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# PRODUCTION CHARACTERISTICS OF A CULTURE OF THE DIATOM *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN ET LEWIN IN A TWO-STAGE CHEMOSTAT

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The advantages and disadvantages of flow and batch microalgae cultivation are discussed. The benefits of the flow cultivation are indicated, in particular in a quasi-continuous mode in a two-stage chemostat. It is proposed to use the culture of the benthic diatom *Cylindrotheca closterium* as a producer of valuable substances since this species has several useful properties of both biological and technological nature. Specifically: 1) *C. closterium* is characterized by relatively high production rates; 2) it efficiently utilizes light energy which removes restrictions on the location of production in areas with a small number of sunny days *per* year; 3) it has a rather low temperature optimum for growth which is significant for the implementation of industrial technologies in Russian Federation; and 4) it has the specific density of cells of more than one, therefore, cells quickly enough sink to the photobioreactor bottom in the absence of the culture aeration (this simplifies the separation of biomass from the culture medium and reduces its cost). The aim of this work is to analyze the production characteristics of the quasi-continuous *C. closterium* culture in the two-stage chemostat. The studies were carried out at a temperature of (20 ± 1) °C and irradiation of 150 μmol quanta·m$^{-2}$·s$^{-1}$. The chemostat for *C. closterium* cultivation consisted of two glass 3-L photobioreactors of the plane-parallel type, each having a working thickness of 5 cm and a working surface of 0.03 m$^2$. The cultivation was carried out on the nutrient medium RS with a constant aeration (the speed was of 1.5 L of air *per* 1 L of culture *per* min). The culture was examined at different dilution rates of the nutrient medium: 0.1; 0.3; 0.5; 0.7; and 0.9 day$^{-1}$. The growth parameters of the batch culture were calculated: the specific growth rate $\mu_b$ = 0.7 day$^{-1}$; the time for doubling the biomass $t_d$ = 0.987 days. The maximum productivity of a one- and two-stage chemostat was registered at the optimal dilution rate of 0.59 day$^{-1}$; the values were 1.348 and 1.498 g·L$^{-1}$·day$^{-1}$, respectively. As found experimentally, *C. closterium* productivity in the flow culture is 2.2 times higher than in the batch culture. The experimental data were used to calculate the maximum specific growth rate $\mu_m$ and the saturation constant $K_S$ with limiting *C. closterium* growth by silicon; the values were 1.05 day$^{-1}$ and 0.028 g·L$^{-1}$, respectively. It was shown that the observed need for silicon in the flow culture ($Y_{fl}$ = 35 mg·g$^{-1}$) is lower by 7.9% than in the batch culture ($Y_b$ = 38 mg·g$^{-1}$). For the diatom *C. closterium*, $\mu_m$, $K_S$, and $Y_{fl}$ are important physiological characteristics; those play the key role in the design of industrial systems for intensive microalgae cultivation.

**Keywords:** *Cylindrotheca closterium*, chemostat, mathematical model

Microalgae are widely used in modern biotechnology [Bozarth et al., 2009]. Their biomass and waste products have found an application in food industry, agriculture, and aquaculture [Creswell, 2010; Sathasivam et al., 2019]. Microalgae play an important role in technologies of wastewater treatment [Abinandan et al., 2018; Wollmann et al., 2019], rehabilitation of water basins by preventing blooming [Kiran et al., 2016], $CO_2$ utilization [Singh, Dhar, 2019], algolization of soils, and green manuring. On their basis, industrial technologies are created for obtaining unique biologically active compounds applied in medicine, perfumery [Lauritano et al., 2016; Lincoln et al., 1990; Patras et al., 2018], *etc.* Microalgae are actively used in various human activity, but their potential is far from being exhausted. Selection of new objects for intensive cultivation from natural populations and production of genetically modified strains with desired properties constantly expand the possibilities for creating new biotechnologies based on microalgae. In this field, there is a considerable progress; however, the search for new producers is ongoing.

Microalgae are known to accumulate many valuable substances precisely under stressful conditions, for example, when their growth is limited by biogenic elements. Accordingly, various methods of two-stage batch cultivation have been developed: at the first stage, the accumulation of biomass occurs, and at the second one, the accumulation of valuable substances, *e. g.*, lipids and carotenoids [Lu et al., 2018; Minyuk et al., 2014; Nagappan et al., 2019]. A significant disadvantage of the two-stage batch cultivation is the death of a considerable part of microalgal cells at the second stage, under stress conditions; this notably reduces the efficiency of obtaining the target product [Minyuk et al., 2014]. The most promising alternative to the two-stage batch cultivation is the flow cultivation, in particular, a usage of a two-stage chemostat with equal or different specific flow rates for each stage.

From the standpoint of obtaining valuable biologically active compounds on an industrial scale, the most promising cultivation objects are benthic species of microalgae. Those are distinguished by several useful properties of both biological and technological nature. Specifically:

1) benthic microalgae are characterized by relatively high production rates [Zheleznova, 2019];
2) those efficiently utilize light energy [Baldisserotto et al., 2019], and this removes restrictions on the location of production in areas with a small number of sunny days *per* year;
3) benthic microalgae are characterized by a rather low temperature optimum for growth [Salleh, McMinn, 2011; Stock et al., 2019], which is significant for the implementation of technologies in Russian Federation;
4) those have the specific density of cells of more than one, therefore, cells quickly enough sink to the photobioreactor bottom in the absence of the culture aeration (this simplifies the separation of biomass from the culture medium, reduces its cost, *etc.*).

In literature, data on intensive cultivation of benthic microalgae are scarce. The studies on benthic diatoms in a flow culture are practically not described as well. Out of many species of benthic microalgae for intensive cultivation on an industrial scale, the diatom *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin, 1964 is of the greatest interest [Gevorgiz et al., 2016; Wang et al., 2018]. It is capable of accumulating valuable polyunsaturated fatty acids and fucoxanthin [Wang et al., 2015; Zheleznova et al., 2017], as well as iodine, iron, and other trace elements in organic form [de la Cuesta, Manley, 2009; Zheleznova et al., 2015a].

The aim of this work is to analyze the production characteristics of a quasi-continuous *C. closterium* culture in a two-stage chemostat.

## MATERIAL AND METHODS

We studied the culture of the diatom *C. closterium* from the IBSS collection of microalgae cultures. For two weeks, the culture obtained from this collection was adapted to the concentrated nutrient medium RS [Zheleznova et al., 2015b] and to conditions of intensive cultivation. The adapted culture was centrifuged (at 1,600 g for 1 min); then, the supernatant was removed, and algal raw mass was used as an inoculum to analyze the production characteristics of a quasi-continuous culture in a chemostat. When working with the chemostat, the nutrient medium RS was used with a 3-fold increase in the concentration of each component; it was prepared on sterile Black Sea water. The composition of the nutrient medium is given in Table 1.

**Table 1.** Composition of the nutrient medium RS [Zheleznova et al., 2015b] used in the experiment

| No. | Component | Content, $g \cdot L^{-1}$ |
|---|---|---|
| 1 | $NaNO_3$ | 2.331 |
| 2 | $NaH_2PO_4 \cdot 2H_2O$ | 0.665 |
| 3 | $Na_2SiO_3 \cdot 9H_2O$ | 1.158 |
| 4 | $Na_2EDTA$ | 0.792 |
| 5 | $FeSO_4 \cdot 7H_2O$ | 0.192 |
| 6 | $CuSO_4 \cdot 5H_2O$ | 0.0006 |
| 7 | $ZnSO_4 \cdot 7H_2O$ | 0.001 32 |
| 8 | $CoCl_2 \cdot 6H_2O$ | 0.0006 |
| 9 | $MnCl_2 \cdot 4H_2O$ | 0.001 08 |
| 10 | $NaMoO_4 \cdot H_2O$ | 0.000 36 |

*C. closterium* production characteristics were studied under optimal light conditions, at optimal temperature and nutrient availability. The chemostat for *C. closterium* cultivation (Fig. 1) consisted of two glass 3-L photobioreactors (the two-stage chemostat) of the plane-parallel type, each having a working thickness of 5 cm. The working volume of the suspension in each photobioreactor was maintained at a constant level, 2 L. The experiment was carried out with uniform day-and-night one-sided irradiation of photobioreactors. As a source of irradiation, luminescent lamps CE-PIL-1-LF 46W/54-765 (Poland) were used. On the working surface of each photobioreactor (0.03 $m^2$), those gave an average of 150 $\mu mol\,quanta \cdot m^{-2} \cdot s^{-1}$ (33 $W \cdot m^{-2}$). To measure irradiation, a TKA-Spectr spectrophotometer (PAR) was applied. Throughout the experiment, the temperature of the algal suspension was maintained at a constant level of (20 ± 1) °C. To provide cells with carbon, the culture in both photobioreactors was aerated (1.5 L of air *per* 1 L of culture *per* min) with a compressor unit. To increase $CO_2$ solubility in the culture medium, air was supplied to the suspension *via* a dispensing nozzle.

