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ULTRASOUND INFLUENCE ON *Scenedesmus acutus* PRODUCTIVITY AND PROTEIN CONTENT

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Summary. Ultrasound influence on *Scenedesmus acutus* productivity and protein content was investigated. Parameters at which a positive effect occurs on biomass accumulation and protein content were assessed. Protein fractional composition and electrophoretic spectrum composition of albumins and globulins, isolated from algal biomass, obtained under conditions of highest ultrasound stimulating effect were determined.

Treatment of algal cells with suitable ultrasound parameters increased yield of biomass with 9–11% and that of protein contents with 5–10%. No difference in the electrophoretic spectra of albumins and globulins, isolated from cells treated or untreated with ultrasound were established.

Keywords: *Scenedesmus acutus*, ultrasound, biomass, protein

Introduction

Productivity of vegetal organisms depends on the interaction between inherited potential and environmental conditions. The fundamental purpose of every exogenic effect concerning yield increase and quality improvement of vegetable biomass is in its essence activation of metabolic processes in plant cells and their direction towards the wanted trend.

Green algae are characterized with the specific trend of their metabolic processes toward formation of preferably nitrogen compounds. This trait, combined with their

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susceptibility to the influence of abiotic agents, can be utilized in the search of new possibilities for enhancement of biomass and protein yield.

Change in protein quantity and quality in microalgal biomass depends on the phase of cell development. This fact was reported by Müller (1961) and later confirmed by the studies of many other authors (Ruppel, 1962; Kanazawa, 1964; Mushak, 1971; Bozhkova at al., 1982; Richmond, 1986; Borowitzka et al., 1988).

Pinevitch et al. (1968) studying the soluble protein complex in *Chlorella* dependent on nitrogen nutrition established that nitrogen starvation of the culture provoked complete inclusion of inorganic nitrogen in the protein. Algal protein is characterized especially by its amino acid composition (Kuzmenko, 1984; Trubachev et al., 1984). Available data on the fractional protein composition according to its solubility in various solvents are comparatively sparse. Kuzmenko (1984b) has studied the quantitative changes in water-, alcohol- and alkaline soluble proteins and the correlations between them. The ratio between easy- and hard soluble fractions during the different phases of exponential growth of the two *Dunaliella* species has been established.

The purposes of our investigation were: 1) To test the influence of ultrasound on *Scenedesmus acutus* and to determine the parameters of its application, which lead to a positive effect on biomass accumulation and protein content. 2) To study the fractional composition of protein and protein content in albumins and globulins, isolated from an algal biomass, produced under conditions of most stimulating ultrasound effect. We are not familiar with similar experiments.

Material and Methods

The investigations were carried out with an algal culture of *Scenedesmus acutus Meyen Tomaselli 8*, grown in intensive laboratory conditions under standard parameters of cultivation; mineral nutrient medium, described by Georgiev et al. (1978), luminescent light 16 000 lx, temperature of suspension 30±1 °C, bubbling with enriched air with 2% CO_2.

Ultrasound treatment was performed on algal suspension of about 1 g/l density with consequent dilution up to 0.5 g/l as an initial material for culture growth. The ultrasound device used was type UD-11 Techpan (Poland) with a frequency of 22 kHz and 5 steps power of 100–320 W.

Algal biomass growth was recorded at every 24th, 48th and 72nd hour after the start of cultivation. A visual observation of the suspension and a microscopic one of the algal cells were performed after the treatment. Total and protein nitrogen content in the biomass were determined after Kheldal and the soluble protein content – after Lowry. Protein fractions were obtained by algal biomass disintegration, using glass pearls up to the complete destruction of the cells. The homogenate, free of any cells, was further treated according to the scheme given in Fig. 1, after the method of

Moureaux and Landry (1968). Electrophoresis of soluble protein – by PAAG - acid and alkaline – was carried out after the method of Davis (1964), Reisfeld et al. (1962). The densitometry of the electrophoregrams was performed on the densitometric device VEB C.T. JENA.

