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# THE CARBON FIXATION EFFICIENCY IN BIOMASS OF *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN & J. C. LEWIN (BACILLARIOPHYCEAE) UNDER THE CONDITIONS OF CUMULATIVE CULTIVATION

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The carbon utilization efficiency is an important characteristic of the cultivated object. Diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & J. C. Lewin is known to use carbon from aquatic environment quite effectively, as it has many unique carbonic anhydrases and carbon transporters. However, the carbon fixation efficiency for many types of diatoms in culture is still unknown. When calculating the carbon fixation efficiency, researchers use different terminology and methods, and it leads to significant difficulties when comparing the carbon fixation efficiency in the biomass of various types of microalgae. The aims of this study are: 1) to update terms and definitions used in literature on the basis of modern concepts of carbon fixation in microalgae biomass, as well as absorption of inorganic carbon by microalgae culture; 2) to evaluate the carbon fixation efficiency in the biomass of *C. closterium* diatom under conditions of cumulative cultivation. *C. closterium* was grown at a temperature of +20 °C on a nutrient medium RS. During the cultivation, the culture was bubbled with air (1.1 L of air per 1 L of culture per minute). The air temperature at the outlet of the suspension was of +19 °C; the maximum productivity of the culture was of 1.254 g·L$^{-1}$·day$^{-1}$. According to the results of the CHN analysis, the proportion of carbon in *C. closterium* dry biomass was of 23 %. Under the conditions of cumulative cultivation in *C. closterium*, the carbon fixation efficiency in biomass was of 90 %. Compared with other algae species, *C. closterium* is characterized by a rather high $CO_2$ fixation efficiency. For example, in green microalga *Chlorella protothecoides* and *Ch. vulgaris*, the $CO_2$ fixation efficiency was of 20 % and 55.3 %, respectively; in cyanobacteria *Spirulina* sp. – of 38 %; in red microalgae *Porphyridium purpureum* – of 69 %. It was observed that to ensure an increase of 1 g of *C. closterium* dry biomass per day at a temperature of +19 °C, a minimum of 0.46 L of $CO_2$, or 1132 L of air, should be consumed. Possibly, it is high carbon fixation efficiency, as well as low carbon fraction in *C. closterium* biomass, that explains the high production indices of this species. Under equal conditions of cultivation in terms of light and carbon availability, the productivity of *C. closterium* can exceed the productivity of other types of microalgae by 5–10 times. So, while *Spirulina* sp. productivity reaches 0.2 g·L$^{-1}$·day$^{-1}$, *C. closterium* productivity is of 1.254 g·L$^{-1}$·day$^{-1}$.

**Keywords:** diatom *Cylindrotheca closterium*, cultivation, carbon fixation efficiency

Marine microalgae are widely used in modern biotechnology as producers of valuable biologically active compounds [11, 14, 20]. Many types of marine microalgae are capable of synthesizing unique pigments, fatty acids, carbohydrates, etc. [9, 14, 15]. Among producers of valuable substances on an industrial scale, benthic diatoms are of particular interest, since they are characterized by high efficiency of utilization of light energy. Besides that, due to high silicon content in the absence of mixing, they quickly settle to the bottom of the photobioreactor, which significantly reduces the cost and facilitates harvesting [2].

Diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & J. C. Lewin, 1964 is one of the most promising cultivation objects for the production of valuable polyunsaturated fatty acids and fucoxanthin on an industrial scale. This is due to the fact that *C. closterium* has sufficiently high production indices [2, 4] and is capable of accumulating fucoxanthin to 2.3–2.6 % of dry weight [18, 21]. The diatom is also characterized by high (up to 10 % of dry weight) content of polyunsaturated fatty acids in biomass [17, 19].

Carbon, along with nitrogen, is the main component of the life of microalgae. The availability of carbon determines chemical composition of cells and rate of biosynthesis of waste products; therefore, the carbon utilization efficiency is an important characteristic of the cultivated object [13]. It is known that diatoms, in comparison with other microalgae, are able to use carbon from the aquatic environment quite effectively, since they have many carbon transporters [12]. However, the carbon fixation efficiency for many types of diatoms in culture is still unknown.

