

The Biomethanization Gas Purification of Using Chlorophyll-Synthesizing Microalgae

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ABSTRACT

The paper shows the possibility of using chlorophyll-synthesizing microalgae of *Chlorella vulgaris* to purify biogas from carbon dioxide (CO₂), hydrogen sulfide (H₂S) and ammonia (NH₃). Experimental dependences of the dynamics of CO₂ uptake by microalgae under the action of H₂S inhibitor and NH₃ activator were presented. A mathematical description of the growth of biomass of microalgae *Chlorella vulgaris*, depending on the concentration of hydrogen sulfide and ammonia, was obtained. The optimal values of hydrogen sulfide and ammonia concentration for the efficient process of carbon dioxide uptake by chlorophyll-synthesizing microalgae *Chlorella vulgaris* from biomethanization gas were established.

Keywords: microalgae *Chlorella vulgaris*, biogas, carbon dioxide, hydrogen sulfide, ammonia, nitrogen oxides, sulfur dioxide.

INTRODUCTION

The rapid development of industry has led to the depletion of world fossil fuel reserves. Therefore, renewable energy technologies have been actively introduced recently, in particular the production of various types of biofuels, the most available of which today is biomethane gas – biogas. The main disadvantage of biogas is the significant amount of impurities, in particular CO₂, H₂S and NH₃. That is why biogas needs purification for two main reasons: the first is to increase its calorific value, and the second is to reduce the equipment damage as well as adverse effects on human health and the environment to the toxic effects of H₂S [Allegue and Hinge, 2014].

To date, the technologies for biogas purification have been developed and are available; they include physical absorption and chemisorption, cryogenic separation, membrane separation. However, physico-chemical treatment methods are not only high in value, as they require a lot of energy, materials and reagents, but also generate a significant amount of wastewater that pollutes the environment [Awe et al. 2017].

In addition, the polluting gases captured in this way are dangerous during storage, transportation and so on. At the same time, there are biological methods of purification, in particular with the use of microalgae, which have photosynthetic activity, which extent eliminates of these problems.

Biological treatment allows transforming pollutants into harmless products of microorganisms and biomass. Microalgae deserve special attention; they are quite effective converters of solar energy with well-organized stages of reduction of CO₂ to a whole complex of biomolecules, including carbohydrates, proteins, lipids, which can be involved in further biotechnological transformation into a variety of target products. Microalgae can grow quite quickly under harder conditions of their unicellular structure. The microalgae of *Chlorella vulgaris* are considered to be one of the most productive. This genus of microalgae has long been known and well studied, which is an undoubted advantage for their use in industry. Along with high biomass productivity, these cells have a number of features that allow them to be considered the most suitable for use in biotechnological processes as a substrate. The advantage of microalgae of

Chlorella vulgaris is its exceptional adaptability to environmental changes.

The use of microalgae to sorb carbon dioxide, which is formed during methane fermentation, will provide a solution to the problem of purification of biogas from hydrogen sulfide and ammonia. Because sulfur and nitrogen are trace elements necessary for the activity of microalgae, they can be consumed in certain concentrations without inhibiting their growth. Moreover, this method of using microalgae is interesting not only for the purification of biogas, but also because it allows obtaining valuable biomass, which is used as raw material in various biotechnological processes.

Many works have been devoted to the study of the influence of carbon dioxide, sulfur dioxide, and nitrogen on the growth of microalgae, and this issue has been sufficiently covered in [Dyachok et al. 2017, 2020, 2021; Manakov and Pobedimskiy, 1990]. At present, the information on the impact on the growth and development of microalgae of associated gases – products of biomethanization, hydrogen sulfide H_2S and ammonia NH_3 is extremely insufficient.

The aim of the work is to establish the optimal values of the concentrations of hydrogen sulfide H_2S and ammonia NH_3 at which the absorption of carbon dioxide CO_2 by microalgae of *Chlorella vulgaris* is the most efficient.

MATERIALS AND METHODS

Literature reports the average chemical composition of biogas. That is why, to study the growth and development of microalgae, the values of concentrations sulfide HS^- anion and ammonium NH_4^+ cation, which correspond to the values of volume pressures of these pollutants in biogas, were taken.