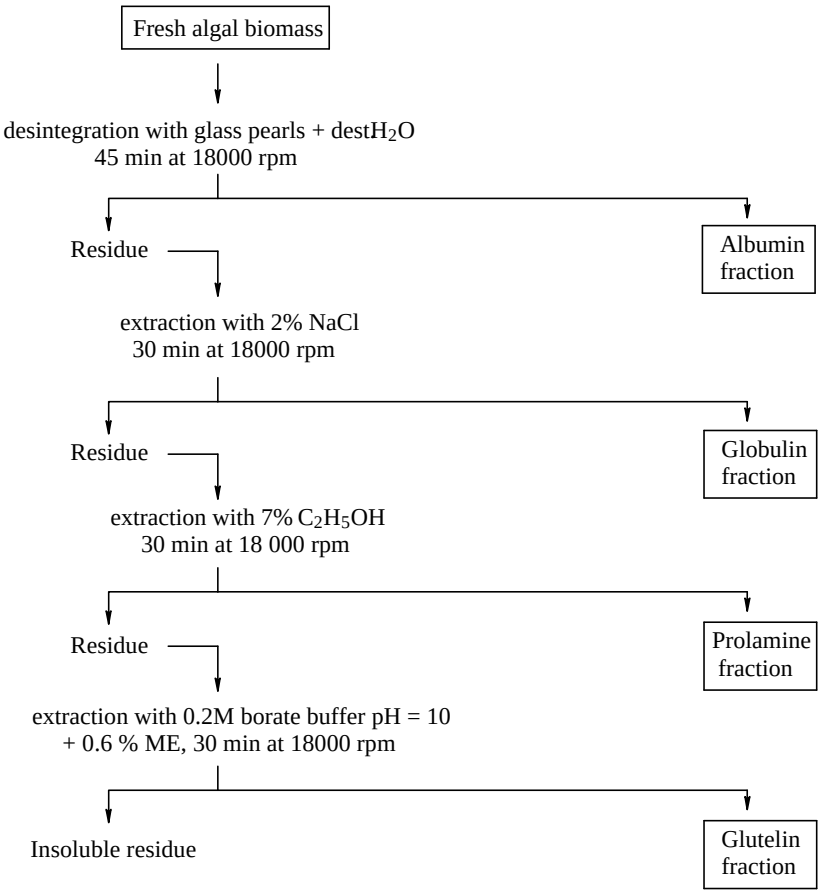

Fig.1. Scheme for protein component extraction from *Scenedesmus acutus* biomass

Result and Discussion

Data in Fig. 2 show that, depending on the strength and the duration of ultrasound effect upon the algal cells, the result is either stimulating or inhibiting. Any increase of the ultrasound power at one and the same duration (15 s, 30 s, 1, 2 and 3 min) results in an increase of the inhibiting effect. The same trend is observed with prolonga-

tion of ultrasound at one and the same power. When higher power is applied (III, IV and V degree), this tendency is notably expressed. The stimulating effect at these powers is observed after up to 15–30 s of ultrasound action on algal cells. Conversely, with reduction of ultrasound power (I and II degree), the duration of the ultrasound action of 30 s is prolonged up to 2 min in order to obtain an increase of algal biomass. Such inverse dependency between power and duration of the action is well known and is underlined by other authors in their research with other sources of radiation (Tiphlova and Karu, 1986; Arutyunyan et al., 1987).

Damaging doses of ultrasound (up to 3 min and III, IV and V degree) result in various degrees of algal cell destruction. Visual observation of this effect shows releasing of cell matter in the cultural medium. The intensity of supernatant pigmentation, from pale green to dark green, corresponds to the degree of cell destruction. Under conditions of intensive cultivation such cells grow very poorly or die.

