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ABOUT THE PROBLEM OF BIOLOGICAL PROCESSES COMPLICATED BY MASS TRANSFER

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Abstract. The carbon dioxide absorption by unicellular microalgae has been studied. The stages of the process have been examined taking into account the peculiarities of cultivation conditions, in particular the presence of cellular and intercellular environment. The constants realizing the mathematical model of carbon dioxide absorption from the air by microalgae cells have been determined. The obtained results allow to predict the absorption kinetics and develop the equipment for gas wastes cleaning while implementation of the industrial process.

Keywords: photosynthesis, microalgae, mathematical model, mass transfer coefficient, biomass, intercellular environment, diffusion.

1. Introduction

Nowadays the humanity has to solve a large number of problems related to anthropogenic impacts on the environment, including a negative impact on the atmosphere of a large number of industrial emissions, namely carbon dioxide (CD). There are many industrial methods of gas emissions cleaning from carbon dioxide, but it is necessary also to take into account the photosynthetic activity of vegetation, which decreases as a result of its increasing destruction [1-2].

Science has proven the ability of nature to absorb CD from the atmosphere by plants and algae and its "handling" in the form of biomass. This process chemism is described by the following reaction:

$$n\mathrm{CO_2} + m\mathrm{H_2O} + \text{solar energy} \rightarrow \mathrm{C}_n(\mathrm{H_2O})_m + n\mathrm{O_2}$$

Microalgae are unicellular, phyto-like organisms, which absorb CD *via* photosynthesis. These organisms have great advantages over usual land plants because microalgae are characterized by high values of growth rates contributing to the rapid transformation of CD into biomass. They can grow and retain all necessary properties in the closed systems, where land plants can not grow. The implementation of industrial methods of CD absorption followed by photosynthesis is an important task of our time. One more important task is solving the problem of expanding the application limits of the obtained biomass. Today the most advisable way is to process it into hydrocarbon products *via* hydrothermal carbonization or to obtain biofuel. The anaerobic fermentation resulting in methane production is also of great interest [3].

There are many methods of CD recovery with the aim of decreasing its amount. The biological method is the most effective one [4]. In the literature there are data about creation of so called "street lamps" – massive reservoirs filled by water and algae. During the day, under the sunlight they process carbon dioxide and other compounds in enormous amounts. The experimental "lamp" absorbs around one ton of CD per year. It is 200 times higher than one tree can do. Thus, such "lamps" are capable of solving the problem of carbon emissions. The disadvantage is that photosynthesis does not proceed in the enclosed space and the reservoir requires lamplight itself despite being called a lamp [5].

So, unicellular algae may be used for the biological cleaning processes of gas emissions from CD. Biological objects are usually very complicated, and the processes occurring in them are influenced by many factors, which are often dependent on each other. The most important of them are: the temperature, degree of aeration, light, carbon dioxide concentration, the content of macro – and microelements, alkaline-acid balance, *etc*. By means of correlation between physico-chemical and biological values we can deeper understand the biological processes in the investigated object.

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The basis of modern kinetic study strategy and description of biological processes, complicated by mass transfer is a separate quantitative study of the influence of kinetic and diffusion factors and finding such mode of the process when the influence of mass transfer is insignificant or can be neglected.

Hence, the aim of this work is to study the temperature influence on the processes accompanying CD absorption, *i.e.* CD transformation into biomass.

2. Experimental

2.1. Theoretical Part

CD absorption by microalgae during its bubbling through the aqueous solution may be conditionally divided to 4 stages:

– *the first stage*: the supply of CD from the primary volume of the solution to the surface of microalgae colony. Quantitatively the process may be described by mass transfer equation:

$$dM/dt = b\,F(C - C_s) \tag{1}$$

where β – coefficient of mass transfer from the primary volume of the solution to the surface of microalgae colony; M – the mass of CD transferred from the primary volume of the solution to the surface of microalgae colony; t – time; F – surface area of mass transfer; C and C_s – concentrations of CD in the primary volume and on the surface of microalgae colony, respectively.