To calculate the carbon fixation efficiency, researchers use different terminology and methods [6, 7, 8], which leads to significant difficulties when comparing the carbon fixation efficiency in the biomass of various types of microalgae. Therefore, the aims of this study are: 1) to update terms and definitions used in literature on the basis of modern concepts of carbon fixation in microalgae biomass, as well as of absorption of inorganic carbon by microalgae culture; 2) to evaluate the carbon fixation efficiency in *C. closterium* diatom biomass under conditions of cumulative cultivation.

## MATERIAL AND METHODS

We used the culture of *Cylindrotheca closterium* from IBSS RAS culture collection. *C. closterium* culture was grown on RS nutrient medium [16], all components of which were increased three times [5], at a constant suspension temperature of $(20 \pm 1)$ °C, in the accumulative cultivation mode in plane-parallel photobioreactors with a working volume of 2 L and a layer of 5 cm, at round-the-clock lighting with CE-PIL-1-LF 46W/54-765 fluorescent lamps. The average light intensity on the working surface of the photobioreactor was of 150 μmol quanta·m$^{-2}$·s$^{-1}$ (33 W per m$^2$). During the cultivation, the culture was bubbled with air (1.1 L of air per 1 L of culture per minute) through a compressor unit. The temperature of the air flow at the outlet of the suspension was of $(19 \pm 0.5)$ °C. A dispersant nozzle was used to increase the solubility of atmospheric $CO_2$ in the culture medium. Culture density at the beginning of cumulative cultivation was of 0.1–0.2 g of dry matter per 1 L.

The culture density was determined by two methods: 1) by the method of iodide oxidation [1]; 2) by direct weighing of *C. closterium* wet weight in polypropylene tubes on an analytical balance with an error of 0.1 mg after cell precipitation by centrifugation (1600g for 2 minutes). To recalculate the obtained data on dry weight, we used the coupling coefficient between dry and wet weight (k = 0.1; n = 20). To determine the carbon fraction in biomass, a suspension of *C. closterium* cells was taken on the 6$^{th}$ day of the experiment, centrifuged for 1–2 minutes at 1600g, and washed twice with isotonic NaCl solution (9 g per L). The crude biomass was then dried at +105 °C for 24 hours to constant weight. The CHN analysis of dry biomass samples was performed using a Flash EA 1112 (Thermo Finnigan, Italy) at the centre of collective usage of the Russian Technological University (Moscow). Acetanilide was used as a standard.

## RESULTS AND DISCUSSION

The maximum density of *C. closterium* culture (B$_{max}$ = 3 g per L) was observed on the 6$^{th}$ day of the experiment (Fig. 1), and the maximum increase (P$_{max}$ = 1.254 g·L$^{-1}$·day$^{-1}$) was observed on the 5$^{th}$ day of the experiment. According to the results of CHN analysis, the proportion of carbon in *C. closterium* dry biomass was of 23 %.
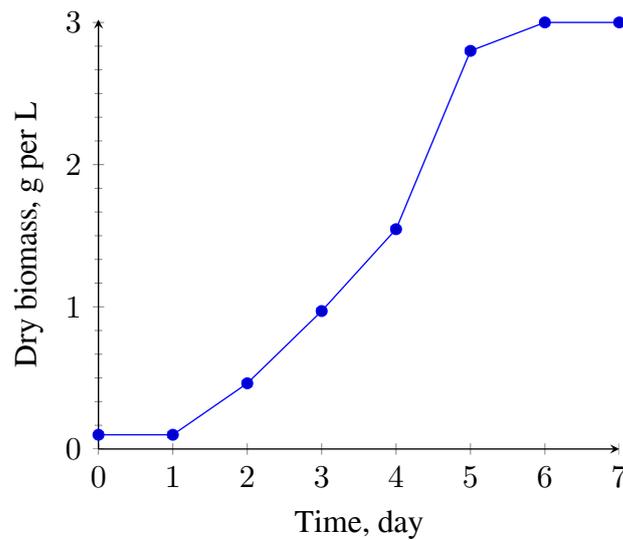
**Fig. 1.** Dynamics of density of *Cylindrotheca closterium* batch culture on a nutrient medium RS [16]