The growth dynamics of *Chlorella vulgaris* microalgae were studied for the corresponding values of the concentration of HS^- anion sulfide by the addition of sodium sulfide Na_2S , and ammonium NH_4^+ cations introduced by the addition of NH_4OH ammonia water. Sodium sulfide and ammonia water were applied once at the beginning of the experiment. Cultivation of microalgae was performed in photobioreactors for 14 days at a temperature of 30 ± 2 °C. In all cases, the pH was within rational values for the cultivation of *Chlorella vulgaris*.

There were five samples in the experiment and control:

- control – the environment for the cultivation of microalgae without HS^- ;
- sample No. 1 – microalgae with the content of HS^- at a concentration of 170 g/m^3 , which corresponds to 0.5 vol.% hydrogen sulfide in biogas;
- sample No. 2 – microalgae with the content of HS^- at a concentration of 340 g/m^3 , which corresponds to 1 vol.% hydrogen sulfide in biogas;
- sample No. 3 – microalgae with HS^- content – at a concentration of 510 g/m^3 , which corresponds to 1.5 vol.% hydrogen sulfide in biogas;
- sample No. 4 – microalgae with HS^- content – at a concentration of 765 g/m^3 , which corresponds to 2 vol.% hydrogen sulfide in biogas;
- sample No. 5 – microalgae with HS^- content – at a concentration of 1147 g/m^3 , which corresponds to 2.5 vol.% hydrogen sulfide in biogas.

The ammonia content in biogas can reach 1 vol.%, depending on the raw material taken for methane fermentation [Bauer et al., 2009]. Therefore, • sample No. 1 NH_4^+ at a concentration of 228 g/m^3 , corresponding to 0.25 vol.%, • sample No. 2 NH_4^+ at a concentration of 455 g/m^3 , corresponding to 0.5 vol.%, • sample No. 3 NH_4^+ at a concentration of 910 g/m^3 , corresponding to 1 vol.%, • sample No. 4 NH_4^+ at a concentration of 1365 g/m^3 , corresponding to 1.5 vol.%, • sample No. 5 NH_4^+ at a concentration of 1820 g/m^3 , corresponding to 2.0 vol.%, respectively, and control • without the addition of NH_4^+ .

The increase in the biomass of microalgae under such conditions was determined by photocolometric method using a blue light filter according to the law of Bouguer-Lambert-Ber [Ofitsiyhy portal..., 2020]. Since the optical density is proportional to the content of microalgae in the culture medium, the obtained experimental data on the accumulation of microalgae biomass as a function of time within the studied values of HS^- ; NH_4^+ concentrations correspond to the values of optical densities. The results of the study at the same initial content of microalgae cells 0.2 mg/ml in photobioreactors are presented in Figures 1 and 2.

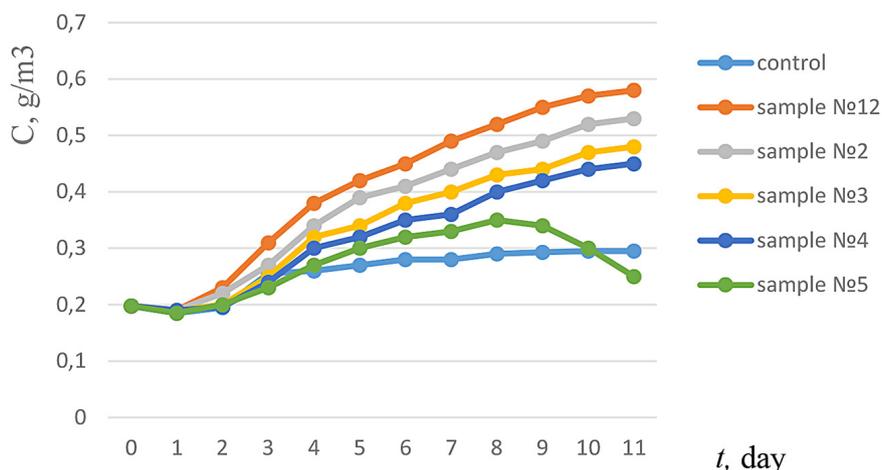


Fig. 1. The dependence of the change in the content of microalgae cells in the cultivation medium over time at appropriate concentrations of HS^-