– *the second stage*: CD diffusion from the surface of microalgae colony through intercellular environment to the surface of cellular membranes. The dissolved CD permeates the membrane surface *via* molecular diffusion and then transfers to the internal volume through cellular membrane. Quantitatively the process of intercellular mass transfer is described by Eq. (2):

$$D_m = e \cdot D \tag{2}$$

where ε – coefficient of microalgae colony porosity; D_m – coefficient of CD diffusion in the intercellular environment of microalgae colony; D – coefficient of CD diffusion in water.

– *the third stage*: CD permeation through cellular membrane to the internal volume of algae cell owing to active and passive transfer. If passive transfer occurs the process has diffusion character and may be represented by Eq. (3):

$$g = -D_c\,grad C \tag{3}$$

where γ – density of CD flow through cellular membrane; C – CD concentration in the intercellular environment; D_c – coefficient of CD diffusion through the cellular membrane.

– *the fourth stage*: photosynthesis. Diffusion comes to the end in the chloroplasts, where CD enters into biochemical photosynthesis reaction. The photosynthesis kinetics is described by Eq. (4):

$$\frac{dC}{dt} = kC_{CO_2}C_{H_2O} \tag{4}$$

where k – rate constant of biochemical photosynthesis.

According to the additivity rule the total coefficient of mass transfer K is determined as:

$$K = \frac{1}{\frac{1}{b} + \frac{l}{D_m} + \frac{d}{D_c} + \frac{d_c}{k}} \tag{5}$$

where l – conditional average size of microalgae colony; δ – membrane thickness.

While permeating the cellular membrane the CD concentration decreases in accordance with the linear law from C_0 to C_i. The rate of CD transfer through the cellular membrane with the area F is described by Eq. (6):

$$\frac{dm}{dt} = u = D_c F \frac{C_0 - C_i}{d} \tag{6}$$

where C_0 and C_i – CD concentrations on the external and internal surface of cellular membrane, respectively.

The rate of biochemical reaction is proportional to the concentration C_i in the internal volume, because the water concentration in the internal volume is a significant and constant value.

$$u_p = kC_i \tag{7}$$

Under the stationary conditions the amount of CD participated in the photosynthesis is equal to the one that permeated the cellular membrane and entered the cell internal volume:

$$b_c(C_0 - C_i)_p = kC_i \tag{8}$$

where b_c – coefficient of mass transfer through cellular membrane.

We determine the value C_i from Eq. (8), substitute it into Eq. (7) and obtain Eq. (9) to determine the rate of photosynthesis reaction:

$$u_p = C_0 \frac{k b_c}{k + b_c} \tag{9}$$

If the rate of photosynthesis reaction is higher than the rate of CD transfer through the cellular membrane

$$k >> b_c \tag{10}$$

then the value b in Eq. (9) may be neglected and the reaction rate is equal to:

$$u_p = b_c C_0 \tag{11}$$

It is obvious from Eq. (11) that the rate of photosynthesis reaction is determined by the rate of CD diffusion (transfer) through the cellular membrane (diffusion kinetics).

If the rate of photosynthesis reaction is considerably lower than the rate of CD transfer through the cellular membrane:

$$k << b_c \tag{12}$$

then the value k in Eq. (9) may be neglected and the reaction rate is equal to:

$$u_p = kC_0 \tag{13}$$

It means that the reaction rate is determined by the rate of CD interaction with water. If the rate of interaction is very high, the CD concentration in the internal volume is equal to zero and Eq. (6) takes the form:

$$\frac{dm}{dt} = u = \frac{D}{d} FC_0 \tag{14}$$

Using the experimental data this simple ratio allows to determine the diffusion coefficient at known value of cellular membrane thickness.

If the reaction rate is determined by the diffusion rate, it is advisable to use components stirring to intensify the process, *i.e.* to intensify the hydrodynamics. If the reaction rate depends on the rate of biochemical interaction, the hydrodynamics intensification will not give a desired effect. In such a case it is necessary to increase the temperature.

The increase in temperature has less effect on the diffusion kinetics compared with the rate of biochemical reaction.

