

PRODUCTIVITY AND BIOCHEMICAL COMPOSITION OF THE GREEN MICROALGA *HAEMATOCOCCUS PLUVIALIS* FLOTOW CNM-AV-05 CULTIVATED IN THE PRESENCE OF SOME ALCOHOLS

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Summary. Some alcohols have stimulating action on the productivity and biochemical composition of the green microalga *Haematococcus pluvialis* Flotow-CNM-AV-05. From all alcohols tested, the best results were obtained by adding 3.0 ml/l isopropanol for the non-axenic culture and 0.5 ml/l for the axenic culture to the algal nutrient medium. This alcohol can be used as a regulator of *Haematococcus pluvialis* growth and biosynthetic activity.

Keywords: *Haematococcus pluvialis*, alcohols, productivity, biochemical composition

Introduction

The green alga *Haematococcus pluvialis* Flotow CNM-AV-05 has particular interest for phycobiotechnology due to its unique ability to synthesize increased amounts of β -carotene and astaxanthin. The limited industrialization of the production of β -carotene and astaxanthin from *Haematococcus* species is caused by the relatively low productivity of the strains used in traditional phototrophic systems [5]. Various strategies have been proposed to solve this problem, one of them being the use of mixotrophic systems, but this procedure has not found practical application in hematococcal biotechnology due to the lability of the chemical composition, especially carotenoids [2].

The productivity and biochemical composition of many algal species is influenced by the presence of alcohols in the nutrient medium – sources of carbon radicals. It has been demonstrated that the addition of alcohols: methyl, ethyl, isopropyl, amyl, mannitol, adonite, sorbitol, inositol, dulcitol to the nutrient medium ensures an obvious increase in porphyridium productivity. Most of the alcohols tested have an effect of stimulating lipogenesis and the process of accumulation of phycobiliproteins. A pronounced positive effect on protein biosynthesis was shown by methyl and isoamyl alcohols [1].

Materials and methods

The object of the research carried out was the strain of green algae *Haematococcus pluvialis* Flotow CNM-AV-05 [8]. It was algologically and bacteriologically purified and cultivated on RD nutrient medium [7]. Young immobile green cells were taken as inoculum in the amount of 0.3g/l in recalculation to the absolutely dry biomass (ADB). Cultivation was carried out at the illumination intensity - 1500lx, temperature - 26°C, pH - 6.8-7.0. The axenic conditions were ensured according to the methods described by T. Dudnicenco [3]. On the second day of cultivating the algal culture, the tested amount of respective alcohol was added to the nutrient medium. On the 7th day of cultivation, the algal cells are separated from the culture liquid and subjected to biochemical analyses. The determination of hematococcal productivity was carried out according to the developed method [3]. The amount of β -carotene was achieved using the value of the optical density of the studied carotenoid at a wavelength equal to 451nm, the extinction coefficient of β -carotene in hexane (2592) and the mass of the investigated sample. Protein was quantified according to the Lowry method [4]. The determination of the effective satellite microflora was carried out by the plate culture method [6]. The statistical-mathematical processing of the experimental data was carried out by standard methods [9].

Results and discussions

From the considerations mentioned above, being current and necessary research aimed at increasing the productivity of the haematococcus and improving the biochemical value of the biomass, a series of experiments were carried out in this direction, using axenic and non-axenic cultures of the green alga strain *H. pluvialis* CNM-AV- 05.

The productivity and chemical composition of *H. pluvialis* was studied on nutrient media with the inclusion of the following alcohols: methanol, ethanol, propanol, isopropanol, butanol, isobutanol, pentanol, hexanol, octanol - in concentrations 0.25 - 8 ml/l; mannitol, dulcitol, glycerol, inositol, adonitol and sorbitol - in concentrations 0.25 - 8 g/l. When hexanol, octanol, amyl and isoamyl alcohol (in concentrations 0.25 – 1.50 ml/l) were added to the nutrient medium, both axenic and nonaxenic algal culture died over 24 hours, which proves their algotoxicity. For this reason, the mentioned alcohols have not been included in the tables below. The results regarding the use of other alcohols are presented in tables 1-3.