Extensive literature is concerned with the study of the carbon nutrition of microalgae cultures, and the researchers use different terminology in their publications, for example, "carbon binding", "carbon utilization", "usage" "assimilation", "adsorption", etc. [6, 7, 8, 12, 13]. In some cases, this leads to an obscure understanding of the processes studied; therefore, to avoid ambiguity, we give definitions of the terms used in this paper.

*The inorganic carbon flow* is the amount of inorganic carbon carried per unit of time. The units are mol per s, kg per s, and L per min.

*The inorganic carbon flow density* is the amount of carbon supplied to a suspension of microalgae (per unit of volume or unit of phase interface area) per unit of time. The units are $mol \cdot L^{-1} \cdot day^{-1}$, $mol \cdot m^{-2} \cdot day^{-1}$, $g \cdot L^{-1} \cdot day^{-1}$, and $g \cdot m^{-2} \cdot day^{-1}$.

In algological practice, a mixture of air and $CO_2$ is usually used to provide algae with carbon; therefore, *the carbon flow density* is expressed in the volume of the air-gas mixture supplied to the microalgae culture per unit of volume of suspension in one minute and is denoted as the ventilation coefficient of the culture, $L \cdot L^{-1} \cdot min^{-1}$. Given the molar volume of the gas at a given temperature, the carbon flow is easily expressed in mass units. For example, when a 2 % gas-air mixture is supplied (*i. e.* taking into account carbon in air of 2.04 % vol.) at the rate of 1 L per 1 L of suspension per minute at a temperature of +20 °C, the carbon flow density is of 10.2 mg per L of suspension in minute.

*The assimilation (binding, utilization) of inorganic carbon by a microalgae culture* is a set of biological processes in a suspension of microalgae, as a result of which inorganic carbon is converted into organic substances. At the same time, bound carbon is a part of organic substances, both biomass itself and exometabolites of microalgae (exopolysaccharides, proteins, etc.).

*The fixation of inorganic carbon by microalgae culture* is a combination of carbon assimilation and carbon-concentrating mechanism in microalgae cells. In addition to bound organic carbon, a certain amount of inorganic carbon is present in biomass. If carbon fixation is considered only in biomass (excluding exometabolites), then the carbon fixation rate ($F_C^b$) is determined by the expression:

$$F_C^b = c \times P \, , \tag{1}$$

where c is the carbon fraction in the biomass;

P is the growth rate of biomass (productivity), $g \cdot L^{-1} \cdot day^{-1}$.

*The inorganic carbon absorption by a microalgae culture* is a combination of the processes of fixation and physico-chemical absorption of inorganic carbon (solubility of $CO_2$ in the culture medium, formation of $HCO_3^-$ and $CO_3^{2-}$).

*The efficiency of carbon absorption by a microalgae culture* is the ratio of the mass of inorganic carbon absorbed by the microalgae culture to the mass of carbon supplied to the microalgae suspension:

$$E_C = (F_0 - F)/F_0 \times 100\% \,, \tag{2}$$

where $F_0$ and F are the carbon flow density at the inlet for the cell suspension and at the outlet, respectively.

*The efficiency of carbon fixation in the microalgae biomass* is the ratio of the mass of carbon fixed in the biomass to the mass of carbon supplied to the suspension of microalgae:

$$E_C^b = F_C^b/F_0 \times 100\% \,, \tag{3}$$

where $F_C^b$ is the rate of carbon fixation in the microalgae biomass;

$F_0$ is the carbon flow density at the inlet for the cell suspension.

When $CO_2$ is used to provide the culture with inorganic carbon, then, taking into account (1), the rate of carbon fixation in biomass ($F_{CO_2}^b$) can be calculated as follows:

$$F_{CO_2}^b = M(CO_2)/M(C) \times F_C^b = 44/12 \times c \times P \,, \tag{4}$$

where $M(CO_2)$ and $M(C)$ are the molar mass of carbon dioxide and carbon, respectively, g per mol.