For the biological processes, the rate of which depends on the rate of biochemical reaction, the temperature effect is described as follows:

$$u = kC^n e^{-\frac{E}{RT}} \tag{15}$$

where E – activation energy; n – reaction molecularity; C – concentration of the reacted compounds; k – rate constant.

The dependence of rate constant k on the temperature T has the form:

$$k = k_0 e^{-\frac{E}{RT}} \tag{16}$$

Finding the logarithm of Eq. (16) we obtain Eq. (17):

$$\lg k = \lg k_0 - \frac{E}{2.3RT} = \lg k_0 - \frac{E}{4.575T} \tag{17}$$

If we designate $\lg k$ as B and $\frac{E}{4.575}$ as A, we obtain Eq. (18) known as Arrhenius equation:

$$\lg k = B - \frac{A}{T} \tag{18}$$

The values A and B are the reaction constants. If we plot the dependence $\lg k = f(\frac{1}{T})$ according to the experimental data, we receive the straight line, the slope angle of which allows to determine the value of A (Fig. 4). Then the activation energy may be determined: $E = 4.575A = 4.575tg\alpha$.

2.2. Practical Part

To study the temperature effect on the CD absorption by microalgae we used three photobioreactors. The used equipment provided a sufficient amount of carbon dioxide for microalgae in the whole volume of photobioreactor. Stirring and lighting favor the process of CD absorption, accompanied by the increasing number of algae cells (biomass). The temperatures of the culture medium in photobioreactors were 293 ± 1, 301 ± 1, and 308 ± 1 K, respectively; cultivation medium pH – 6.5. The samples of algae biomass were withdrawn in the definite range of days. Biomass concentration was determined by photocolorimetric method [7].

The experimental researches of the CD concentration that influences the rate of biomass growth were conducted using three photobioreactors. The first reactor with microalgae in the nutrient medium was a check one; the air was bubbled in the second reactor and carbon dioxide – in the third one. The cultivation temperature was 293 ± 1 K, medium pH was 6.5.

3. Results and Discussion

When CD is bubbled through the aqueous solution in the reactor, the CD supply from the primary volume to the surface of microalgae colony is very intensive. Hence the coefficient of mass transfer b is a considerable value and its inverse value (see Eq. (6)) may be neglected. Taking into account the value of colony porosity (e = 0.4), the convective mass transfer of carbon dioxide in the intercellular volume is possible and the second coefficient in the denominator of Eq. (5) may be neglected as well. CD transfer through the cellular membrane and photosynthesis reaction are accompanied by biomass growth proportional to the amount of absorbed CD:

$$\frac{dN}{dt} \sim \frac{dM}{dt} \sim \frac{dm_{CO_2}}{dt} \tag{20}$$

where $\frac{dN}{dt}$ – rate of algae cells growth in the medium; $\frac{dM}{dt}$ – rate of algae mass growth in the medium; $\frac{dm_{CO_2}}{dt}$ – rate of CD absorption.

Thus the total value of mass transfer coefficient K is proportional to the sum of both values in the denominator of Eq. (5), *i.e.* to the growth factor of biomass k_m:

$$k_m \sim K \tag{21}$$

The kinetics results of biomass growth while absorbing CD at different temperatures are represented in Fig. 1. These curves of usual S-like shape allow to observe four periods of growth taking place in a certain sequence and expressed to a greater or lesser extent: initial or lag-phase, exponential growth phase, stationary phase and phase of culture sedimentation. One can see from Fig. 1 that kinetics of growth essentially depends on temperature.

The obtained results are well described by Eq. (22) [1]:

$$N = N_0 e^{-k_m t} \tag{22}$$

The equation in the logarithmic coordinates represents the straight line, the slope angle of which allows to determine the growth factor k_m at every temperature: $\ln N = \ln N_0 + k_m t$

The results of transformations are represented in Fig. 2.

The values of biomass growth factor at the studied temperatures of 293, 301 and 308 K were 0.1, 0.12 and 0.15 day^{-1}, respectively. Using the obtained results we determined the dependence of growth factor on the temperature (Eq. (23), Fig. 3).

$$k_m = 11.35 \cdot 10^{-4} e^{-\frac{2073}{T}} \tag{23}$$

The activation energy E = 4.57 kJ/mol (Fig. 4) is calculated according to Eq. (21).