Table 1

The productivity of *H. pluvialis* at cultivation in the presence of some alcohols

No.	Alcohol	Alcohol concentration	Nonaxenic culture			Axenic culture	
			ADB		The effectiveness of the satellite microflora (mln/ml) X±m _x	ADB	
			(g/l)	(% of control)		(g/l)	(% of control)
			X±m _x	X±m _x		X±m _x	X±m _x
	Control	0	2.2±0.1	100	20.4±0.9	2.1±0.0	100
1.	Methanol	0.25ml/l	2.0±0.1	90.9	14.2±0.8**	1.7±0.0*	81.0
		0.5	1.9±0.0*	86.4	14.6±1.1**	1.5±0.0**	71.4
		1.0	1.5±0.1**	68.2	14.5±1.1**	1.3±0.0**	61.9
2.	Ethanol	0.25	2.2±0.1	100	41.5±1.0**	2.1±0.1	100
		0.5	2.2±0.1	100	48.9±1.6**	2.1±0.0	100
		1.0	2.2±0.0	100	40.7±1.5**	2.1±0.1	100
3.	Propanol	0.5	2.4±0.2	109.1	80.3±1.3**	2.2±0.1	104.8
		1.0	2.4±0.1	109.1	80.2±1.5**	2.2±0.0	104.8
		1.5	2.4±0.1	109.1	81.5±1.8**	2.2±0.1	104.8
4.	Isopropanol	0.25	2.4±0.1	109.1	42.8±1.4**	3.0±0.1**	142.9
		0.5	2.8±0.2	127.3	46.9±3.4**	3.2±0.2**	152.4
		1.0	2.8±0.2	127.3	70.5±2.4**	3.1±0.2**	147.6
		2.5	3.0±0.1**	136.4	91.6±2.6**	3.0±0.2*	144.8
		3.0	3.1±0.3*	140.9	201.7±2.5**	2.9±0.3	138.1
		3.5	2.0±0.3	90.9	180.9±2.5**	2.7±0.2*	128.6
5.	Butanol	0.25	1.8±0.1*	81.8	159.8±3.1**	1.5±0.1**	71.4
		0.5	1.6±0.0**	72.7	180.6±3.4**	1.2±0.0**	57.1
		1.0	1.2±0.0**	54.6	241.3±3.6**	1.1±0.0**	52.4
6.	Isobutanol	0.25	1.9±0.0*	86.4	123.7±3.4**	1.8±0.0	85.7
		0.5	1.7±0.1*	77.3	168.9±2.5**	1.5±0.0**	71.4
		1.0	1.5±0.1**	68.2	190.7±2.8**	1.4±0.1**	66.7
7.	Pentanol	0.25	2.0±0.3	90.9	146.3±9.1**	1.9±0.1*	90.5
		0.5	1.6±0.0**	72.7	189.6±8.2**	1.9±0.1*	90.5
		1.0	1.4±0.1**	63.6	239.5±5.6**	1.9±0.0*	90.5
	Control	0	2.4±0.3	100	16.5±1.4	2.3±0.2	100
8.	Glycerol	2.0mg/l	3.2±0.3	133.3	28.5±0.4**	3.2±0.2*	142.7
		2.5	3.3±0.2	137.5	30.6±3.4**	3.4±0.2*	147.3
		3.0	3.1±0.1	129.2	29.5±1.1**	3.3±0.3	145.3
9.	Ethylene	0.25g/l	2.4±0.1	100	20.5±0.2	2.3±0.2	100