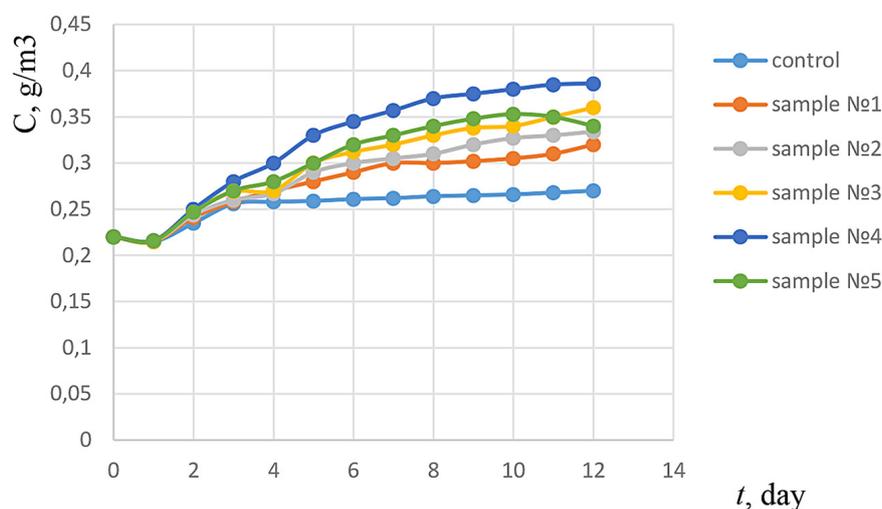


Fig. 2. The dependence of the change in the content of microalgae over time at appropriate concentrations of NH_4^+

RESULTS

The growth microalgae of *Chlorella vulgaris* during 14 days at different contents of HS^- anion sulfide is illustrated in Figure 1. The graph in the figure shows that the first day can be considered a period of adaptation, after which sample growth begins to grow rapidly. However, this does not continue for all samples until the end of the experiment – 14 days. The control from the second day passes the phase of exponential growth and from the fourth curve of control goes to the plateau, to the stationary phase of growth.

The largest increase in biomass was observed in sample 1, then in sample 2. Samples 3 and 4 are almost at the same level. Sample 5, after a slight increase in growth, decreases and ultimately

cell death is observed, accompanied by a down movement of the curve.

Thus, the largest increase in biomass is characteristic of the cultures grown in a medium with a concentration of sulfide anion 390 g/m^3 , which is twice as high as under control conditions. Higher values of the concentration of sulfide anion in the environment lead to a decrease in biomass growth, up to negative – sample 5. At the beginning of the study, the *Chlorella vulgaris* cells were not adapted, and sulfide anion acts as a growth inhibitor – which cannot be said microalgae in control. The control goes through a phase of adaptation and cell growth goes to the plateau, because sulfur, which was contained in the nutrient, is depleted. Accordingly, growth is reduced, including in the absence of this

macronutrient. In the samples with a higher value of sulfur in the form of sulfide anions, on the contrary, the content of the inhibiting substance, i.e. sulfides decreases over time, as they are oxidized to sulfites and sulfates, which are the main assimilative form for the microalgae of *Chlorella vulgaris*.

The lower the concentration of sulfides, the faster most of them will turn into sulfites and sulfates. Therefore, the growth of cells of sample 1 after adaptation quickly reaches the plateau, while samples 2, 3 and 4 continue to divide more slowly. By the end of the experiment, the largest relative cell growth was observed for sample 1, because the highest concentration of assimilable sulfur in the form of sulfites and sulfates, and sulfur is an important and necessary element for normal cell division. In the same sample, the largest increase in microalgae biomass is observed, which is probably a rational value of HS⁻ sulfide anion concentration for CO₂ absorption and increase in *Chlorella vulgaris* biomass, and this concentration value does not adversely affect the development of microalgae.

Ammonia formed as a result of biomethanization can also be used as an energy material necessary for the activity of microalgae cells. The required energy will be formed as a result of enzymatic reactions of oxidation of ammonia and ammonium salts and later nitric acids.

This assumption is confirmed by the obtained data presented in Figure 2. The figure shows that the NH₄⁺ cations contribute to the absorption of carbon dioxide, which is reflected in the growth of biomass of microalgae. The increase in the

concentration of microalgae cells significantly depends on the concentration of NH₄⁺ cations that act as activators of the process of carbon dioxide absorption of CO₂. From the first to the fourth sample, an increase in microalgae is observed that is much faster than in the control sample in which no other cations were added, only microalgae and nutrient.

