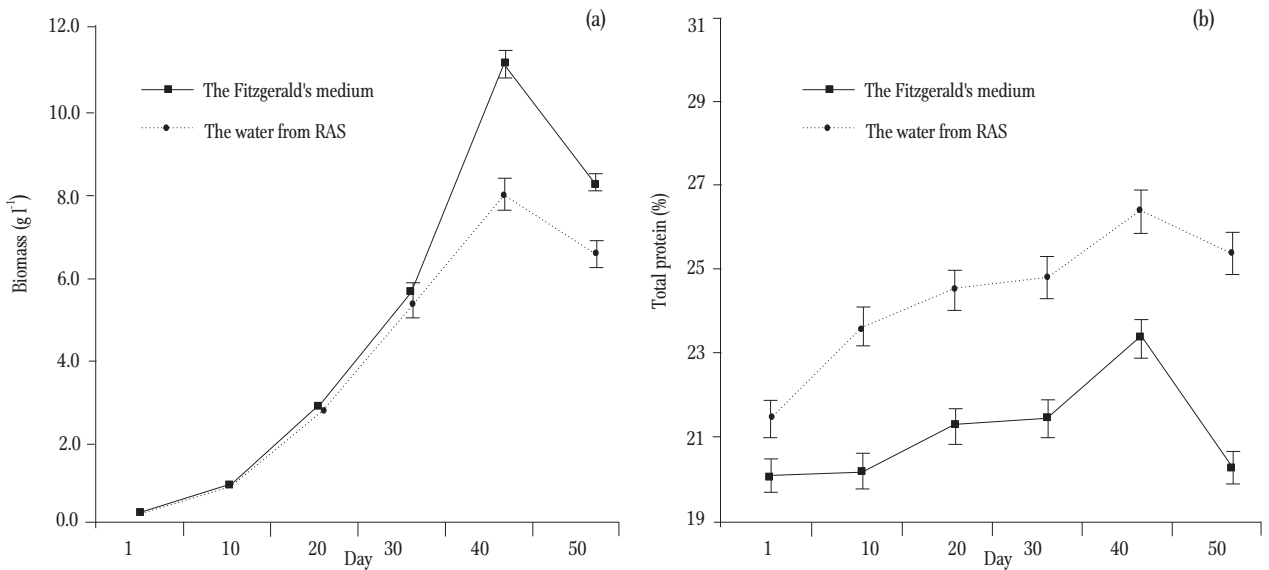


Figure 2. The changes of biomass (a) and total protein (b) in control (Fitzgerald's medium) and cultures with water from RAS of *D. armatus*.

Table 1

Amino acid composition of *D. armatus* biomass (mean \pm SD)

Amino acid	RAS water (mg g ⁻¹)	Fitzgerald's medium (mg g ⁻¹)
Lysine (Lys)	0.148 \pm 0.008	0.106 \pm 0.014
Histidine (His)	0.038 \pm 0.003	0.021 \pm 0.009
Arginine (Arg)	0.146 \pm 0.009	0.086 \pm 0.011
Asparagine (Asp)	0.322 \pm 0.030 ^a	0.243 \pm 0.021 ^b
Threonine (Thr)	0.160 \pm 0.010	0.119 \pm 0.004
Serine (Ser)	0.153 \pm 0.018	0.117 \pm 0.031
Glutamic acid (Glu)	0.350 \pm 0.008 ^a	0.258 \pm 0.030 ^b
Proline (Pro)	0.139 \pm 0.045	0.116 \pm 0.006
Glycine (Gly)	0.183 \pm 0.007	0.152 \pm 0.023
Alanine (Ala)	0.245 \pm 0.016	0.207 \pm 0.026
Cysteine (Cys)	0.010 \pm 0.002	0.006 \pm 0.001
Valine (Val)	0.084 \pm 0.013	0.067 \pm 0.017
Methionine (Met)	0.008 \pm 0.001	0.006 \pm 0.001
Isoleucine (Ile)	0.045 \pm 0.005	0.036 \pm 0.007
Leucine (Leu)	0.189 \pm 0.024 ^a	0.152 \pm 0.043 ^b
Tyrosine (Tyr)	0.083 \pm 0.021	0.058 \pm 0.018
Phenylalanine (Phe)	0.114 \pm 0.038	0.088 \pm 0.011
Total amino acids	2.416 \pm 0.048	1.840 \pm 0.037

