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Activity, patterns, and localization of carbonic acid enzymes in algae used in wastewater treatment.

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Abstract. This article presents the results on the presence, activity, localization, properties and functional role of the carbonic anhydrase enzyme involved in the carbon metabolism of microalgae - euglena (which has a specific nutritional feature similar to heterotrophic and protozoa) Chlamydomonas and Dunaliella grown in intensive and mass culture, depending on the activity of various forms of carbonic anhydrase on the concentration of CO2 and nitrogen in the gas phase and medium. As analyzes have shown, all studied species and strains of algae have carbonic anhydrase activity, depending on the type of strain and cultivation conditions, 0,10-3,60 per mg of dry matter or 12-416 per chlorophyll unit. The lowest activity of the carbonic anhydrase enzyme was observed in the Dunalilla minute algae grown in an open-air semi-production facility. In laboratory conditions grown in glass vessels, the minimum enzyme activity is observed in Chlamydomonas Reinhardii UA-5-16. The study of the localization of carbonic anhydrase in various cellular fractions of algae showed that in Dunaliella and Chlamydomonadal algae. The enzyme activity is mainly localized in the soluble fraction and gives similar values for both the homogeneous and the fraction of soluble proteins. In eugelena algae, a certain part (up to 40%) of carbonic anhydrase activity is localized in the membrane-bound fraction.

Keywords: Water grass, Chlorella, Growing water grass, Enzymes, Carboangdride, Enzyme location, Membrane-bound enzymes, Cytoplasmic images, Wastewater treatment water grass.

Introduction

The intensity of photosynthesis in algae, similarly to plants, primarily depends on the concentration of CO_2 supplied to the culture air [1]. For example, a high yield of algae biomass was obtained when the content of CO_2 in the air was up to 5,8%. However, a decrease in this concentration in the supplied gas mixture to 0,2% significantly reduces the growth and development, and then the productivity of chlorella.

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In the case of mass cultivation of algae in open pools, special collectors, and in a series of cultivation, the introduced culture suspension is usually bubbled with a mixture of 1-5% CO_2 in a mixture with air[2]. It was found that an important factor affecting the photosynthetic productivity of microalgae during their intensive cultivation is their optimal supply of carbon dioxide.

At limiting CO_2 concentrations, especially at a high light intensity, carbonic anhydrase plays an essential role in the biochemical adaptation of photosynthetic cells, which catalyzes the reversible hydration of carbon dioxide and participates in the regulation of photosynthetic and respiratory metabolism of the culture[3,12,4]. The study of the value and function of carbonic anhydrase is of interest both for understanding the mechanisms of the complex process of photosynthesis and in connection with the search for ways to optimize the conditions for carbon nutrition of photosynthetic organisms. Elucidation of such a fixation factor and the involvement of carbon dioxide in the biosphere cycle is of great interest at the present time. For, the impending danger of global warming., The accumulation of excessive concentration of carbon dioxide in the atmosphere poses a threat to the ecology and the environment.

Many works have been devoted to the study of the activity, localization, properties, structure, and functional role of carbonic anhydrase in unicellular green algae and algae - microphytes [5,6,7,8,9,10,11,13].

Despite a significant number of works devoted to the study of carbonic anhydrase of microalgae, there were no data on the presence of this enzyme in euglena, which has a specific feature in terms of nutrition types similar to heterotrophic and protozoa, Chlamydomonas and Dunaliella grown in mass culture. Therefore, the task of our research was to elucidate the regulation of carbon nutrition, the activity and localization of carbonic anhydrase in the cells of Chlamydomonas, euglena algae, and Dunaliella has grown in intensive and mass culture, as well as the dependence of the activity of various forms of carbonic anhydrase on the concentrations of CO_2 and nitrogen in the gas phase and medium.

Cultivation Of Microalgae And Measurement Of Carbonic Anhydrase Activity And Its Methods

The objects of the study were 6 species and strains of microalgae, which are representatives of two divisions: green - Chlorophyta and euglena - Euglenophyta and 3 genera: Sceenodesmus Meyen, Ankistrdesmus Corda, Dunaliella Teod, Chlamydomonas Breb., Euglena Ehrenb. Algological pure cultures were obtained from the collection of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan.