Attention should also be paid to the sample No. 5, which in comparison with others, goes to the plateau after a slight increase and then begins to decline, indicating a detrimental concentration of NH₄⁺ cations, for the growth and development of microalgae.

Therefore, the most favorable according to experimental studies for the growth and development of the *Chlorella vulgaris* microalgae, and for the absorption of carbon dioxide are the values of concentration: HS⁻ 0.17 g/m³ and NH₄⁺ 910 g/m³, which corresponds to 0.5 vol.% hydrogen sulfide and 1.0 vol.% ammonia in biogas.

It is logical to assume that the synergy effect will occur under the presence of the found optimal values of NH₄⁺; HS⁻ concentrations. This assumption was confirmed by the experiment, which consisted in the following: the following values of concentrations were loaded into the photobioreactor under conditions similar to those previously described – ● sample No. 1 HS⁻ at a concentration of 170 g/m³ and NH₄⁺ – 1365 g/m³, corresponding to 0.5 vol.% hydrogen sulfide and 1.5 vol.% ammonia in biogas, ● sample No. 2 HS⁻ at a concentration of 170 g/m³ and NH₄⁺ 1365 g/m³, which corresponds to 0,5 vol.% hydrogen sulfide and 1.0 vol.% ammonia in biogas, ● sample No. 3 HS⁻ at a concentration of 340 g/m³

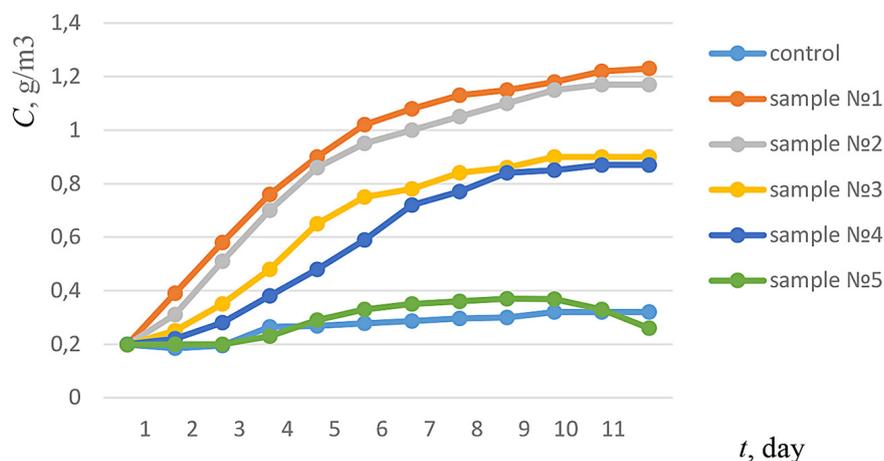


Fig. 3. Combined effect on the growth of microalgae biomass of HS⁻ and NH₄⁺ ions

and NH_4^+ – 1820 g/m³, which corresponds to 1.0 vol.% hydrogen sulfide and 1.5 vol.% ammonia in biogas, ● sample No. 4 HS⁻ at a concentration of 340 g/m³ and NH_4^+ – 1620 g/m³, corresponding to 1.0 vol.% hydrogen sulfide and 1.5 vol.% ammonia in biogas, ● sample No. 5 HS⁻ at a concentration of 1147 g/m³ and NH_4^+ – 1820 g/m³, corresponding to 2.5 vol.% hydrogen sulfide and 2.0 vol.% ammonia in biogas. The results of this experiment are presented in Figure 3.

On the basis of the data of experimental studies and mathematical processing of the obtained results, it is seen that the maximum increase of chlorophyll-synthesizing microalgae *Chlorella* is achieved by the ratio of NH_4^+ ; HS⁻ concentrations, which were added to the first sample, namely 1.5 vol.% ammonia and 0.5 vol.% hydrogen sulfide in biogas. Figure 3 also shows that the sample No. 2 has a rapid increase compared to others. Sample No. 2 contains 1.0 vol.% Ammonia and 0.5 vol.% hydrogen sulfide in biogas. Moreover, the increase in microalgae in both samples far exceeds the increase in the individual action of hydrogen sulfide and ammonia in biogas (Figures 1 and 2). At concentrations of 1 vol.% hydrogen sulfide and the two best values of ammonia concentrations of 1 vol.% and 2 vol.%, although lower than in previous studies, 0.5 vol.% hydrogen sulfide and vol.% and 2 vol.% ammonia in biogas.