Data in rows with different letter indexes differ significantly statistically ($P \leq 0.01$)

conditions (Fig. 2). From day 10, the amplification of growing processes in the investigated culture was observed. The most intensive growth of *D. armatus* biomass was noted between days 10 and 40 of cultivation, when its maximal productivity was also

noted. Beginning from day 40 of the experiment, the amount of biomass in both media starts to decrease because of the algal culture transition to the degradation phase. Parallel with biomass growth, biosynthesis is activated in the exponential phase.

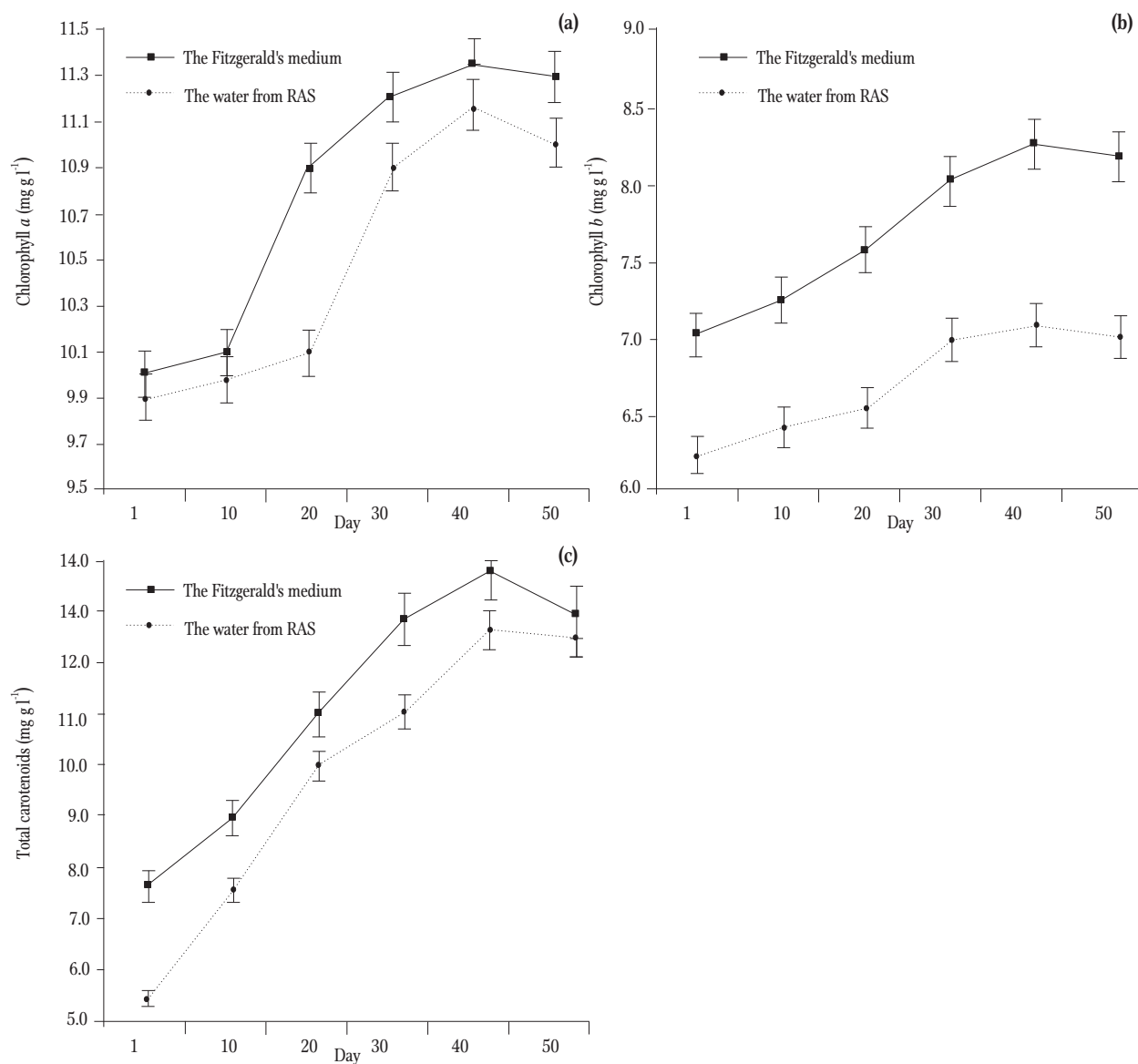


Figure 3. The changes of chlorophyll a (a), chlorophyll b (b), and total carotenoids (c) in control (Fitzgerald's medium) and cultures with water from RAS of *D. armatus*.

A gradual increase of protein content in *D. armatus* cells was noted from days 10 to 40 of cultivation in both RAS water and in Fitzgerald's medium, and on day 40 of the experiment it reached maximal values of 26 and 23%, respectively. Simultaneously, 17 proteinogenic amino acids, including 9 replaceable and 8 essential ones, were detected and identified in *D. armatus* biomass cultivated in waste water (Table 1). Among the essential amino acids, tryptophan was absent. The spectrum of amino acids in *D. armatus* biomass that was cultivated in both culture media

did not differ in qualitative composition, but did so with regard to quantitative indicators.

The highest possible content of aspartic and glutamic acids was observed in *D. armatus* biomass that grew in both media with amounts ranging from 0.320 to 0.350 mg g⁻¹ in waste water and from 0.240 to 0.260 mg g⁻¹ in Fitzgerald's medium. Among the essential amino acids, the highest content was of leucine, but the amount of it in the *D. armatus* biomass cultivated in waste water was 0.189 mg g⁻¹ in comparison to 0.152 mg g⁻¹ in Fitzgerald's medium.