	glycol	0.5	2.2±0.1	91.7	21.4±0.3	2.2±0.0	95.7
		1.0	2.1±0.0	87.5	26.8±0.1**	2.1±0.0	91.3
10.	Mannitol	1.5	2.7±0.2	114.1	33.9±0.2**	2.9±0.1	127.8
		2.0	3.1±0.0	129.2	38.4±0.4**	3.0±0.1	130.1
		2.5	2.9±0.0	122.6	41.2±0.3**	2.8±0.1	132.2
11.	Dulcitol	1.5	3.1±0.0	128.5	60.3±0.4**	3.0±0.1	131.7
		2.0	3.2±0.0*	132.6	60.5±3.1**	3.1±0.0*	136.0
		2.5	3.1±0.0	130.6	59.6±2.8**	3.0±0.2	130.1
12.	Inositol	1.5	2.8±0.0	116.7	34.5±1.3**	2.8±0.2	122.1
		2.0	3.1±0.1	129.2	39.5±3.2**	3.0±0.1	130.1
		2.5	2.9±0.0	120.8	61.2±2.9**	2.6±0.2	114.2
13.	Adonitol	0.1	2.5±0.0	104.2	78.4±2.5**	2.7±0.2	117.4
		0.5	2.9±0.1	120.8	90.2±3.4**	2.9±0.1	127.5
		1.0	2.7±0.0	114.1	85.6±4.5**	2.8±0.1	122.0
14.	Sorbitol	1.0	2.4±0.0	100	96.3±4.6**	2.6±0.1	113.0
		1.5	2.8±0.0	116.7	94.1±4.3**	2.8±0.2	122.0
		2.0	2.5±0.1	104.2	98.2±3.5**	2.6±0.1	114.2

*T_{real}>T_{95%}; **T_{real}>T_{99%}

Table 2

The content of protein and β-carotene in the biomass of the nonaxenic culture of *H. pluvialis* at the cultivation in the presence of some alcohols

No.	Alcohol	Alcohol concentration	Protein		β-carotene	
			% from ADB X±m _x	% of control	Raw content synthesized per day (mg/l) X±m _x	% of control
	Control	0	27.4±0.2	100	10.7±0.2	100
1.	Methanol	0.25ml/l	26.4±0.3**	96.2	9.1±0.1**	85.1
		0.5	26.1±0.4**	95.1	8.4±0.2**	78.5
		1.0	23.9±0.2**	87.1	6.4±0.1**	59.8
2.	Ethanol	0.25	27.4±0.2	100.0	10.7±0.1	100
		0.5	27.4±0.2	99.8	10.7±0.1	100
		1.0	27.3±0.1	99.7	10.7±0.1	100
3.	Propanol	0.5	27.7±0.1	101.0	9.6±0.2*	89.7
		1.0	27.6±0.1	100.6	9.6±0.2*	89.7
		1.5	27.9±0.1	101.7	9.6±0.2*	89.7
4.	Isopropanol	0.25	27.6±0.1	100.5	11.7±0.1*	109.4
		0.5	27.4±0.2	100.1	14.4±0.2**	134.6
		1.0	27.2±0.2	100.5	14.4±0.1**	134.6
		2.5	29.6±0.3**	107.8	16.7±0.3**	156.1
		3.0	32.4±0.1**	118.3	17.7±0.3**	165.4
		3.5	30.1±0.2*	109.7	10.9±0.1	101.9
5.	Butanol	0.25	21.3±0.5**	77.7	5.7±5.7**	53.3
		0.5	19.0±0.1**	69.2	4.1±0.1**	38.3
		1.0	17.6±0.6**	64.2	3.9±0.0**	36.5
6.	Isobutanol	0.25	18.3±0.1**	66.8	7.1±0.1**	66.4
		0.5	15.2±0.1**	55.5	6.3±0.0**	58.9
		1.0	12.6±0.1**	46.1	5.4±0.1**	50.5
7.	Pentanol	0.25	19.1±0.1**	69.6	5.8±0.0**	54.2