At concentrations of 2.5 vol.% hydrogen sulfide and 2 vol.% ammonia, the death of the microalgae *Chlorella vulgaris* is observed. The defining parameter that characterizes the growth of microalgae $\delta\mu$ is the specific growth rate:

$$\delta_k = \delta C_f / C \times \delta t \quad (1)$$

where: C – concentration of microalgae, $\delta\mu$ – the specific growth rate or specific growth rate (s⁻¹). The growth rate was determined from the kinetic equation:

$$\delta C / \delta t = \mu \times C \quad (2)$$

According to equation (2), the growth factor characterizes the relative increase in the density of microalgae per unit time. If $\delta\mu$ remains unchanged for a certain time, then such an increase is called exponential, and the corresponding period of time – the exponential phase of growth. Integrating equation (2), a constant integration is found provided that at the initial time $t = 0$ there is an initial density of cells of microalgae C_0 .

$$C = C_0 \times \exp(\mu t) \quad (3)$$

Since the logarithmic dependence of the concentration of microalgae cells on time during the period of exponential growth is a linear dependence, it enables to determine the growth coefficient μ as the tangent of the angle of inclination of the experimental line. Therefore, substituting the experimental data in equation (3) the dependences $\ln C = f(t)$ are obtained, which are shown in (Fig. 4).

After mathematical processing of the results of experimental research and the obtained calculated data, the sample No. 1, $\mu = 0,1107 \text{ d}^{-1}$, and a sample No. 2, $\mu = 0.1086 \text{ d}^{-1}$ (Fig. 4) have the highest value of the growth factor. The values of the coefficients of addition for other samples are presented in Table 1.

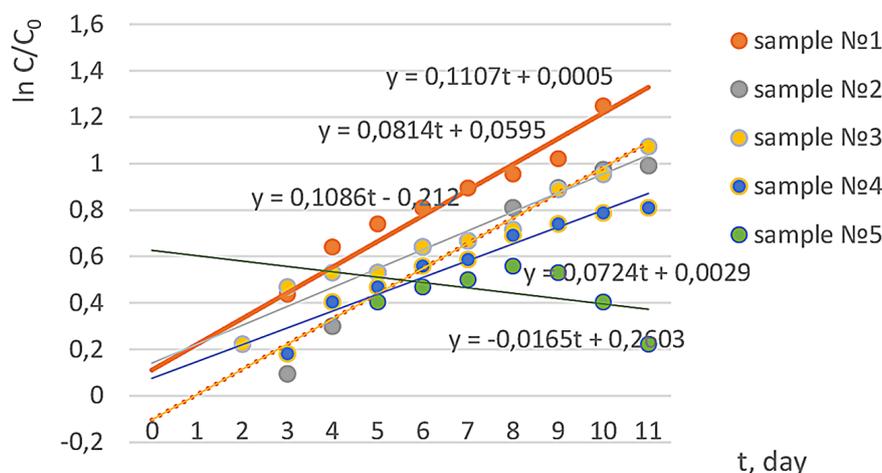


Fig. 4. Dependence of the change in the logarithm of the microalgae content on time (at the corresponding concentrations of activators NH_4^+ and the inhibitor HS^-)

Table 1. Experimental data and calculated values

Sample	μ growth coefficient, d ⁻¹	HS ⁻ vol.% concentration, mg/m ³	NH ₄ ⁺ vol.% concentration, mg/m ³
Control	0.0292	-	-
Sample No. 1	0.1107	0.5	1.5
Sample No. 2	0.1086	0.5	1.0
Sample No. 3	0.0814	1.0	1.5
Sample No. 4	0.0724	1.0	1.0
Sample No. 5	-0.0165	2.5	2.0

CONCLUSIONS

It was found that sulfide ions HS⁻ inhibit the growth of *Chlorella vulgaris* regardless of the initial concentration of microalgae. Over time, sulfides are oxidized to sulfates, which in turn stimulate proliferation, because they are the main assimilative form of sulfur. The optimal concentration of HS⁻ sulfide ions in the cultivation medium of *Chlorella vulgaris* is the concentration corresponding to 0.5–1.0 vol.% hydrogen sulfide in biogas. The use of microalgae *Chlorella vulgaris* for purification of biogas from hydrogen sulfide is possible by diluting biogas, in particular ammonia, in order to achieve the optimal values of hydrogen sulfide concentration.

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