From formulas (1) and (4), the limiting ratio follows, indicating the minimum $CO_2$ flow density ($g \cdot L^{-1} \cdot day^{-1}$), which is necessary to ensure a given microalgae growth rate:

$$F_C^b = M(C)/M(CO_2) \times F_{CO_2}^b = c \times P \,,$$

*i. e.*

$$P = 0.273/c \times F_{CO_2}^b \,. \tag{5}$$

For those cases when a gas-air mixture with a given percentage of $CO_2$ is fed into the suspension, expression (5) is converted to the following:

$$P = \frac{0.273}{c} \frac{M(CO_2)}{V_{CO_2}(T)} \frac{\nu}{100} 1440 F_{GA}^{min} \,, \tag{5a}$$

where $V_{CO_2}$(T) is the molar volume of gas at a given temperature, L per mol;

ν is the proportion of $CO_2$ in the gas-air mixture, % vol.;

$F_{GA}^{min}$ is the minimum gas-air mixture flow density necessary to ensure a given microalgae growth rate, $L \cdot L^{-1} \cdot min^{-1}$.

At a temperature different from +273 °K, the gas volume is equal to:

$$V_{CO_2}(T) = \frac{V_0}{T_0}T = 0.082T \,, \tag{6}$$

where $V_0$ is the molar volume of gas under normal conditions ($V_0$ = 22.4 L per mol at $T_0$ = +273 °K).

Using expressions (3), (5a), and (6), we can calculate *the efficiency of carbon fixation in the biomass* of any species of cultivated algae by the formula:

$$E_C^b = \frac{c \times P}{0.273 \frac{M(CO_2)}{0.082T} \frac{\nu}{100} 1440 F_{GA}} 100\% \, , \tag{7}$$

where $F_{GA}$ is the gas-air mixture flow density at the inlet for the cell suspension, $L \cdot L^{-1} \cdot min^{-1}$.

Concerning our experiment with the cultivation of *C. closterium* under the conditions of cumulative cultivation, *the rate of carbon fixation* from the 4th to the 5th day of the experiment was the following: $F_C^b = 0.23 \times 1.254 = 0.29$ g·L$^{-1}$·day$^{-1}$.

*The efficiency of carbon fixation in biomass* was:

$$E_C^b = \frac{0.23 \times 1.254}{0.273 \frac{44}{0.082 \times (273+19)} \frac{0.0405}{100} 1440 \times 1.1} 100\% \approx 90\% \, .$$

For comparison: green microalga *Chlorella protothecoides* and *Ch. vulgaris* are characterized by the efficiency of $CO_2$ fixation in biomass of 20 % and 55.3 %, respectively; cyanobacteria *Spirulina* sp. – of 38 % [7, 10]; red microalga *Porphyridium purpureum* – of 69 % [6]. So, compared with other algae species, *C. closterium* is characterized by a rather high $CO_2$ fixation efficiency.

It is important to note that in calculating the carbon fixation efficiency by a microalgae culture, many researchers do not take into account increase in gas volume with increasing temperature in accordance with formula (6). Typically, the calculations use values for standard conditions, although the experimental conditions with a microalgae culture are not standard; it leads to erroneous results. Thus, if experiments with *C. closterium* culture are carried out at a temperature of +30 °C, and the calculation of the carbon fixation efficiency is made for standard conditions (0 °C), then the values obtained can be significantly underestimated. This can be easily verified by substituting the temperature values in formula (7). For the standard temperature (0 °C), the carbon fixation efficiency $E_C^b$ is of 84 %, and for +30 °C it is of 93 %.

In practice of intensive microalgae cultivation, it is often necessary to calculate the cost of $CO_2$ for obtaining a unit of biomass. Assuming that all carbon entering the microalgae suspension is converted to organic matter, it is possible to estimate the cost of $CO_2$ using the limiting ratio (5a). It should be noted that the concentration of carbon dioxide in the atmosphere in 2018 according to the World Meteorological Organization reached 0.0405 % vol. [3].