The detailed analysis of the obtained experimental results, in particular the rate of biomass growth *vs.* time at different temperatures is represented in Fig. 5. We observe the significant effect of temperature on the rate. The maximum growth is observed at 309 K for 2 days. At higher temperatures microalgae perish and at lower temperatures the growth rate approaches the maximum value, which indicates the enzymatic mechanism of the process. Under the mentioned conditions the rate of biomass growth depends not only on CD concentration but on the amount of enzymes as well. At the constant set of enzymes the biomass growth is constant too (Fig. 5).

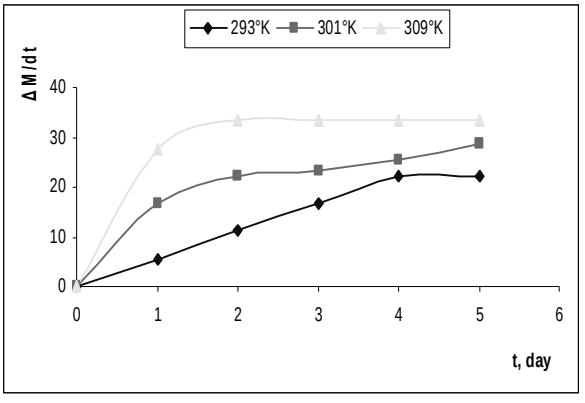

Fig. 1. Kinetic curves of algae concentration growth depending on time at different temperatures

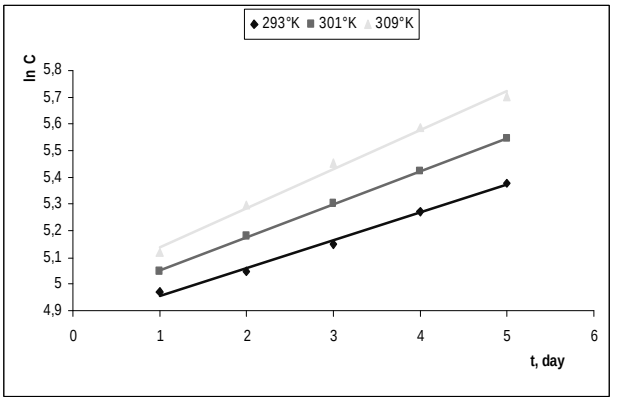

Fig. 2. Kinetic curves of logarithm of algae concentration growth depending on time at different temperatures