The productivity of algae cultures was also estimated using indicators of pigment accumulation, in particular chlorophyll *a*, *b*, and carotenoids. The dependence of photosynthetic efficiency on mineral nutrition elements was determined by their necessity in the formation of both the photosynthetic apparatus and its renewal during the lifetime of the culture. During *D. armatus* cultivation in waste water, a gradual increase in the amounts of chlorophyll *a* and *b* was noted, and they reached maximal values at the stationary phase of culture growth (Fig. 3). Beginning on day 10 of cultivation, a gradual increase in the amount of pigments was observed. On day 40 of the experiment, the chlorophyll *a* and *b* content in *D. armatus* cells was at the highest possible levels of 11.17 and 7.07 mg g⁻¹ of dry weight of chlorophyll *a* and *b*, respectively. Upon further cultivation in both culture media, a slight decrease in the amount of these pigments was noted.

The maximal content of carotenoids matches the stationary phase of *D. armatus* culture growth. Thus, on day 40 of cultivation this parameter reached 12.05 and 13.79 mg g⁻¹ of dry weight during cultivation of algal cultures in waste water and in synthetic medium, respectively. Upon further cultivation, the amount of carotenoids in *D. armatus* cells increased slightly, but it does not significantly differ from the maximal values that were noted on day 40 of cultivation. The pigment contents in *D. armatus* cells depended on both the growth phase and the cultivation conditions. During this time, the dynamics of carotenoid accumulation correlated with dynamics that are observed while determining the amount of chlorophyll *a* and *b* and the biomass amount. Therefore, the degree of biomass increase in RAS water was slightly lower compared to the biomass increase in Fitzgerald's medium. This regularity was also noted when studying the accumulation indexes of carotenoids and photosynthetic pigments, particularly chlorophyll *a* and *b*. Simultaneously, the quantitative content of total proteins in *D. armatus* algal cells that were grown in waste water was 3% higher in comparison to the culture grown in the synthetic medium. This also applies to the study of the quantitative characteristics of individual amino acids.