At the first stage of the experiment, the culture was grown in a batch mode, and at the second stage, in a quasi-continuous one. *C. closterium* proportional-flow quasi-continuous cultivation was carried out in a single-flow mode as follows:

1) for yield, a part of the working volume was withdrawn daily from the second photobioreactor (the second stage of the chemostat);

2) the same part of the working volume was withdrawn from the first photobioreactor (the first stage of the chemostat) and transferred to the second photobioreactor – to restore the working volume in the second photobioreactor;
3) the working volume in the first photobioreactor was restored by adding fresh nutrient medium. The culture was diluted every day.

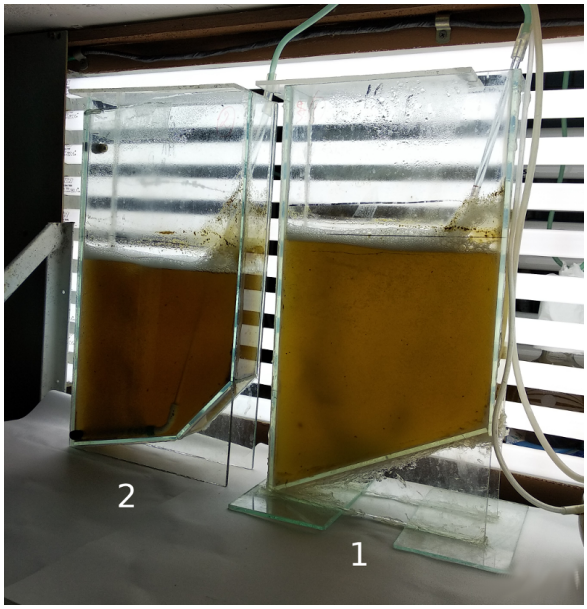


**Fig. 1.** *Cylindrotheca closterium* culture in a two-stage chemostat (stages are indicated by numbers)

After the batch cultivation, since the 6$^{th}$ day of the experiment, the culture was grown in the quasi-continuous mode with a flow rate of the nutrient medium through the working volume of the microalgal suspension of 0.6 L·day$^{-1}$ (dilution rate, or specific flow rate, $\omega$ = 0.6 / 2 = 0.3 day$^{-1}$). From the 17$^{th}$ to the 27$^{th}$ day of the experiment, the specific flow rate was set as 0.1 day$^{-1}$; from the 27$^{th}$ to the 35$^{th}$, 0.5 day$^{-1}$; from the 35$^{th}$ to the 40$^{th}$, 0.7 day$^{-1}$; and from the 40$^{th}$ to 44$^{th}$, 0.9 day$^{-1}$.

To determine the culture density, two methods were applied – the method of iodate oxidation [Gevorgiz et al., 2015] and direct weighing of *C. closterium* raw mass in polypropylene test tubes on an analytical balance with an error of 0.1 mg after cell sedimentation by centrifugation (at 1,600 g for 2 min). To calculate the data obtained for wet mass in terms of dry, the conversion factor (k = 0.1) was used [Zheleznova, Gevorgiz, 2020].

## RESULTS AND DISCUSSION

The dynamics of the density of *C. closterium* culture in the batch and flow modes of cultivation is shown in Fig. 2. In the experiment, the adapted culture was used; therefore, there was no lag phase for an accumulation curve. The exponential growth phase lasted four days. In the stationary phase, the culture density practically did not change. Upon transition to the quasi-continuous mode of cultivation, rapid adaptation to the conditions of flow cultivation was observed. With a change in the specific flow rate, the transition processes lasted for a short time (no more than 2–3 days), and the culture density value quickly reached a new stationary dynamic equilibrium (Fig. 2).

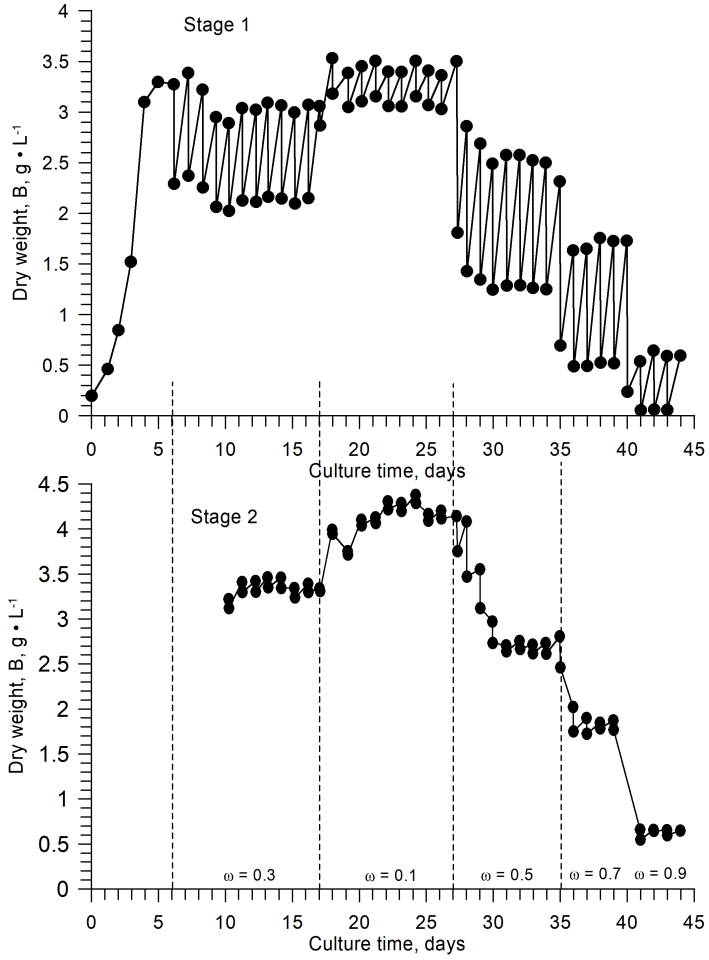


**Fig. 2.** Dynamics of *Cylindrotheca closterium* density in the batch culture and in the two-stage chemostat at different dilution rates $\omega$ (the boundaries are indicated by the dotted lines)

In both the first and second chemostat stages, at a specific flow rate of 0.1 day$^{-1}$, insignificant cell agglutination was recorded; it caused difficulties in sampling when measuring the culture density and contributed to an increase in data scatter. In both chemostat stages, almost at any flow rate, cells were evenly distributed throughout the entire working volume. At a specific flow rate of 0.9 day$^{-1}$, a near-wall culture growth was observed, and the aeration was not enough to mix the suspension. Even after mixing the culture with a manual stirrer, some biomass quickly sank to the photobioreactor bottom or walls.

**Growth parameters.** At the beginning of the batch cultivation, when introducing inoculum into the nutrient medium, the culture density ($B_0$) was 0.2 g·L$^{-1}$. In the batch culture, there was no lag phase, since it was adapted to the experimental conditions earlier. The exponential phase of the accumulation curve was characterized by a constant specific growth rate and was described with high accuracy ($R^2$ = 0.99) by equation (1) (Fig. 3):

$$\ln B = \mu_b (t - t_0) + \ln B_0; \qquad \ln B = 0.7\, t - 1.61, \tag{1}$$

where $\mu_b$ is the specific growth rate in the batch culture, day$^{-1}$;

$B_0$ is the culture density at the initial moment of time $t_0$ ($t_0$ = 0).