Thus, substituting the data of our experiment in (5a), we see the following: to ensure an increase of 1 g of *C. closterium* dry biomass per day at a temperature of +19 °C, it is necessary to consume a minimum of 0.46 L of $CO_2$, or 1132 L of air.

**Conclusion.** Under equal cultivation conditions in terms of light and carbon availability, the productivity of *C. closterium* can exceed the productivity of other types of microalgae by 5–10 times. So, while *Spirulina* sp. productivity reaches 0.2 g·L$^{-1}$·day$^{-1}$, *C. closterium* productivity is of 1.254 g·L$^{-1}$·day$^{-1}$. Possibly, it is high carbon fixation efficiency, as well as low carbon fraction in *C. closterium* biomass that explains the high production indices of this species.

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## ЭФФЕКТИВНОСТЬ ФИКСАЦИИ УГЛЕРОДА В БИОМАССЕ *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN & J. C. LEWIN (BACILLARIOPHYCEAE) В УСЛОВИЯХ НАКОПИТЕЛЬНОГО КУЛЬТИВИРОВАНИЯ

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Эффективность утилизации углерода является важной характеристикой объекта культивирования. Известно, что диатомовая водоросль *Cylindrotheca closterium* (Ehrenberg) Reimann & J. C. Lewin может эффективно использовать углерод из водной среды, так как имеет множество уникальных карбоноангидраз и углеродных транспортёров. Между тем эффективность фиксации углерода

для многих видов диатомей в культуре по-прежнему неизвестна. Для её расчёта ряд авторов используют разную терминологию и способы, что приводит к значительным трудностям при сравнении эффективности фиксации углерода в биомассе различных видов микроводорослей. Цели работы: 1) на основе современных представлений о фиксации углерода в биомассе микроводорослей, а также о поглощении неорганического углерода культурой микроводорослей актуализировать используемые в литературе термины и определения; 2) оценить эффективность фиксации углерода в биомассе диатомеи *C. closterium* в условиях накопительного культивирования. Культуру *C. closterium* выращивали при температуре +20 °C в интенсивном режиме на питательной среде RS. В процессе выращивания культуру барботировали воздухом (1,1 л воздуха на 1 л культуры в минуту). Температура воздуха на выходе из суспензии составляла +19 °C, максимальная продуктивность культуры — 1,254 г·л$^{-1}$·сут$^{-1}$. По результатам CHN-анализа, доля углерода в сухой биомассе *C. closterium* составляла 23 %. В условиях накопительного культивирования у *C. closterium* эффективность фиксации углерода в биомассе достигла 90 %. По сравнению с другими видами водорослей *C. closterium* характеризуется достаточно высокой эффективностью фиксации $CO_2$. Так, у зелёных микроводорослей *Chlorella protothecoides* и *Ch. vulgaris* эффективность фиксации $CO_2$ составляет 20 % и 55,3 % соответственно, у цианобактерии *Spirulina* sp. — 38 %, у красной микроводоросли *Porphyridium purpureum* — 69 %. Отмечено, что для обеспечения прироста 1 г сухой биомассы *C. closterium* в сутки при температуре +19 °C необходимо затратить минимум 0,46 л $CO_2$, или 1132 л воздуха. Возможно, именно высокая эффективность фиксации углерода, а также низкая доля углерода в биомассе *C. closterium* позволяют объяснить высокие продукционные показатели этого вида. В равных условиях культивирования по свету и обеспеченности углеродом продуктивность *C. closterium* может превышать продуктивность других видов микроводорослей в 5–10 раз. Так, у *Spirulina* sp. продуктивность достигает 0,2 г·л$^{-1}$·сут$^{-1}$, у *C. closterium* — 1,254 г·л$^{-1}$·сут$^{-1}$.

**Ключевые слова:** диатомовая водоросль *Cylindrotheca closterium*, культивирование, эффективность фиксации углерода