Cultivation of microalgae. Under laboratory conditions, algae were grown in glass vessels proposed by the Institute of Plant Physiology, Russian Academy of Sciences [16]. The vessels were placed in a thermostatic chamber, where thermoregulation was carried out with a ultrathermostat (UT-15). Mixing air containing 1–2% carbon dioxide was bubbled with a microcompressor. The light source was LK-40 DRL-250 and 400 lamps. Lighting of different intensity was created by changing the distance between the lamps and culture vessels. Illumination was measured with a Yu-16 luxmeter and was expressed in kilo lux (klx) and W/m^2.

In the open air, algae were cultivated in a horizontal tray-type installation with a volume of 1000 liters. An installation with a volume of 1000 liters is a rectangular 10 m long, 0.8 m wide, and 10-12 cm deep, lined with plastic wrap or plastic. A dividing board is mounted in the middle of the installation, the ends of which do not reach the side short sides by 30-40 cm. The board is attached to the sides of the installation with transverse stripes. Mixing in installations is carried out using a pump 36 MTs 4-12, and CO_2 is supplied from a cylinder through a fitting welded under the impeller of the pipe.

When growing microalgae used nutrient media - O_4 [17,18,19].

Measurement of carbonic anhydrase activity. Disintegration of cells was carried out in a phosphate buffer containing 0.06 m Na_2HPO_4, 5 mm cysteine, and 1 mm EDTA (pH 8,3). Fractionation of the cell-free homogenate into fractions of soluble proteins and insoluble cellular components was carried out by the method of stepwise centrifugation sequentially at 3, 5, 14, and 18 thousand q, respectively, 5, 10, 20, and 60 min at 2° C [5], and also by centrifugation at 18 × 103 q for 60 min [15].

The carbonic anhydrase activity of intact microalgal cells in the supernatant and in insoluble cellular components was measured by the electrometric method [5,15] by changing the initial pH value from 7,8 to 7,3 in the reaction of CO_2 hydration. The shift in pH from 7,8 to 7,3 as a function of time on a linear section was recorded with an ionometer (OP-267, Hungary) connected to a recorder and a universal polarograph

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OH-105 (Hungary). The reaction was started by the rapid introduction of 2 ml of a saturated solution of carbon dioxide into an equal volume of the reaction mixture containing intact cells of microalgae, soluble proteins, and insoluble cellular components in phosphate buffer (0,06 M Na_2HPO_4, 5 mm cystine, 1 mm EDTA). The rate of non-enzymatic reaction was determined by adding a saturated solution of CO_2 to 2 ml of phosphate buffer pH 8,3. Enzymatic activity was calculated in Wilbur-Anderson units using the formula:

$$E = 10 \quad \frac{T_0}{T-1} \qquad (1)$$

There are:
T_0 is the time (s) of the change in the pH of the nonenzymatic reaction,
T is the time (s) of the change in the pH of the enzymatic reaction, and μmole CO_2 per mg dry weight, chlorophyll unit and per mg protein were calculated.

Activity, Localization And Change In The Activity Of Carbon And Graphite Enzymes In Relation To Co_2 And Nitrogen

Studies have shown (Table 1) that all studied species and strains of algae have carbonic anhydrase activity. The activity level of this enzyme in the cells of different Englena species was close to each other (E. Gracilis – 0,16 per mg of dry matter or 10,0 per chlorophyll unit, in E. Proxima, respectively, 0,22 and 11,0), as well as in different species of Chlamydomonas (C.Reinhardii - 449 – 0,20 or 18,0, and C.Reinhardii UA-5-16 – 0,10 or 12,0). The enzyme activity in Dunaliella minute in intensive culture was 0,62 and 40,0 units. respectively.
Comparison of the carbonic anhydrase activity in intact cells per unit of chlorophyll and mg of dry matter shows that the level of its activity in the studied species and strains of Dunaliella significantly exceeds the activity of this enzyme in euglena and Chlamydomonas algae.

Table 1. Comparison of the activity of carbonic anhydrase of some microalgae on the concentration of carbon dioxide

Culture	Concentration CO_2, %	CA activity per mg of dry matter	CA activity per unit chlorophyll
Dunaliellaminuta	0,03	2,71	200
D. minute	2,0	0,62	40
D. minute*	0,03	3,60	416
D. minute*	2,0	0,91	60
Euglena proximaUA-4-19	0,03	1,75	120
E.proximaUA-4-19	2,0	0,22	11
E. gracilisUA-4-17	0,03	1,35	108
E. gracilisUA-4-17	2,0	0,16	10
Chlamydomonas reinhardii-449	0,03	0,78	50
Chl. Reinhardii-449	2,0	0,20	18
Chl. Reinhardii-UA-5-16	0,03	0,46	20
Chl. Reinhardii UA-5-16	2,0	0,10	12

Note: * - grown in the open air, in other cases in laboratory conditions.