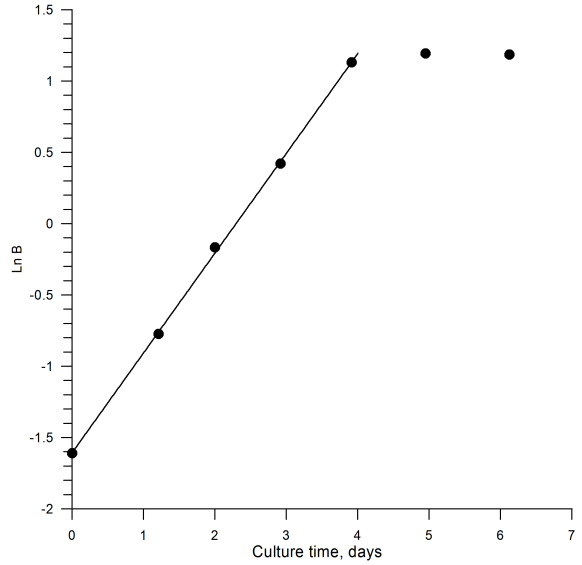


**Fig. 3.** Dynamics of *Cylindrotheca closterium* density in the batch culture in semi-logarithmic coordinates. Approximation of experimental points by equation (1), $R^2$ = 0.99. The specific growth rate $\mu_b$ = 0.7 day$^{-1}$

The biomass doubling time in the exponential growth phase ($t_d$) was:

$$t_d = \ln 2 \frac{t - t_0}{\ln B - \ln B_0} = \frac{\ln 2}{\mu_b} = \frac{0.693}{0.7} = 0.987. \quad (2)$$

Reverse doubling time (Fig. 3) was as follows:

$$\log_2 B = \frac{1}{t_d}(t - t_0) + \log_2 B_0, \qquad \frac{1}{t_d} = \frac{\mu_b}{\ln 2} = \frac{0.7}{0.693} = 1.01. \quad (3)$$

On the 5$^{th}$ day of the experiment, the culture reached the stationary growth phase. In this phase, the culture density remained the same ($B_m$ = 3.3 g·L$^{-1}$) until the transition to the quasi-continuous mode of cultivation (Fig. 2). For 5 days of the batch cultivation, the yield was $B_b(5) = B_m - B_0$ = 3.1 g·L$^{-1}$. Accordingly, the average productivity of the batch culture $\overline{P}_b$ = 0.62 g·L$^{-1}$·day$^{-1}$.

Quantitative requirements for nutrients in the actively growing *C. closterium* culture were experimentally determined by us earlier [Zheleznova et al., 2015b]. Based on these data and applying formula (4), economic coefficients ($Y_{ec}$) were calculated for several biogenic elements (Table 2):

$$Y_{ec} = \frac{1}{Y_b}, \quad (4)$$

where $Y_b$ is the observed requirement for a biogenic element in the batch culture.

**Table 2.** Observed requirements for *Cylindrotheca closterium* in the batch culture ($Y_b$) according to the data from [Zheleznova, Gevorgiz, 2014] and calculated values of economic coefficients ($Y_{ec}$) according to formula (4) for some nutrients

| No. | Biogenic element | $Y_b$, mg·g$^{-1}$ | $Y_{ec}$, g·g$^{-1}$ |
|---|---|---|---|
| 1 | Nitrogen | 64 ± 1 | 15.6 |
| 2 | Silicon | 38.2 ± 0.01 | 26.2 |
| 3 | Phosphorus | 17 ± 1 | 60.3 |
| 4 | Iron | 45 ± 0.2 | 22.2 |

To determine the dependence of *C. closterium* specific growth rate on the concentration of the limiting substrate in the nutrient medium, based on the data from [Zheleznova, Gevorgiz, 2014], the saturation constant ($K_S$) was calculated. Analyzing the composition and ratios of biogenic elements in the nutrient medium F [Guillard, 1975; Guillard, Ryther, 1962], as well as the ratio of *C. closterium* requirements from Table 2, it can be concluded as follows: when this alga is cultivated on the nutrient medium F, as was done in [Zheleznova, Gevorgiz, 2014], the limiting growth factor is the concentration of silicon in this medium. Therefore, the saturation constant was calculated precisely for silicon (Fig. 4):

$$P = P_m \frac{S}{K_S + S}, \quad P = 1.81 \frac{S}{0.028 + S}; \qquad \mu = \mu_m \frac{S}{K_S + S}, \quad \mu = \mu_m \frac{S}{0.028 + S}, \tag{5}$$

where $S$ is the concentration of the limiting substrate (silicon) in the nutrient medium, g·L$^{-1}$.

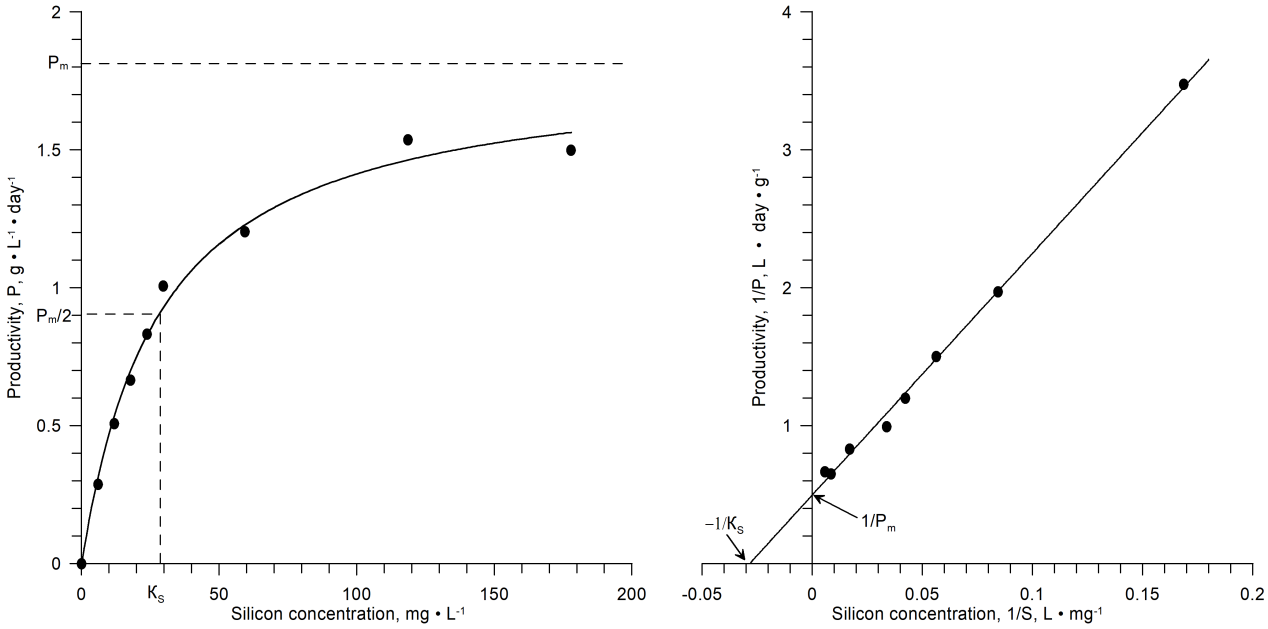


**Fig. 4.** Dependence of *Cylindrotheca closterium* growth rate on the silicon concentration in the nutrient medium. $P_m$ = 1.81 g·L$^{-1}$·day$^{-1}$; $K_S$ = 0.028 g·L$^{-1}$. Calculation by equation (5) according to the experimental data from [Zheleznova, Gevorgiz, 2014]

**Flow culture.** The chemostat theory was developed for continuous cultures of heterotrophic microorganisms in the second half of the XX century and is detailed in [Herbert et al., 1956; Maxon, 1955; Methods in Microbiology, 1970; Pirt, 1978; Theoretical and Methodological Basis of Continuous Culture of Microorganism, 1968]. In some cases, it is applicable to lower phototrophs as well. However, since their growth requires light energy, which, along with biogenic elements, can limit cell growth, the chemostat theory needs amendments and clarifications for phototrophs. This is especially true for dense cultures of microalgae, when the accumulation curve is characterized by the presence of not only an exponential growth phase, but also a long phase of growth deceleration (phase of negative growth acceleration). Of particular note is the need for significant additions and changes in the chemostat theory, when: 1) natural light is used, and a change in the growth-limiting factor is observed during the day; 2) it is difficult or impractical to organize a continuous flow of a nutrient medium; *etc.* In such a case, a batch

culture (quasi-continuous one) is often used in practice. Interestingly, the method of quasi-continuous cultivation is a certain generalization of various techniques of the flow cultivation of microorganisms. However, the publications on modeling processes in a quasi-continuous culture are quite rare [Fencl, 1968; Trenkenshu, 2005].