Discussion

Representatives of *Desmodesmus* sp. are used for obtaining various products from natural and processed algal masses that are rich in vitamins, proteins, carbohydrates, and macro- and microelements; they are also used in aquaculture as food for mollusks, fish, and crustaceans (Becker 2007, Ladygina 2007). *Desmodesmus* sp. species are considered to be one of the most promising sources of raw materials thanks to their ability to grow in different types of waste waters and their high productivity (Imran et al. 2014). Algal biomass growth rates are dependent primarily on culture medium composition and cultivation conditions (light intensity, photoperiod, temperature, etc.). Regulating the mineral elements provided to microalgae is also necessary in order to achieve highly productive growing cultures (Chubchikova et al. 2013). Therefore, analyzing changes in cultivation parameters, including pH and total mineralization is quite important (Imran et al. 2014). By continuously monitoring the physicochemical indicators of culture media, it is possible to establish the optimal duration of *D. armatus* cultivation that lasts for 40 days.

In the first days of *D. armatus* cultivation in both culture media, low growth activity was noted, which is obviously caused by the microalgae adapting to new conditions. The duration of the lag phase was five days. The activation of growth processes was observed from day 10 of cultivation, and in this period the biomass of the algae doubled. These observations do not contradict data obtained for other species of the genus, particularly *Scenedesmus obliquus* (Toyub et al. 2008). The intensification of growth processes in both culture media from days 10 to 30 is an indication of the culture adapting excellently to the new conditions in the waste water. This trend was facilitated by the availability of mineral nutrition components (Oturyna et al. 2010).

After day 30, the biomass in the waste water medium was lower in comparison with that in Fitzgerald's medium. This was because the waste water was exhausted faster since its chemical composition differed from that in the artificial medium. The protein

content of the algal mass cultivated in waste water is higher compared to that in algal mass from Fitzgerald's medium. Meanwhile, the quantitative characteristics of the amino acid composition was similar for both media, and were typical of the content of total proteins. In this case, one of the key factors was the quantity and form of available nitrogen (González López et al. 2010, Crofcheck et al. 2012). Thus, there were no differences in the qualitative content of the amino acids in the algal masses between the two culture media.

Each type of algae contains a complex of pigments in differing ratios. Carotenoids (astaxanthin, canthaxanthin, keto-carotenoids) (Pirastru et al. 2012) and chlorophyll *a* and *b* (Sánchez et al. 2008) accumulate in the biomass of *Desmodesmus sp.* species. The pigment dynamics in algae culture growth cycles stems from the features of accumulative cultivation. So, during long-term cultivation, the increasing of cell numbers leads to the exhaustion of the culture medium and shortages of certain minerals. At the same time, microalgae cultures are shaded by their own high densities, which gradually leads to the features of monoculture *D. armatus* development that was established previously. Starting on day 40 of the experiment, the algae culture began approaching the degradation phase, and its growth and productivity were limited by decreases in available nutrients and the accumulation of metabolic products in the culture fluid.

Cultivating *D. armatus* in RAS waste water permits obtaining active growing cultures characterized by constant biomass growth and high contents of total proteins, complex proteinogenic amino acids, and the main photosynthetic pigments. At the same time, the effectiveness of cultivation in RAS waste water is not significantly different from that in artificial Fitzgerald's medium. The culture obtained in this way can be used for further processing or transferred into fresh culture media. Using RAS waste water as a culture medium, on the one hand, helps to reduce significantly the costs of algal mass biotechnology and, on the other, it cleans the water of nutrients.

Conclusion

Therefore, RAS waste water can be used as a culture medium for *D. armatus* cultivation. This approach facilitates obtaining active-growing cultures that are characterized by constant biomass growth and high contents of total proteins and the main photosynthetic pigments.

Author contributions. M.M., L.C. and I.M. conceived of and implemented the experiment; I.M. and L.C. performed the experiment, analyzed the data and wrote the paper.

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