The level of carbonic anhydrase activity, in contrast to the enzymes of the Calvin cycle, depends on the concentration of CO2 in the medium and increases at low concentrations (Pronina, 1981). Our studies have shown that the activity of carbonic anhydrase and the above algae at medium (2,0) and low CO2 concentrations (0,03%) under conditions of the same light intensity (30 W / m2) and temperature (28˚C ± 2) with a decrease CO2 concentration up to 0,03% (after 1-3 days) leads to an increase in the enzyme activity in all studied forms of algae. Similar results were obtained when comparing the Cabo anhydrase activity in chlorella Scenedesmus [21,9], as well as Dunaliella when grown at a low (0,03%) concentration of carbon dioxide[20].

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Significant increases in enzyme activity (5 times per chlorophyll unit) at low carbon dioxide concentrations (0,03%) are observed in Dunaliella, especially during mass cultivation (more than 6 times per chlorophyll unit). It also turned out that the activity of this enzyme in Dunaliella is more sensitive to carbon dioxide nutrition and, CO_2– dependent form of carbonic anhydrase (soluble form of carbonic anhydrase - sCA) in Dunaliella is synthesized more than in other studied algae (Table 2).

In euglena algae with a low CO_2 content in the gas phase, in contrast to Chlamydomonas, a significant increase in carbonic anhydrase activity is observed. The mechanism of adaptation to CO_2-limited conditions in euglena differs from Chlamydomonas and not only soluble form of carbonic anhydrase (sCA) participates in adaptation to these conditions, but also a membrane-bound form of carbonic anhydrase (MaCA) (Table 2) [21].

The study of the localization of carbonic anhydrase in various cellular fractions showed (Table 3) that almost all of its activity in algal Chlamydomonas is associated with the soluble fraction. The localization of carbonic anhydrase in Chlamydomonas in the soluble fraction is also evidenced by the fact that the activity of this enzyme, calculated per mg of dry matter, gives similar values for both the homogeneous and the fraction of soluble proteins. In contrast to Chlamydomonas, in euglena algae, a certain part (30-40%) of carbonic anhydrase activity is concentrated in the membrane-bound fraction.

Table 2. Dependence of soluble (sCA) and membrane-bound (MsCA) carbonic anhydrase on carbon dioxide concentration (CA activity per mg of dry matter)

Culture	% CO_2	2	0,03	2			0,03
	hour	2	4	6	8	10	12
sCA							
Chlorella sp.C*		2,0	8,3	5,9	2,0	2,1	6,0
D. minute		1,3	6,5	5,5	1,5	1,6	5,4
E. proxima UA-4-19		0,6	3,9	,3,	0,8	1,0	2,8
Chl. Reinhardii UA-5-16		0,4	2,0	1,6	0,5	0,7	1,5
MsCA							
E. Proxima UA-4-19		0,3	0,8	0,4	0,4	0,3	0,9
Chl. Reinhardii UA-5-16		0,4	0,5	0,4	0,4	0,4	0,5

More specific conclusions regarding the intracellular distribution of carbonic anhydrase can be made when calculating the total activity of the enzyme in cellular fractions [22]. Our data indicate that carbonic anhydrase in Chlamydomonas cells of algae is indeed localized in the fractions of soluble proteins (Table 4), and in euglena cells, both in soluble and membrane-bound fractions, it is somewhat higher in comparison with its activity in homogenate. Similar results were obtained by other researchers [5] when studying the localization of this enzyme in Scenedesmus. It is possible that this is due to the suppression of the enzyme activity in homogeneous physiologically active compounds of an indole or phenolic nature. MCA in euglena can be localized in the chloroplast of cells. It is known that in chlorella MCA is localized in the chloroplast and is strongly associated with the membrane system of this compartment[8].