Fig. 2 shows also that the inhibiting effect of the sound occurs during the 24th hour of algal development and is getting stronger up to the 72nd hour of cultivation. A stimulating effect has not been observed at the 24th hour, but such an effect is slightly expressed at the 48th hour, and is stronger at the 72nd hour. Consequently, the stimulating effect of sonization increases with the duration of time. Presumably, one of the causes of the observed changes is the accelerated growth and development of cells. This is evident from the microscopy of algal cells. In an inoculate with about 90% degree of synchronized cells, after 24 hours' cultivation, the only cells observed in the control are those in the phase of maturity. In treated with ultrasound cells, autospores, released during the division, were predominant. The division of cells was completely accomplished in the variants with most strongly expressed stimulative effect.

The values of biomass yield and protein nitrogen content after the 72nd hour of cultivation and under the influence of stimulating ultrasound doses are given in Table 1. Highest growth of biomass was produced during sonization with a power of the I degree for a duration of 2 min. This growth is 11.2% higher than that of the control. The values, obtained during sonization with a II degree power and 30 s or 1 min duration, are almost the same and are about 9% higher than those of the control.

Data of total protein content and nonprotein nitrogen content, given in Table 1, show that in all variants, without exceptions, the total protein and protein nitrogen content is greater than that of the control, and the nonprotein content is lower than that of the control. The quantity of protein nitrogen is 5–10% higher in each variant as compared to those of the control. The relatively highest percentage of protein nitrogen (10.4%) is observed in cells, treated with ultrasound power of the II degree for a duration of sonization of 1 min.

Protein fraction distribution is plotted on Table 2. It shows that the protein in the examined biomass is divided into water-, alcohol- and alkaline soluble fractions and insoluble protein. These results confirm Kuzmenko's research (1984b) about the dif-

Fig.2. Influence of various ultrasound doses on *Scenedesmus acutus* growth dinamics. *a* – 24th hour, *b* – 48th hour, *c* – 72nd hour, *C* – control, *1* – 15 s, *2* – 30 s, *3* – 1 min, *4* – 2 min, *5* – 3 min, I–V – degrees of ultrasound power

Table 1. Biomass yield and nitrogen content in *Scenedesmus acutus* after 72nd hours cultivation under ultrasound stimulative doses

Variants		Algal biomass		Nitrogen (% from dry biomass)			
Power degree	Time	mg/ml	% to the control	Total	Protein	Non-protein	Protein (% to the control)
Control		7.57 ± 0.25	100.0	10.13 ± 0.07	8.58 ± 0.11	1.55	100.0
I d.	1 min	8.20 ± 0.33	108.3	10.45 ± 0.05	8.97 ± 0.06	1.48	104.5
	2 min	8.42 ± 0.26	111.2	10.54 ± 0.04	9.20 ± 0.10	1.34	107.2
II d.	30 s	8.28 ± 0.22	109.3	10.47 ± 0.04	9.05 ± 0.19	1.42	105.5
	1 min	8.27 ± 0.13	109.2	10.86 ± 0.04	9.48 ± 0.51	1.48	110.4
III d.	30 s	7.98 ± 0.23	105.4	10.57 ± 0.07	9.16 ± 0.32	1.41	106.8
IV d.	15 s	7.98 ± 0.23	105.4	10.42 ± 0.10	9.23 ± 0.22	1.19	107.6
V d.	15 s	7.89 ± 0.23	104.2	10.31 ± 0.08	9.12 ± 0.11	1.19	106.3

Table 2. Ultrasoud influence on protein fractions in *Scenedesmus acutus* biomass (the cells are treated by II power degree for 2 min)

Protein fractions	Control			Variant		
	Protein mg in probe	% total protein	% dry biomass	Protein mg in probe	% total protein	% dry biomass
Albumins	100.25	41	19	105.50	42	22
Globulins	11.56	5	2	11.69	5	2
Prolamins	7.56	3	1	8.63	3	2
Glutelins	7.75	3	1	8.12	3	2
Insoluble residue	116.88	48	22	110.00	44	23

ferent solubility of protein fractions present in algal biomass. The major quantity of protein is distributed between water-soluble protein fraction albumins (41–42%) and insoluble protein residue (44–48%). The remaining protein fractions – globulins, prolamins and glutelins, represent about a 1/10 part of the albumins and about 3–4% of the total protein in the biomass.