		0.5	14.3±0.0**	52.2	4.3±0.0**	40.2
		1.0	13.2±0.1**	48.4	4.0±0.1**	37.4
	Control	0	26.3±0.1	100	11.7±0.2	100
8.	Glycocol	2.0mg/l	31.5±0.6**	117.0	16.5±0.2**	141.0
		2.5	33.6±0.1**	124.8	17.9±0.2**	153.0
		3.0	32.7±0.1**	121.5	15.5±0.1**	132.5
9.	Ethylene glycol	0.25g/l	26.8±0.1	99.7	11.7±0.1	100
		0.5	27.1±0.2	100.7	10.1±0.1**	115.8
		1.0	26.7±0.2	99.2	9.6±0.2**	82.1
10.	Mannitol	1.5	29.7±0.8**	110.4	13.5±0.3**	115.4
		2.0	30.3±0.2**	112.8	15.9±0.4**	136.0
		2.5	28.9±0.2**	107.4	14.1±0.2**	120.5
11.	Dulcit	1.5	27.9±0.9**	103.5	15.1±0.3**	129.1
		2.0	30.1±0.3**	111.8	15.5±0.2**	132.5
		2.5	29.4±0.5**	109.2	15.1±0.1**	129.1
12.	Inositol	1.5	28.2±0.6**	104.8	13.6±0.2**	116.2
		2.0	31.4±0.5**	116.7	14.6±0.3**	124.8
		2.5	28.9±0.8**	107.4	13.7±0.0**	117.1
13.	Adonit	0.1	27.1±0.8*	100.7	12.5±0.1*	106.8
		0.5	27.0±0.7	100.5	14.1±0.1**	120.5
		1.0	26.9±0.7	100.0	13.1±0.2**	112.0
14.	Sorbitol	1.0	26.5±0.5	98.7	11.7±0.2	100
		1.5	26.3±0.4	97.8	14.4±0.1**	123.1
		2.0	26.7±0.4	99.2	12.1±0.1	103.4

*Treal>T_{95%}; **Treal>T_{99%}

Table 3

Protein and β-carotene content in the biomass of the axenic culture of *H. pluvialis* at the cultivation in the presence of some alcohols

No.	Alcohol	Alcohol concentration	Proteină		β-caroten	
			% from ADB X±m _x	% of control	conținut brut sintetizat pe zi (mg/l) X±m _x	% l of control
	Control	0	28.2±0.1	100	9.9±0.2	100
1.	Methanol	0.25ml/l	26.4±0.6*	93.6	7.5±0.1**	75.8
		0.5	23.1±0.8**	82.2	6.4±0.2**	64.7
		1.0	20.5±0.2**	72.7	5.4±0.1**	54.6
2.	Ethanol	0.25	28.2±0.1	99.9	9.9±0.1	100
		0.5	28.1±0.3	99.9	9.6±0.2	103.1
		1.0	28.1±0.2	99.9	9.6±0.2	103.1
3.	Propanol	0.5	28.4±0.4	100.3	10.7±0.1*	108.1
		1.0	28.6±0.5	101.0	10.7±0.1*	108.1
		1.5	28.5±0.1	101.6	10.7±0.1*	108.1
4.	Isopropanol	0.25	28.9±0.1	102.2	15.4±0.1**	155.6
		0.5	30.2±0.1**	107.7	17.3±0.2**	174.8
		1.0	29.4±0.1**	104.2	17.2±0.1**	173.7
		2.5	28.1±0.6	99.2	15.4±0.2**	155.6
		3.0	28.1±0.6	99.6	14.9±0.1**	150.5
		3.5	28.0±0.4	99.3	13.1±0.1**	132.3

5.	Butanol	0.25	18.2±0.5**	64.6	6.0±0.1**	60.6
		0.5	17.6±0.5**	62.4	4.6±0.1**	46.5
		1.0	17.4±0.3**	61.7	4.2±0.0**	42.4
6.	Isobutanol	0.25	27.4±0.5*	97.1	6.4±0.0**	64.7
		0.5	25.1±0.6**	89.1	5.1±0.0**	51.5
		1.0	23.6±0.2**	83.7	4.4±0.0**	44.4
7.	Pentanol	0.25	20.3±0.1**	72.1	6.0±0.0**	60.6
		0.5	17.2±0.2**	61.2	6.0±0.1**	60.6
		1.0	15.1±0.3**	53.6	6.0±0.0**	60.6
	Control	0	27.4±0.4	100	10.5±0.1	100
8.	Glycocol	2.0mg/l	32.8±0.4**	119.5	15.1±0.1**	152.5
		2.5	33.4±0.3**	121.9	17.0±0.2**	161.9
		3.0	31.5±0.5**	115.1	16.0±0.1**	152.4
9.	Ethylene glycol	0.25g/l	25.20±.2**	92.0	10.5±0.1	100
		0.5	22.8±0.3**	83.2	10.4±0.1	99.1
		1.0	21.0±0.4**	76.7	9.6±0.1	91.4
10.	Mannitol	1.5	27.5±0.3	100.3	14.5±0.2**	138.1
		2.0	27.6±0.2	100.8	14.5±0.2**	138.1
		2.5	27.5±0.5	112.6	13.2±0.1**	125.7
11.	Dulcit	1.5	29.3±0.3**	106.8	13.7±0.2**	130.5
		2.0	31.0±0.6**	113.1	14.2±0.2**	135.2
		2.5	26.2±0.5	102.8	14.1±0.2**	134.3
12.	Inositol	1.5	28.1±0.3	102.5	12.8±0.1**	121.9
		2.0	28.1±0.4	103.5	14.1±0.2**	134.2
		2.5	27.9±0.2	101.7	12.3±0.1**	117.1
13.	Adonit	0.1	27.5±0.3	100.3	12.3±0.2**	117.1
		0.5	27.9±0.5	101.7	13.7±0.1**	130.5
		1.0	28.0±0.4	102.4	13.2±0.2**	125.7
14.	Sorbitol	1.0	27.2±0.2	99.4	11.9±0.1**	113.3
		1.5	27.0±0.3	98.7	13.2±0.2**	125.7
		2.0	27.3±0.1	99.6	11.9±0.1**	113.3