In our experiment, the exponential growth phase of the accumulation curve was accurately described by equation for exponential growth (see equation (1) and Fig. 3); that is, cell growth was practically not limited by light conditions. Apparently, there was no change in the growth-limiting factor in this area. This circumstance allows us to apply an approach, which is similar to that used when working with heterotrophic microorganisms, to derive equations for the density dynamics of a quasi-continuous culture. We will analyze a quasi-continuous culture in a proportional-flow single-flow multistage chemostat with the same working volume $V$ (L) in each stage and the same volume w (L), which is withdrawn from each stage during the exchange procedure. The ratio of the algal mass in the *i*-th stage of the chemostat $m_i$ (g) to the working volume $V$ (L) determines the actual density of the culture in this stage (g·L$^{-1}$):

$$B_i = \frac{m_i}{V}. \tag{6}$$

As a result of the exchange procedure, the volume w, which contains the algal mass $m_i^*$, is withdrawn from the *i*-th stage of the chemostat. The remaining volume ($V$ – w) contains the algal mass $m_i^{**}$. Therefore, the following equalities can be written:

$$m_i = m_i^* + m_i^{**}; \quad m_i^* = \mathrm{w} \cdot B_i; \quad m_i^{**} = V \cdot B_i - \mathrm{w} \cdot B_i = (V - \mathrm{w}) \cdot B_i. \tag{7}$$

In a multistage chemostat, after withdrawing the volume w, the *i*-th stage receives the same volume of suspension from the previous stage. So, the culture density in the *i*-th stage after the exchange ($B_i^{(-)}$) is equal to:

$$B_i^{(-)} = \frac{m_{i-1}^* + m_i^{**}}{V} = \frac{\mathrm{w} \cdot B_{i-1} + (V - \mathrm{w}) \cdot B_i}{V}, \tag{8}$$

where $m_{i-1}^*$ is the algal mass from the previous stage, which is introduced into the current stage during the exchange;

$B_{i-1}$ is the culture density in the previous chemostat stage.

Therefore:

$$\frac{\mathrm{w}}{V} = \frac{B_i - B_i^{(-)}}{B_i - B_{i-1}} = \frac{m_i^* - m_{i-1}^*}{m_i - m_{i-1}}; \tag{9}$$

it means that the ratio of the volume during the exchange procedure is equal to the ratio of the algal mass withdrawn during the exchange from the current stage to the difference in the algal masses in the current and previous chemostat stages.

The ratio of the culture density before and after the exchange indicates the value of the culture dilution ($\theta_i$); it shows how many times the algal mass and the culture density decrease in the *i*-th stage:

$$\theta_i = \frac{B_i}{B_i^{(-)}} = \frac{V}{V - \mathrm{w}\left(1 - \frac{B_{i-1}}{B_i}\right)}. \tag{10}$$

Worth noting as follows: if the culture is diluted with a nutrient medium ($B_{i-1} = 0$), then, expression for the first stage of the chemostat is obtained, similar to that in the publication [Trenkenshu, 2005]:

$$\theta_1 = \frac{B_1}{B_1^{(-)}} = \frac{V}{V - \mathrm{w}}.$$

From expressions (9) and (10), it follows:

$$\frac{\mathrm{w}}{V} = \frac{B_i - B_i^{(-)}}{B_i - B_{i-1}} = \frac{B_i - \frac{1}{\theta_i} B_i}{B_i - B_{i-1}}. \tag{11}$$

In a quasi-continuous culture, the change in the algal mass ($\Delta m_i$) is determined by two processes – an increase in the mass driven by algal growth and a decrease in the mass due to the difference in inflow and outflow with a part of the suspension during the exchange procedure:

$$\underbrace{m_{i+1} - (m_{i-1}^{*} + m_i^{**})}_{\text{growth}} + \underbrace{m_{i-1}^{*}}_{\text{inflow}} - \underbrace{(m_i - m_i^{**})}_{\text{decrease with outflow}} = m_{i+1} - m_i = \Delta m_i, \tag{12}$$

where $m_i$ is the algal mass before the exchange;

$m_{i+1}$ is the algal mass before the exchange at the next step of the quasi-continuous cultivation;

$m_{i-1}^{*}$ is the mass of the alga introduced into the *i*-th stage from the previous one during the exchange;

$m_{i-1}^{**}$ is the algal mass that remains in the *i*-th stage after withdrawing a part of the suspension with volume w.

Considering (6), (7), and (8), we can write the change in the culture density ($\Delta B_i$):

$$\underbrace{[B_{i+1} - B_i^{(-)}]}_{\text{increase}} - \underbrace{[B_i - B_i^{(-)}]}_{\text{decrease}} = B_{i+1} - B_i = \Delta B_i, \tag{13}$$

where $B_i$ is the culture density before the exchange (current density);

$B_i^{(-)}$ is the culture density after the exchange;

$B_{i+1}$ is the culture density before the exchange at the next step of the quasi-continuous cultivation.

In microbiological practice, the algal growth in a culture is described by two quantitative characteristics – growth rate (productivity) and relative (specific) growth rate. Over the time interval between exchange procedures $\Delta t$ (days), the culture density in the *i*-th stage increases to the value $B_{i+1}$. Therefore, on this time interval, the average growth rate is (g·L$^{-1}$·day$^{-1}$):

$$\overline{P}_i = \frac{B_{i+1} - B_i^{(-)}}{\Delta t}, \tag{14}$$

and the average specific growth rate is (day$^{-1}$):

$$\overline{\mu}_i = \frac{B_{i+1} - B_i^{(-)}}{B_i^{(-)} \cdot \Delta t}. \tag{15}$$

The decrease in density is related to the dilution value of the culture, which depends on both the algal mass withdrawn from the current chemostat stage during the exchange procedure and the algal mass introduced into the working volume from the previous chemostat stage. In one exchange procedure, the volume w is withdrawn from the working volume over the time interval $\Delta t$. Therefore, the culture flow rate ($F$) in the current chemostat stage is equal to (L·day$^{-1}$):

$$F = \frac{\mathrm{w}}{\Delta t},$$

and the relative (specific) flow rate $\omega$ is equal to (day$^{-1}$):

$$\omega = \frac{F}{V} = \frac{\mathrm{w}}{V \cdot \Delta t}. \tag{16}$$

Taking into account (10) and (16), we can express the value of the specific flow rate of the nutrient medium for the *i*-th stage:

$$\omega \left(1 - \frac{B_{i-1}}{B_i}\right) = \left(1 - \frac{1}{\theta_i}\right) \frac{1}{\Delta t}; \qquad \omega = \frac{1 - \dfrac{1}{\theta_i}}{1 - \dfrac{B_{i-1}}{B_i}} \frac{1}{\Delta t}. \tag{17}$$

It should be kept in mind as follows: (16) allows us to calculate for the *i*-th stage the specific flow rate of the algal suspension (or a nutrient medium for the first stage), which is the same in all the chemostat stages. Expression (17) indicates what the specific flow rate will be when the culture density in the *i*-th stage decreases by $\theta_i$ times, taking into account the culture density in the previous chemostat stage.

The value of the change in the culture density (13) over the time interval $\Delta t$ can be expressed in terms of the growth rate and the flow rate. From (11), (13), and (16), it follows:

$$\frac{\Delta B_i}{\Delta t} = \frac{B_{i+1} - B_i^{(-)}}{\Delta t} - \frac{B_i - B_i^{(-)}}{\Delta t} = \overline{P}_i - \omega\,(B_i - B_{i-1}). \tag{18}$$

Thus, the rate of change in the density of the quasi-continuous culture in the current chemostat stage is determined by the average growth rate ($\overline{P}_i$), the rate of introduction of the alga from the previous stage ($\omega \cdot B_{i-1}$), and the rate of withdrawal of the alga during the exchange ($\omega \cdot B_i$).

Then, we express the average growth rate $\overline{P}_i$ in units of the culture density (biomass) in the *i*-th stage of the chemostat:

$$\overline{P}_i = \tilde{\mu}_i \cdot B_i, \tag{19}$$

where $\tilde{\mu}_i$ is the coefficient reflecting the ratio of the biomass growth $B_i$ over the time interval $\Delta t$.

At its core, this coefficient is a relative growth rate, similar to (15), with the difference that the growth rate refers not to the culture density after the exchange, but to the culture density before the exchange – to $B_i$. The comparison of (14), (19), and (10) allows to see that $\overline{\mu}_i = \tilde{\mu}_i \cdot \theta_i$. The closer $\theta_i$ to 1 and the shorter the time interval $\Delta t$ between culture dilutions, the smaller the difference between these values.

Substituting (19) into (18), we obtain the following equation:

$$\frac{\Delta B_i}{\Delta t} = \tilde{\mu}_i \cdot B_i - \omega\,(B_i - B_{i-1}) = \left[\tilde{\mu}_i - \omega\left(1 - \frac{B_{i-1}}{B_i}\right)\right] B_i. \tag{20}$$

The solution of this equation makes it possible to describe the dynamics of the density of the quasi-continuous culture in a multistage chemostat. In a particular case, when $\Delta t \to 0$, $\tilde{\mu}_i \to \mu_i$, $\theta_i \to 1$ (a continuous flow), we obtain differential equations identical to those published in [Fencl, 1968; Pirt, 1978].