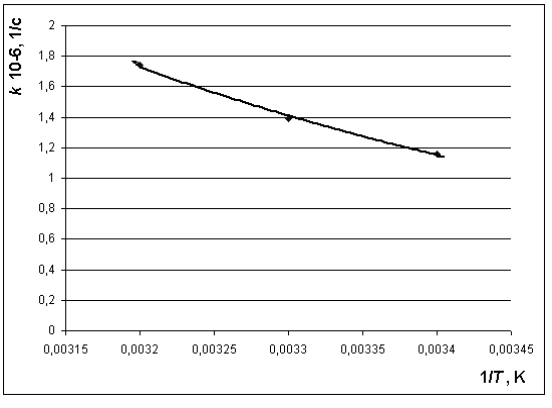

Fig. 3. Growth factor k_m *vs.* $1/T$

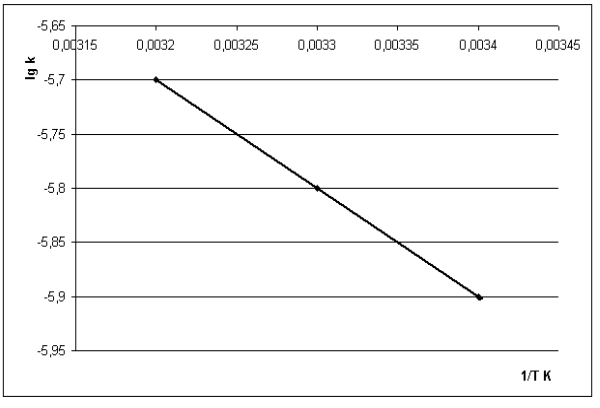

Fig. 4. Logarithm of growth factor k_m *vs.* $1/T$

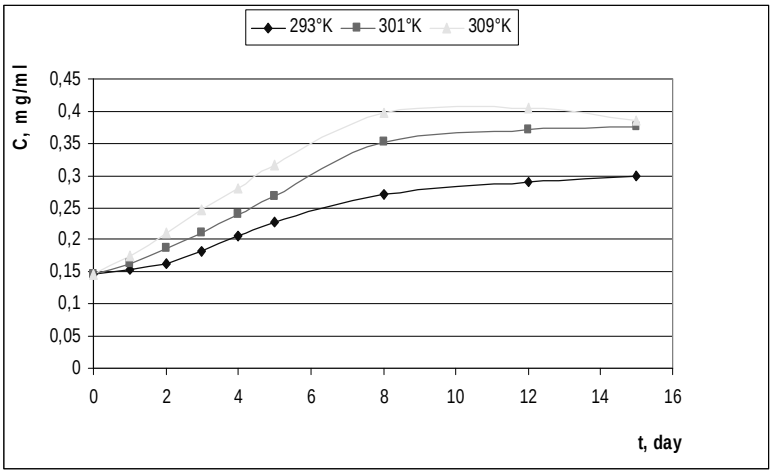

Fig. 5. Rate of biomass growth *vs*. time

The kinetics of enzymatic reactions is described by Michaelis-Menten equation (Eq. 24):

$$U = U_{\max} \frac{[S]}{k_M + [S]}. \quad (24)$$

where k_M – Michaelis constant characterizing the rate of enzymatic reaction depending on substrate concentration under stationary conditions; U_{max} – maximum rate of enzymatic reaction; S – substrate concentration (in our case concentration of carbon dioxide).

This equation connects the reaction rate and substrate (CD) concentration. In such a case the enzymatic transfer of carbon dioxide through cellular membrane (active transfer) and photosynthesis biochemical reaction catalyzed by corresponding enzymes take place. Biomass growth is proportional to the amount of absorbed CD. Based on the experimental results we assume that the determined Michaelis constant is equal to the sum of two latter values in denominator of Eq. (5). Then:

$$K \sim k_m \sim k_M \quad (25)$$

While analyzing Eq. (22) it should be noted that $k_m = k_1 - k_2$, where k_1 – proportionality constant that determines the increase in cell concentration per time unit and depends on environment conditions (temperature, presence of nutrients, *etc*.); k_2 – proportionality constant that determins the intensity of alga cells death. Analytically this condition is expressed by Eq. (26):

$$\frac{dN}{dt} = k_1 N - k_2 N = k_m N \quad (26)$$

Dividing the variables we obtain:

$$\int_{N_0}^{N} \frac{dN}{N_0} = k \int_0^t dt \quad (27)$$

Solving Eq. (27) we obtain the dependence of cells concentration in the cultivation medium on time: $N = N(dt)$, *i.e*. Eq. (22).

If $k_1 > k_2$, $k > 0$, then the growth of cells number in the system is endless $N(t) \to \infty$ at $t \to \infty$; if $k_1 < k_2$, the colony will die in time $N(t) \to 0$ at $t \to \infty$; and only in one case, if $k_1 = k_2$, the number of alga cells is constant $N = N_0$; where N_0 – cells concentration at the initial time $t = 0$.

Using the data of CD absorption dynamics we plotted the kinetic curves of microalgae biomass growth presented in Fig. 6. Since bubbling is absence in the check reactor, microalgae association and agglomeration followed by settling are observed. The curve describing their growth is down-directed. The residual amount of microalgae continues to divide and the curve becomes up-directed. When the air or CD is bubbled in the reactor, the formed associates are sustained in the bubbling medium and both curves are up-directed (Fig. 6).