*Treal>T95%; **Treal>T99%

The results of the experiments (Tab.1) confirm the fact that isopropanol, glycocol, dulcitol, mannitol, inositol and adonit have a stimulating effect on algal productivity: by 21-41% in the case of non-axenic culture and by 27-52% in the case of more axenic culture much to the witness. The most effective of the alcohols tested is isopropanol in a concentration of 3.0ml/l in the case of the non-axenic culture (increasing by 40.9% the accumulation of biomass compared to the control) and in a concentration of 0.5ml/l in the case of the axenic culture (increasing by 52.4% the accumulation of the biomass). These differences between the results obtained for the axenic and nonaxenic cultures are due to the competition for this nutrient substrate between the algal cells and the bacterial cells of the nonaxenic culture, while in the case of the axenic culture, this competition is missing and the volume of isopropanol necessary to ensure an obvious increase in productivity is 6 times lower. In the case of the use of methanol, butanol, isobutanol, pentanol and ethylene glycol, with the increase of their concentration in the nutrient medium, a decrease in algal productivity is observed (Tab. 2 and 3).

From the point of view of chemical composition, the action of alcohols is different. Thus, the studied alcohols do not stimulate protein synthesis, with the exception of glycocol in a concentration of 2.5mg/l. Under its influence, the amount of protein increases by 24.8% compared to the control in the case of the non-axenic culture and by 22.0% in the case of the axenic culture.

Methanol, butanol, isobutanol, pentanol and ethylene glycol have a negative effect on protein synthesis both in the case of axenic and non-axenic cultures (Tab. 2. and 3).

Regarding carotenogenesis, an increase in it was highlighted when cultivating *H. pluvialis* in axenic and nonaxenic conditions in the presence of alcohols: isopropanol, glycol, mannitol, dulcitol, inositol, adonitol and sorbitol. The other alcohols have an inhibitory or neutral effect because, possibly, the enzyme equipment of the algae transforms these substances into intermediate compounds, including substances that negatively influence the carotenogenesis process.

Isopropanol has a more beneficial effect on carotenogenesis, as in the case of stimulating ADB synthesis. Thus, in the case of the non-axenic culture when isopropanol is added to the nutrient medium in a concentration of 3ml/l, an increase in the gross content of β -carotene synthesized per day is observed by 65.4% compared to the control, and in the case of the axenic culture – the presence of this alcohol in the cultivation medium in a concentration of 0.5ml/l – increases the content of β -carotene synthesized per day by 74.8% compared to the control.

Conclusions

Generalizing the results obtained regarding the effect of alcohols on the productivity and biochemical composition of *H. pluvialis*, it follows that isopropanol in a concentration of 3.0ml/l in the nutrient medium for the non-axenic culture and 0.5ml/l for the axenic culture can be applied to direct the quality biomass of this alga.

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EFFECT OF X-RAY RADIATION ON THE VIABILITY OF EUROPEAN BEECH SEEDS

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