For the first stage of the chemostat, when the culture is diluted with a nutrient medium ($B_{i-1} = 0$), expression (20) is transformed into:

$$\frac{\Delta B_1}{\Delta t} = \tilde{\mu}_1 \cdot B_1 - \omega \cdot B_1 = (\tilde{\mu}_1 - \omega) B_1. \tag{21}$$

It is completely identical to expression obtained earlier for a quasi-continuous culture in a one-stage chemostat [Trenkenshu, 2005].

In our experiment, the transition processes with various changes in the flow rate of the nutrient medium lasted for a short time (Fig. 2). Accordingly, we will consider only established processes (stationary dynamic equilibrium) – when the culture density remains the same over time in each chemostat stage ($\Delta B_i = 0$). For the conditions of stationary dynamic equilibrium, from (20), expressions for the relative growth rate and culture productivity in the *i*-th stage of the chemostat follow:

$$\tilde{\mu}_i = \omega\left(1 - \frac{B_{i-1}}{B_i}\right); \qquad \overline{P}_i = \omega\,(B_i - B_{i-1}). \tag{22}$$

Whence follow the relationship of culture densities in the neighboring chemostat stages and the dilution rate in the *i*-th stage:

$$B_i = \frac{\omega}{\omega - \tilde{\mu}_i} B_{i-1}; \qquad \omega = \tilde{\mu}_i \frac{B_i}{B_i - B_{i-1}} = \frac{\overline{P}_i}{B_i - B_{i-1}}. \tag{23}$$

It is to be noted that for the conditions of stationary dynamic equilibrium, the following equalities will be valid:

$$B_i = \frac{\omega}{\omega - \tilde{\mu}_i} B_{i-1} = q_i \cdot B_{i-1}; \quad \tilde{\mu}_i = \omega\left(1 - \frac{1}{q_i}\right) = \omega \cdot \epsilon_i; \quad \tilde{\mu}_i = \frac{\epsilon_i}{\epsilon_{i-1}} \tilde{\mu}_{i-1}, \tag{24}$$

where $q_i$, $\epsilon_i$, and $\epsilon_{i-1}$ are some constant values that are easily determined experimentally.

Indeed, the coefficient of relationship between the culture density in the first and second chemostat stages $q_2$, experimentally obtained by us, is constant at any flow rate (Fig. 5). It can be seen from the figure that even at the maximum flow rate, when a near-wall growth is recorded, the relationship between the culture density in the first and second stages is quite well described by expression (23) at $q_2 = \mathrm{tg}\alpha = 1.11$.

The expression for the culture productivity in the *i*-th stage (22) can be represented as:

$$B_i = \frac{\overline{P}_i}{\omega} + B_{i-1}. \tag{25}$$

In stationary dynamic equilibrium, a culture always has $\overline{P}_i > 0$ and $\omega > 0$. Therefore, for any mode of quasi-continuous cultivation in a multistage chemostat, $B_i > B_{i-1}$. If the culture is diluted with a nutrient medium ($B_{i-1} = 0$), it follows from expression (22):

$$\tilde{\mu}_1 = \omega; \qquad \overline{P}_1 = \omega \cdot B_1. \tag{26}$$

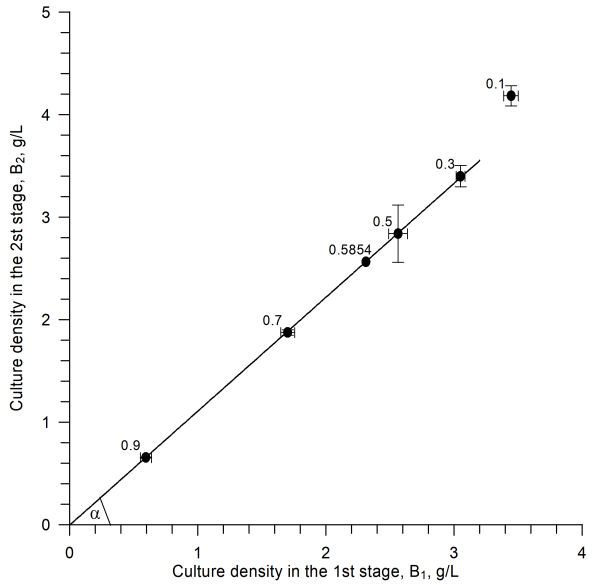


**Fig. 5.** The relationship between culture density in the first and second chemostat stages under conditions of stationary dynamic equilibrium, tg$\alpha$ = 1.11 [see expression (23)]. The dilution rate, $\omega$, is indicated by numbers. The value for $\omega$ = 0.1 was not taken into account in the calculations due to the manifestation of cell agglutination

To determine the dependence of the concentration of the growth-limiting biogenic element in the current chemostat stage on the flow rates and the substrate consumption by cells during growth, we will follow the logic of deriving equation (20). By analogy with (7), such expressions can be written:

$$s_i = s_i^* + s_i^{**}; \quad s_i^* = \mathrm{w} \cdot S_i; \quad s_i^{**} = V \cdot S_i - \mathrm{w} \cdot S_i = (V - \mathrm{w}) \cdot S_i, \tag{27}$$

where $s_i$ is the mass of the limiting substrate in the working volume of the *i*-th stage of the chemostat, g;

$s_i^*$ and $s_i^{**}$ are the masses of the limiting substrate in the withdrawn volume w during the exchange procedure and in the remaining volume ($V$ – w), respectively;

$S_i$ is the concentration of the limiting substrate in the *i*-th stage of the chemostat, g·L$^{-1}$.

The concentration of the limiting substrate in the *i*-th stage after the exchange ($S_i^{(-)}$) will be equal to:

$$S_i^{(-)} = \frac{s_{i-1}^* + s_i^{**}}{V} = \frac{\mathrm{w} \cdot S_{i-1} + (V - \mathrm{w}) \cdot S_i}{V}. \tag{28}$$

From this expression, it follows:

$$\frac{\mathrm{w}}{V} = \frac{S_i^{(-)} - S_i}{S_{i-1} - S_i} = \frac{s_{i-1}^* - s_i^*}{s_{i-1} - s_i}. \tag{29}$$

The change in the mass of the limiting substrate ($\Delta s_i$) can be expressed as follows:

$$\underbrace{s^*_{i-1}}_{\text{increase}} - \underbrace{(s_i - s^{**}_i)}_{\text{decrease with outflow}} - \underbrace{((s^*_{i-1} + s^{**}_i) - s_{i+1})}_{\text{decrease due to growth}} = s_{i+1} - s_i = \Delta s_i, \tag{30}$$

where $s_i$ is the mass of the limiting substrate before the exchange;

$s_{i+1}$ is the mass of the limiting substrate before the exchange at the next step of the quasi-continuous cultivation;

$s^*_{i-1}$ is the mass of the substrate introduced into the *i*-th stage from the previous one during the exchange;

$s^{**}_{i-1}$ is the mass of the substrate that remains in the *i*-th stage after withdrawing a part of the suspension with volume w.

Taking into account (27), (29), and (30), we write the change in the substrate concentration in the *i*-th stage of the chemostat ($\Delta S_i$):

$$\underbrace{[S^{(-)}_i - S_i]}_{\text{increase}} - \underbrace{[S^{(-)}_i - S_{i+1}]}_{\text{decrease}} = S_{i+1} - S_i = \Delta S_i, \tag{31}$$

where $S_i$ is the substrate concentration before the exchange procedure;

$S^{(-)}_i$ is the substrate concentration after the exchange;

$S_{i+1}$ is the substrate concentration before the exchange at the next step of the quasi-continuous cultivation.

The value of the change in the substrate concentration (31) over the time interval $\Delta t$ can be expressed in terms of the substrate consumption rate and the flow rate. From (29), (31), and (16), it follows:

$$\frac{\Delta S_i}{\Delta t} = \frac{S^{(-)}_i - S_i}{\Delta t} - \frac{S^{(-)}_i - S_{i+1}}{\Delta t} = \omega\,(S_{i-1} - S_i) - \overline{P}_{S_i}, \tag{32}$$

where $\overline{P}_{S_i}$ is the average rate of consumption of the limiting substrate by cells over the time interval $\Delta t$.

The ratio of silicon in *C. closterium* biomass varies insignificantly under different cultivation conditions. Therefore, it can be assumed that $Y_b$ value is constant at any flow rate. In this case, the substrate consumption rate $\overline{P}_{S_i}$ will be proportional to the microalgal growth rate $\overline{P}_i$. Accordingly, taking into account (19), expression (32) is represented as:

$$\frac{\Delta S_i}{\Delta t} = \omega\,(S_{i-1} - S_i) - Y_b \cdot \tilde{\mu}_i \cdot B_i. \tag{33}$$

The solution of this equation will describe the dynamics of the concentration of the growth-limiting substrate of a quasi-continuous culture in a multistage chemostat. For a particular case (with a continuous flow of a nutrient medium), this equation is given in [Fencl, 1968; Pirt, 1978].