Unlimited in time exponential growth of microalgae culture is possible only in the case of constant addition of all necessary components (nutrients, aeration, light, *etc*.) and removal of metabolic products. The environment contains limited initial amount of nutrients, so microalgae grow till the content of some necessary component achieves its critical value. After this the growth becomes slower. If we observe the microalgae growth in the aqueous medium, the growth rate varies in time. The curves describing the dependence of live cells or microalga concentration on time are represented in Fig. 1.

Finding the logarithm of Eq. (22) in the system of coordinate $\frac{1}{U} = f(\frac{1}{S})$ we determined the maximum rate of microalgae growth U_{max} and Michaelis constant k_M in three photobioreactors. For the first (check) reactor $U_{max} = 0.07$ mg/ml·day, $k_M = 3.5 \cdot 10^{-4}$ mg/ml; for the second (bubbled with air) reactor $U_{max} = 0.07$ mg/ml·day, $k_M = 4.2 \cdot 10^{-4}$ mg/ml; for the third (bubbled with CD) $U_{max} = 0.104$ mg/ml·day, $k_M = 4.2 \cdot 10^{-2}$ mg/ml (Fig. 7).

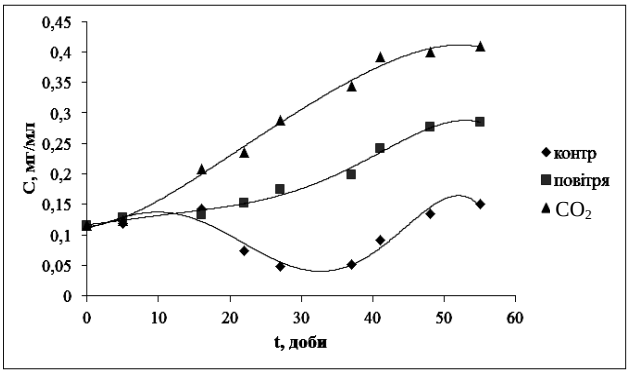

Fig. 6. Curves of microalgae biomass growth

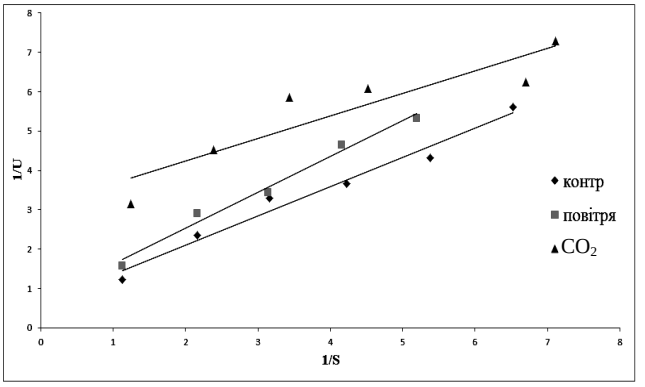

Fig. 7. Dependence of inverse rate growth on substrate concentration

4. Conclusions

The kinetics of carbon dioxide absorption by unicellular microalgae was studied. The enzymatic mechanism of the process was established. The activation energy of the photosynthesis reaction under the experimental conditions, as well as the rates of biomass growth without air, under air and carbon dioxide bubbling, were determined. The maximum rate of algae growth and Michaelis-Menten constant were determined for all three cases.

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ЩОДО ПРОБЛЕМ ВИВЧЕННЯ БІОЛОГІЧНИХ ПРОЦЕСІВ, УСКЛАДНЕНИХ МАСООБМІНОМ

Анотація. *Представлено результати вивчення процесу поглинання вуглекислого газу одноклітинними мікроводоростями. З'ясовано стадії перебігу процесу, враховуючи особливості умов культивування, зокрема наявність клітинного та міжклітинного середовища. Встановлено константи, які конкретизують математичну модель поглинання вуглекислого газу з повітря клітинами мікроводоростей. Отримані рішення дають можливість прогнозувати кінетику процесу поглинання вуглекислого газу та проектувати обладнання для здійснення технологічного процесу очищення газових викидів за умов реалізації біотехнології на практиці.*

Ключові слова: *фотосинтез, мікроводорості, математична модель, коефіцієнт масопереносу, біомаса, міжклітинне середовище, дифузія.*