Table 3 Comparative characteristics of carbonic anhydrase activity in homogeneous and fractions of soluble and membrane-bound proteins of microalgae

Culture	Homogenate *	Soluble protein		Insoluble	
		U	% of homogenate activity	U	% of homogenate activity
Chl. Reinhardii-449	0,40	0,38	95	0,04	0,1
Chl. Reinhardii UA-5-16	0,37	0,35	94,6	0,03	0,8
E. gracilis UA-4-17	0,24	0,18	75	0,11	45,8

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E. Proxima UA-4-19	0,26	0,20	76	0,10	42,3
Chlorella pyrenoidosa 82**	1,41	0,00	0,00	1,26	89,4
Scenedesmus obliquus**	0,30	0,35	116,7	0,04	13,3

Note: * - Units of enzyme activity per mg of dry matter; ** - Literature data [5].

K. Palkvist et al. (1990) revealed the presence of several forms of carbonic anhydrase in algae: soluble (sCA), membrane-bound carbonic anhydrase - of cytoplasmic (CCA) and thylakoid (TCA) membranes, as well as carbonic anhydrase of intact cells (ICA) and their functions should be considered with taking into account its possible topology of the compartment[14]. The activity of carbonic anhydrase in algae not only changes depending on carbon dioxide nutrition, but also on nitrogen nutrition [23]. The study of the adaptive response of various forms of carbonic anhydrase activity in response to nitrogen starvation conditions in Chlamydomonas and Euglena showed (Table 69) that within 1-3 days, regardless of the concentration of CO2, the activity of carbonic anhydrase in inactive cells increased by 2-2,5 times, which is consistent with the data obtained by other researchers [23] when studying the effect of nitrogen starvation on chlorella.

Table 4 Localization of carbonic anhydrase activity in microalgal cells

Culture	Total carbonic anhydrase activity, rel. unit's enzymatic activity		
	homogenate	soluble carbonic anhydrase fraction (sCA)	insoluble fraction (membrane bound) carbonic anhydrase (MsKA)
Chl. Reinhardii 449	4,9	4,6	0,5
Chl. Reinhardii UA-5-16	5,0	4,6	0,4
E. gracilis UA-4-17	4,5	3,4	2,1
E. proxima UA-4-19	4,7	3,6	1,8
Chlorella pyrenoidosa 82**	15,8	0,4	13,7
Scenedesmus obliquus**	4,7	5,3	0,6

Note: * - Literature data [5].

At the same time, the activity of homogenate, including sCA, increased by 50 and 25%, respectively, in Chlamydomonas. The activity of membrane-bound carbonic anhydrase, and, conversely, increased by 22% in comparison with cells grown in a complete nutrient medium.

Apparently, membrane-bound carbonic anhydrase, which is associated with the induction of Chlamydomonas in cells, is involved in the adaptation of enzyme systems to changes in nitrogen nutrition conditions.

Table 5. Effect of nitrogen starvation on the activity of various forms of carbonic anhydrase in microalgae cells

Variants	**Intact CA**		**Homogenous CA**			**Soluble CA**			**Membrane-coupled CA**		
	0,03 % CO_2	2,0 % CO_2	0,03% CO_2								
	Per mg dry matter		Per mg dry matter	per unit of chlorophyll	per mg of protein	per mg dry matter	per unit of chlorophyll	per mg of protein	per mg dry matter	per unit of chlorophyll	per mg of protein

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Chlamydomonas Reinhardii UA-5-16											
Norm	0,54	0,23	0,27	9,16	0,17	0,24	6,0	0,11	0,007	0,8	0,01
Starvation	1,22	0,70	0,13	5,5	7,1	0,10	4,0	5,5	0,01	0,9	1,25
Euglenagracilis UA-4-17											
Norm	1,23	0,30	-	-	-	-	-	-	-	-	-
Starvation	2,23	1,41	-	-	-	-	-	-	-	-	-

Conclusion

As a result of the research carried out, the following solutions can be distinguished:

1. Comparatively studied activities, localization, and regulation of carbonic anhydrase in a number of microalgae showed that in photosynthetic cells there is a rather complexly organized carbonic anhydrase system, including soluble and membrane-bound forms of the enzyme, the presence, and ratio of which depends on the genotype of plants and the conditions of their cultivation.
2. Soluble carbonic anhydrase is involved in the adaptation of algae to CO_2 limitation, insoluble in euglena, and insoluble in nitrogen starvation. The data obtained make it possible to optimize the growth of microalgae in terms of carbon nutrition.
3. Thus, the research results make it possible to assess the mode of saving carbon dioxide. So when growing Dunaliella, it is recommended to use carbon dioxide of at least 2%, and when cultivating Chlamydomonas and euglena – 0,03-2%.

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