For the conditions of stationary dynamic equilibrium, we can express $B_i$ from (33):

$$B_i = \frac{\omega}{\tilde{\mu}_i}\frac{(S_{i-1} - S_i)}{Y_b}; \qquad \tilde{\mu}_i = \frac{\omega}{B_i}\frac{(S_{i-1} - S_i)}{Y_b} = \omega\left(\frac{S_{i-1}}{S_i} - 1\right). \tag{34}$$

We calculate $S_i(\tilde{\mu}_i)$ from (5):

$$S_i(\tilde{\mu}_i) = \frac{K_S \cdot \tilde{\mu}_i}{\mu_m - \tilde{\mu}_i}. \tag{35}$$

Then, we substitute (24) and (35) into (34):

$$B_i = \frac{\omega}{\tilde{\mu}_i}\frac{1}{Y_b}\left(S_{i-1} - \frac{K_S \cdot \tilde{\mu}_i}{\mu_m - \tilde{\mu}_i}\right) = \frac{\omega}{\omega \cdot \epsilon_i}\frac{1}{Y_b}\left(S_{i-1} - \frac{K_S \cdot \omega \cdot \epsilon_i}{\mu_m - \omega \cdot \epsilon_i}\right). \tag{36}$$

Whence we determine the dependence of the culture density in the *i*-th stage on the dilution rate:

$$B_i(\omega) = \frac{1}{\epsilon_i}\frac{1}{Y_b}\left(S_{i-1} - \frac{K_S \cdot \omega}{\frac{1}{\epsilon_i}\mu_m - \omega}\right). \tag{37}$$

In a particular case, for the first stage of the chemostat, when the culture is diluted with a nutrient medium ($S_{i-1} = S_0$; $\epsilon_i = 1$), considering (26), we write:

$$B_1(\omega) = \frac{1}{Y_b}\left(S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega}\right). \tag{38}$$

Substituting into the latter expression the numerical values of the observed requirement for silicon from Table 2, the saturation constant from (5), and silicon concentration in the nutrient medium RS $S_0$ = 0.115 g·L$^{-1}$, we calculate $\mu_m$ using the method of least squares based on the experimental data. Fig. 6 shows the correspondence between the experimental data and the theoretical curve (38) with the calculated value $\mu_m$ = 1.05 day$^{-1}$ ($R^2$ = 0.97). When calculating, the value of the culture density at $\omega$ = 0.9 day$^{-1}$ was not taken into account. As mentioned above, at the flow rate given, a near-wall growth of *C. closterium* was recorded.

Substituting the value $\mu_m$ = 1.05 day$^{-1}$ into (38), for the conditions of our experiment, we obtain the dependence of the culture density in the first stage of the chemostat on the specific flow rate:

$$B_1(\omega) = \frac{1}{0.035}\left(0.115 - \frac{0.028 \cdot \omega}{1.05 - \omega}\right). \tag{39}$$

Worth noting as follows. Table 2 indicates the value of the observed requirement for silicon $Y_b$ = 38.2 mg·g$^{-1}$. However, according to the results of our calculations, in a flow culture, the value of the observed requirement $Y_{fl}$ = 35.0 mg·g$^{-1}$. The underestimation can be explained by the manifestation of autoselection in microorganisms in a flow culture [Gitelzon et al., 1973]. Accordingly, for a batch culture, the need for silicon $Y_b$ = 38.2 mg·g$^{-1}$; for a flow culture, $Y_{fl}$ = 35.0 mg·g$^{-1}$.

Analyzing expression (38), one can notice that at a certain critical dilution rate $\omega_{cr}$, the value $B_1$ turns into zero (Fig. 6). In our experiment, the critical dilution rate (see Fig. 6) was as follows:

$$\omega_{cr}(B_1) = \mu_m \frac{S_0 - Y_{fl} \cdot B_1}{S_0 + K_S - Y_{fl} \cdot B_1}; \quad \omega_{cr}(0) = \mu_m \frac{S_0}{S_0 + K_S} = \frac{1.05 \cdot 0.115}{0.115 + 0.028} = 0.84 \text{ day}^{-1}.$$

Therefore, taking into account (26) for the conditions of our experiment, the limit value $\tilde{\mu}_1 = 0.84$ day$^{-1}$. In comparison with the specific growth rate $\mu_b = 0.7$ day$^{-1}$ obtained in a batch culture [see (1)], this value is somewhat higher, which is associated with autoselection in a flow culture [Gitelzon et al., 1973].

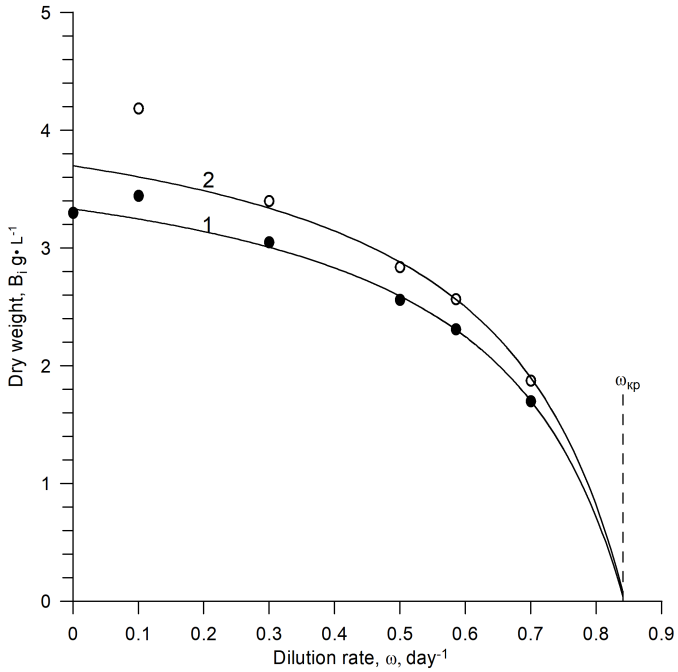


**Fig. 6.** The dependence of culture density on the dilution rate $\omega$ under conditions of stationary dynamic equilibrium: 1, the first stage of the chemostat, calculation according to equation (38) ($R^2 = 0.97$); 2, the second stage, calculation according to equation (45) ($R^2 = 0.96$). In the calculations, the value for $\omega = 0.9$ day$^{-1}$ was not taken into account (see text for an explanation). The dotted line denotes the critical dilution rate $\omega_{cr} = 0.84$ day$^{-1}$

Thus, for the conditions of our experiment, in a batch culture, the specific growth rate $\mu_b = 0.7$ day$^{-1}$, its limit value in a flow culture $\tilde{\mu}_1 = 0.84$ day$^{-1}$, and its maximum possible value when limiting *C. closterium* growth by silicon $\mu_m = 1.05$ day$^{-1}$. Interestingly, the maximum value $\mu_m$ is included in expression (5) and is a certain species-specific characteristic of the substrate-dependent *C. closterium* growth (determined by the genetics of the species) when the growth is limited by silicon.

For the second stage of the chemostat, it is impossible to obtain the dependence $B_2(\omega)$ from (37) in an explicit form (as it is possible for the first stage), since $\epsilon_2 \neq 1$. To obtain the dependence $B_2(\omega)$, we use the relationship (23). When substituting (23) into (38), we get the dependence of the culture density in the second stage on $\omega$ and $\mu_2$:

$$B_2(\omega, \mu_2) = \frac{\omega}{\omega - \tilde{\mu}_2} \frac{1}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right). \tag{40}$$

Since the slope angle for $q_i$ of the linear dependence (24) is constant and can be easily determined from the experimental data, we can write for the second stage of the chemostat:

$$B_2(\omega) = \frac{q_2}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right). \tag{41}$$

Similarly for the *i*-th stage ($i \geq 2$):

$$B_i(\omega, \mu_2, \ldots \mu_i) = \frac{\omega^{i-1}}{\prod\limits_{k=2}^{i} (\omega - \tilde{\mu}_k)} \frac{1}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right). \tag{42}$$

If $q_i$ is a constant value for any pair of neighboring stages, then the product $q_2 \cdot q_3 \cdot \ldots \cdot q_i$ will be a constant value as well. Hence:

$$B_i(\omega) = \frac{\prod\limits_{k=2}^{i} q_k}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right). \tag{43}$$

According to our experimental data (Fig. 5), the slope of $B_2$ dependence on $B_1$ is equal to:

$$q_2 = \frac{\omega}{\omega - \tilde{\mu}_2} = \operatorname{tg} \alpha = 1.11. \tag{44}$$

Therefore, for the conditions of our experiment, the dependence of the culture density in the second stage of the chemostat on the dilution rate can be represented as follows:

$$B_2(\omega) = 1.11 \frac{1}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right) = \frac{1.11}{0.035} \left( 0.115 - \frac{0.028 \cdot \omega}{1.05 - \omega} \right). \tag{45}$$

Fig. 6 shows the dependence of the culture density in the second stage of the chemostat on the specific flow rate and the correspondence between the experimental data and the theoretical curve (45).