A more obvious notion about the correlation between individual protein fractions is given in Fig. 3. It gives an idea about ultrasound effect on the distribution of protein fractions. In biomass treated with ultrasound, albumin content is 1.16% higher and insoluble protein residue content is 3.85% lower than that of the control. No substantial differences between the remaining fractions have been observed. The total quantity of extractable proteins in biomass' cells treated with ultrasound is higher (56.05%) than that of the control (52.7%).

Considering the above mentioned, cells of the investigated alga evidently contain all protein fractions identified in higher plants. However, contrary to them, albumins

Fig. 3. Influence of ultrasound on protein fractional composition in *Scenedesmus acutus* biomass. *a* – control, *b* – variant; I – albumin, II – globulin, III – prolamin, IV – glutalin, V – insoluble protein

and insoluble protein residue are prevalent in algal cells. Changes, observed in the composition of protein under the influence of the applied action, concern both principal fractions. The higher content of albumin can be a result of a more intensive formation of all proteins or of some individual proteins. For a further elucidation of this question they were submitted to an electrophoretic division. The greatest number of protein bands with best separation occurred under alkaline electrophoresis. Not a single band moved towards the cathode, despite the application of different quantity of protein. It is evident, consequently, that albumins are composed of acid proteins both in the investigated alga and in higher plants.

Electrophoregrams of albumins, isolated from the controls (*1*) and from cells treated with ultrasound (*2*) are plotted on Fig. 4, and their densitometric records are plotted on Fig. 5. The electrophoregrams show 9 protein bands with a different colour intensity. The most intensive one is the group of 3 bands, fastly moving towards the anode, but insufficiently separated from one another. Another band with a high intensity and moderate mobility is also observed. The number of protein bands in the control corresponds to that of the cells treated with ultrasound. There are no quantitative differences between them. The effect of the applied ultrasound action was consequently the same on all of the individual proteins, recorded by the applied method.

Fig. 4. Photos of albumin electrophoregrams, isolated from cells: untreated (*1*) and treated (*2*) with ultrasound

Fig. 6 shows the densitometric records of the globulins' electrophoregrams, isolated from control (1) and ultrasound treated (2) cells. Both albumins and globulins are composed of acid proteins. Unlike the former, however, globulins don't penetrate in a 7.5% gel. The electrophoretic spectrum is from a 5% gel. They are, probably,

Fig. 5. Densitograms of albumins isolated from cells: untreated (1) and treated (2) with ultrasound

Fig. 6. Densitograms of globulins isolated from cells: untreated (1) and treated (2) with ultrasound

high-molecular proteins, which can be observed in two bands, drastically different in their electrophoretic mobility towards the anode. The first band, situated near the start, is slowly mobile, while the other one is with fast mobility. The intensity of both bands is not very high, despite the highest possible quantity of applied protein. Both of them show no difference between the number and the colour intensity of protein bands, isolated from control and from ultrasound treated cells.

Thus, under the influence of ultrasound, total albumin content increases; globulin content remains unchanged and the electrophoretic spectrum of the native proteins from the albumin and globulin fractions remain also unchanged. Those results comprise a basis for future investigations and applications.

Conclusions

Ultrasound treatment of *Scenedesmus acutus* cells under suitable parameters of sonization results in a 9–11% increase of biomass' yield.

All stimulating biomass production doses, applied for ultrasound treatment, result in a 5–10% increase of protein content.

Scenedesmus acutus cells contain all protein fractions, identified in higher plants. Albumins and insoluble protein residue are prevalent.

Under the influence of ultrasound treatment the content of albumins increases with 1.16%, and that of insoluble protein residue decreases with 3.85%. The total quantity of extractable proteins is higher in the biomass, treated with ultrasound. No difference has been established in electrophoretic albumin and globulin spectra, isolated from untreated and treated with ultrasound cells. Nine anode protein bands have been observed in the albumin's fraction and two – in those of the globulins.

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