To determine the yield value in different modes of cultivation, we calculate the total productivity of the culture in *i* chemostat stages under conditions of stationary dynamic equilibrium. As follows from expression for the productivity of the *i*-th stage (22), the total productivity of all the chemostat stages $R_i$ (the entire cultivation system) is equal to:

$$R_i = \sum_{k=1}^{i} \overline{P}_k = \omega \cdot B_i. \tag{46}$$

Therefore, under conditions of stationary dynamic equilibrium, the yield ($H_i$) of a cultivation system consisting of *i* stages for a fixed period of time is (g·L$^{-1}$):

$$H_i(t) = R_i \cdot t = \omega \cdot B_i \cdot t, \tag{47}$$

where $t$ is the cultivation time, day.

To determine the productivity of a multistage chemostat, we substitute expression (43) into (46):

$$R_i(\omega) = \omega \frac{\prod\limits_{k=2}^{i} q_k}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right). \tag{48}$$

For a particular case, $R_1(\omega)$ and $R_2(\omega)$, we have:

$$R_1(\omega) = \omega \frac{1}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right); \qquad R_2(\omega) = \omega \frac{q_2}{Y_{fl}} \left( S_0 - \frac{K_S \cdot \omega}{\mu_m - \omega} \right). \tag{49}$$

A similar expression for a one-stage chemostat was obtained in [Pirt, 1978].

Substituting the experimental values and $q_2$ = 1.11 [see (44)] into (49), we have:

$$R_1(\omega) = \omega \frac{1}{0.035} \left( 0.115 - \frac{0.028 \cdot \omega}{1.05 - \omega} \right); \qquad R_2(\omega) = \omega \frac{1.11}{0.035} \left( 0.115 - \frac{0.028 \cdot \omega}{1.05 - \omega} \right). \tag{50}$$

Thus, the dependences of the productivity of a one- and two-stage chemostat on the specific flow rate were obtained. Fig. 7 shows the correspondence between the experimental data and theoretical curves (50).

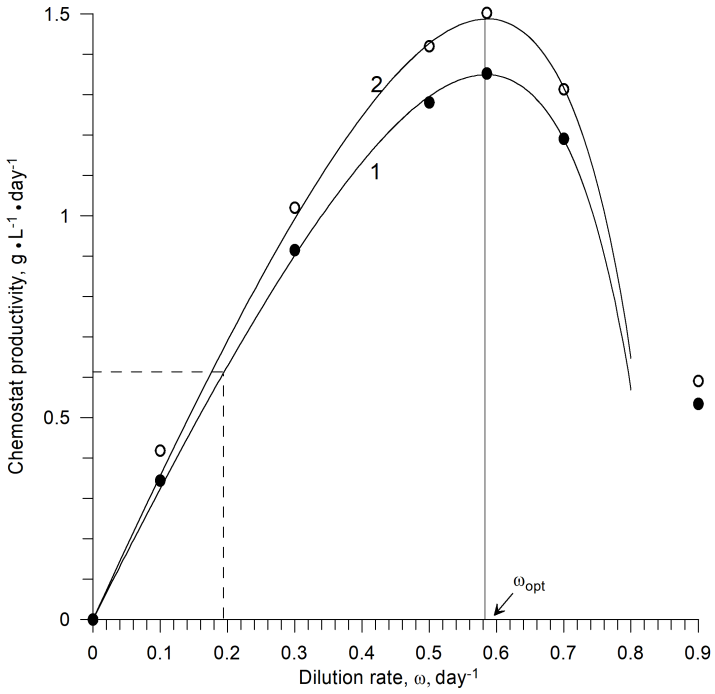


**Fig. 7.** Dependence of the chemostat productivity under conditions of stationary dynamic equilibrium on the dilution rate: 1 and 2 denote calculation according to equation (50) ($R^2$ = 0.98) for a one- and two-stage chemostat, respectively. In the calculations, the value for $\omega$ = 0.9 day$^{-1}$ was not taken into account (see text for an explanation). The arrow indicates the optimal value of the dilution rate $\omega_{opt}$ = 0.59, at which the maximum productivity is achieved. For a comparative assessment, the dotted line shows the productivity of the batch culture [see (55) and (56)]

Then, we determine the optimal value of the specific flow rate $\omega_{opt}$, at which the productivity of a multistage chemostat is maximum. To do this, we differentiate function (48) with respect to $\omega$:

$$R_i'(\omega) = \frac{\prod_{k=2}^{i} q_k}{Y_{fl}} \frac{(K_S + S_0)\omega^2 - 2\mu_m\omega(K_S + S_0) + \mu_m^2 S_0}{(\mu_m - \omega)^2}. \tag{51}$$

For $R_i'(\omega) = 0$, we get a quadratic equation:

$$\omega^2 - 2\mu_m\omega + \frac{\mu_m^2 S_0}{K_S + S_0} = 0. \tag{52}$$

One of the roots of this equation has no sense, since the condition $\mu_m > \omega$ must be satisfied. Another root is the optimal value of the specific flow rate, at which the maximum productivity of the entire multistage cultivation system is achieved:

$$\omega_{opt} = \mu_m \left(1 - \sqrt{\frac{K_S}{S_0 + K_S}}\right). \tag{53}$$

It should be noted that in the latter expression, $\omega_{opt}$ value does not depend on the number of stages. So, (53) can be applied to determine the optimal specific flow rate for both a one-stage and a multistage chemostat.

Then, we calculate the optimal specific flow rate for the conditions of our experiment:

$$\omega_{opt} = 1.05 \left(1 - \sqrt{\frac{0.028}{0.115 + 0.028}}\right) = 0.59 \text{ day}^{-1}. \tag{54}$$

Moreover, using formulas (39), (45), and (50), we determine the culture density and productivity for each stage of the chemostat at $\omega_{opt}$ = 0.59 day$^{-1}$. Applying (47), we calculate the yield value for a one- and two-stage chemostat at the optimal flow rate for four days of cultivation. The calculation results are given in Table 3.

Let us compare the yield value obtained for a fixed period of time under conditions of batch and flow cultivation. The batch cultivation lasted five days, and we carry out our calculations for $t_b$ = 5 days. If the batch culture at the moment of reaching the maximum density $B_m$ (the initial moment of the stationary growth phase) is diluted down to the initial density $B_0$, the growth in the batch culture will continue, and the maximum density will be reached again in a period of time $t_b$. Under conditions when cycles of dilution of the batch culture are repeated at intervals $t_b$, the batch culture is involved in a flow quasi-continuous cultivation with a time interval between dilution procedures $t - t_0 = t_b$. The average productivity, relative growth rate, and yield over this period can be given as:

$$\overline{P}_b = \frac{B_m - B_0}{t - t_0} = \frac{B_b}{t_b}; \qquad \tilde{\mu}_b = \frac{1}{B_m}\frac{B_m - B_0}{t - t_0} = \frac{\overline{P}_b}{B_m}; \qquad B_b = \overline{P}_b \cdot t_b. \tag{55}$$

On the other hand, considering (46) and (47), for a quasi-continuous culture, the average productivity, relative growth rate, and yield are as follows:

$$R_i = \omega \cdot B_i; \qquad \tilde{\mu}_1 = \omega; \qquad H_{fl} = R_i \cdot t_b = \omega \cdot B_i \cdot t_b. \tag{56}$$

Comparing the productivity of two quasi-continuous cultures, (55) and (56), at the same relative flow rate under conditions of stationary dynamic equilibrium ($\omega = \tilde{\mu}_1 = \tilde{\mu}_b$), we have:

$$\frac{R_i}{\overline{P}_b} = \frac{\omega \cdot B_i}{\tilde{\mu}_b \cdot B_m} = \frac{B_i}{B_m} < 1.$$

Therefore, for the conditions $\tilde{\mu}_b = \omega$, batch cultivation is more profitable. However, with an increase in the flow rate, $\tilde{\mu}_b < \omega \le \omega_{opt}$, a flow culture becomes more profitable. Moreover, the maximum benefit of a one-stage chemostat is achieved at the optimal flow rate $\omega_{opt}$ (Fig. 7).

To calculate the productivity of the batch culture, we substitute the numerical values from our experiment into (55). As a result, we get: $\overline{P}_b$ = 0.62 g·L$^{-1}$·day$^{-1}$; $\tilde{\mu}_b$ = 0.19 day$^{-1}$; $B$ = 3.1 g·L$^{-1}$. To determine the chemostat productivity for each of the stationary states, we apply expression (56). The results of the calculation are given in Table 3.

**Table 3.** *Cylindrotheca closterium* production characteristics in a one- and two-stage chemostat under stationary dynamic equilibrium conditions: $\omega$, the dilution rate, day$^{-1}$; $B_1$, $B_2$, the culture density in the first and second stages, respectively, g·L$^{-1}$; $\tilde{\mu}_1$, $\tilde{\mu}_2$, specific growth rate in the first and second stages; $R_1$, $R_2$, productivity of a one- and two-stage chemostat, g·L$^{-1}$·day$^{-1}$; $\overline{P}_2$, productivity of the chemostat second stage; $H_1(5)$, $H_2(5)$, yield received in a one- and two-stage chemostat over a period of time $t_b$ = 5 days, g

| $\omega$ | $B_1$ | $\tilde{\mu}_1$ | $R_1$ | $H_1(5)$ | $B_2$ | $\tilde{\mu}_2$ | $\overline{P}_2 = R_2 - R_1$ | $R_2$ | $H_2(5)$ |
|---|---|---|---|---|---|---|---|---|---|
| 0.1 | 3.44 | 0.1 | 0.344 | 1.72 | 4.19 | 0.02 | 0.074 | 0.419 | 2.09 |
| 0.3 | 3.05 | 0.3 | 0.915 | 4.58 | 3.40 | 0.03 | 0.106 | 1.021 | 5.10 |
| 0.5 | 2.56 | 0.5 | 1.281 | 6.40 | 2.84 | 0.05 | 0.139 | 1.420 | 7.10 |
| $\omega_{opt}$ = 0.59 | 2.29 | 0.59 | 1.348 | 6.76 | 2.54 | 0.06 | 0.150 | 1.498 | 7.51 |
| 0.7 | 1.70 | 0.7 | 1.191 | 5.95 | 1.88 | 0.07 | 0.123 | 1.314 | 6.57 |
| 0.9 | 0.59 | 0.9 | 0.534 | 2.67 | 0.66 | 0.09 | 0.057 | 0.591 | 2.96 |

The comparison of the calculation results shows as follows: at $\omega$ = 0.1 over a time interval $t_b$, the flow culture gives a lower yield than the batch one. Flow cultivation becomes profitable at higher flow rates. The yield is maximum at optimal flow rate: $R_1(0.59)$ = 1.35 g·L$^{-1}$·day$^{-1}$; $H_1(5)$ = 6.76 g·L$^{-1}$. Accordingly, *C. closterium* cultivation in a one-stage chemostat is 2.2 times more profitable than the batch cultivation.

Let us compare the productivity of one- and two-stage chemostat under condition that the same volume of limiting substrate enters the cultivation system *per* unit volume of the microalgal suspension. The working volume of each stage of the chemostat is equal to $V$; therefore, the total volume of the microalgal suspension in a two-stage chemostat is $2V$. Hence, in order to comply with the comparison condition, it is necessary to double the working volume of a one-stage chemostat or to reduce the specific flow rate by half. Taking into account (24) and (46), we write:

$$\frac{R_2}{R_1} = \frac{\omega B_2}{\frac{\omega}{2} B_1} = \frac{2B_2}{B_1} = 2 \cdot q_2 > 1.$$

Whence it follows that under the above conditions, a two-stage cultivation system is more productive than a one-stage one. The data on the productivity of a one- and two-stage chemostat are given in Table 3.

**Conclusion.** Our studies have shown that the culture of the diatom alga *Cylindrotheca closterium* is characterized by sufficiently high productivity rates both in batch and flow culture. In the batch culture, the specific growth rate $\mu_b$ = 0.7 day$^{-1}$, and the time for doubling the biomass $t_d$ = 0.987 days. In the flow culture, at a critical dilution rate, the limit value of the specific culture growth rate $\tilde{\mu}_1$ is 0.84 day$^{-1}$.

For the conditions of our experiment, in a one- and two-stage chemostat, the maximum productivity is recorded at an optimal flow rate $\omega_{opt}$ = 0.59 day$^{-1}$ ($R_1$ = 1.348 g·L$^{-1}$·day$^{-1}$ and $R_2$ = 1.498 g·L$^{-1}$·day$^{-1}$, respectively). As shown experimentally, in the flow culture, *C. closterium* productivity is 2.2 times higher than in the batch one.

Based on the experimental data, two parameters of the Monod equation were determined – the maximum specific growth rate $\mu_m$ and the saturation constant $K_S$ – with limiting *C. closterium* growth by silicon. The values were 1.05 day$^{-1}$ and 0.028 g·L$^{-1}$, respectively. Moreover, the observed need of the alga for silicon in the flow culture was calculated, $Y_{fl}$ = 35 mg·g$^{-1}$. As shown, the observed need for silicon in the flow culture is lower than in the batch one by 7.9%. Interestingly, $\mu_m$, $K_S$, and $Y_{fl}$ values are important physiological characteristics for the diatom *C. closterium*; those play the key role in the design of industrial systems for intensive microalgae cultivation.

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# ПРОДУКЦИОННЫЕ ХАРАКТЕРИСТИКИ КУЛЬТУРЫ ДИАТОМОВОЙ ВОДОРОСЛИ *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN ET LEWIN В ДВУХСТУПЕНЧАТОМ ХЕМОСТАТЕ

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В работе рассмотрены преимущества и недостатки проточного и накопительного культивирования микроводорослей. Указаны достоинства проточного культивирования, в частности в квазинепрерывном режиме в двухступенчатом хемостате. В качестве продуцента ценных веществ предложено использовать культуру бентосной диатомовой водоросли *Cylindrotheca closterium*, которая обладает многими полезными свойствами как биологического характера, так и технологического: 1) характеризуется достаточно высокими продукционными показателями; 2) эффективно утилизирует световую энергию, что снимает ограничения на размещение производства в регионах с малым количеством солнечных дней в году; 3) характеризуется довольно низким температурным оптимумом роста, что актуально для реализации промышленных технологий на территории Российской Федерации; 4) имеет удельную плотность клеток больше единицы, поэтому они достаточно быстро оседают на дно фотобиореактора при отсутствии перемешивания культуры, что упрощает отделение биомассы от культуральной среды и снижает её себестоимость. Цель работы — изучить продукционные характеристики квазинепрерывной культуры *C. closterium* в двухступенчатом хемостате. Исследования проводили при температуре (20 ± 1) °C и облучённости 150 мкмоль квантов·м$^{-2}$·с$^{-1}$. Хемостатная установка для культивирования *C. closterium* состояла из двух стеклянных фотобиореакторов плоскопараллельного типа объёмом 3 л с рабочей толщиной 5 см и рабочей поверхностью каждого фотобиореактора 0,03 м$^2$. Культуру выращивали на питательной среде RS. Перемешивание осуществляли посредством барботажа воздухом (скорость — 1,5 л воздуха на 1 л культуры в мин). Культуру исследовали при различных скоростях протока питательной среды — 0,1; 0,3; 0,5; 0,7; 0,9 сут$^{-1}$. Рассчитаны параметры роста накопительной культуры: удельная скорость роста $\mu_н$ = 0,7 сут$^{-1}$; время удвоения биомассы $t_d$ = 0,987 сут.

Максимальная продуктивность одно- и двухступенчатого хемостата была отмечена при оптимальной скорости протока 0,59 сут$^{-1}$ и составила 1,348 и 1,498 г·л$^{-1}$·сут$^{-1}$ соответственно. Экспериментально показано, что в проточной культуре продуктивность *C. closterium* выше в 2,2 раза, чем в накопительной. На основе экспериментальных данных проведён расчёт максимальной удельной скорости роста $\mu_m$ и константы насыщения $K_S$ при лимитировании роста *C. closterium* кремнием; значения составили 1,05 сут$^{-1}$ и 0,028 г·л$^{-1}$ соответственно. Показано, что наблюдаемая потребность в кремнии в проточной культуре ($Y_{\text{пр}}$ = 35 мг·г$^{-1}$) ниже на 7,9 %, чем в накопительной ($Y_{\text{н}}$ = 38 мг·г$^{-1}$). Отмечено, что величи́ны $\mu_m$, $K_S$ и $Y_{\text{пр}}$ являются важными физиологическими характеристиками диатомовой водоросли *C. closterium* и играют ключевую роль при проектировании промышленных систем для интенсивного культивирования микроводорослей.

**Ключевые слова:** *Cylindrotheca closterium*, хемостат, математическая модель