

TO THE PROBLEMS OF WASTEWATER TREATMENT
FROM MINERAL POLLUTANTS WITH THE USE OF MICROALGAE

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Abstract. This article presents the results of experimental studies on the use of *Chlorella* microalgae for wastewater treatment from the most common mineral pollutants. The influence of the mineral pollutants, namely anions HSO_3^- , NO_3^- , H_2PO_4^- on the dynamics of wastewater treatment has been established. Analytical dependences of microalgae biomass growth rate depending on pollutant concentration HSO_3^- , NO_3^- and H_2PO_4^- are obtained. The values of biomass growth coefficients at the corresponding pollutant concentrations are determined. Based on the obtained experimental research results, mathematical models have been constructed that allow predicting the maximum values of mineral pollutants concentrations at which effective wastewater treatment by chlorophyllsynthesizing microalgae is possible. The technological scheme of wastewater treatment from mineral pollutants and the mechanism of the obtained application biomass are proposed.

Key words: sulfur anion (HSO_3^-), nitrogen anion (NO_3^-), phosphorus anion (H_2PO_4^-), *Chlorella*, chlorophyllsynthesizing microalgae, wastewater treatment, mineral pollutants.

1. Introduction

Due to the expansion of production and the imperfection of treatment technologies, environmental pollution has reached its peak. The hydrosphere is significantly affected by pollutants. Currently, the biological method of wastewater treatment is the promising one (Manakov et al., 1990). The disadvantage of biological purification methods such as activated sludge, granular sludge, biofilm is that the necessary condition is oxygen enrichment, mixing and, as a

consequence, carbon dioxide evolution. The advantage of our technology is that the sorption of sulfur anion (HSO_3^-), nitrogen anion (NO_3^-), phosphorus anion (H_2PO_4^-) and carbon dioxide (CO_2) by *Chlorella* microalgae occurs with the release of oxygen as the product of photosynthesis.

The problem of the current system of biological wastewater treatment in aerotanks is the enormous cost of oxygen for the bacterial processes of organic and mineral substances removal in wastewater, and carbon dioxide (CO_2), as a product of bacteria living abilities, is released into the air. It means that the present existing treatment plants are the oxygen consumers and air pollutants with carbon dioxide (CO_2). It should also be noted that the biological feature of the bacteria is that they are narrowly specialized, that is, there is no one type of bacteria that could purify the entire spectrum of contaminants present in wastewater (Hubskey, 2007).

The proposed technology provides a method of wastewater treatment with different concentrations of contaminants using a specific strain of microalgae. Ecologically, this is justified by the fact that microalgae consume carbon dioxide (CO_2) and release oxygen for their living abilities (Statsenko, Vynohradova, 1972). Evolutionary microalgae are at a higher level of development than bacteria, and therefore many of their species are universal consumers of many organic and mineral substances which are pollutants of wastewater.

In the burning of fuel (solid, liquid, gaseous), besides carbon dioxide, sulfur, nitrogen and phosphorus oxides are released into the air. With precipitation, these pollutants return to the hydrosphere in the form of anions

and cause acidification of reservoirs. Therefore, these anions were chosen for the experimental study.

The literature contains little data on the effect of anions HSO_3^- , NO_3^- and H_2PO_4^- on the dynamics of wastewater treatment by chlorophyllsynthesizing microalgae (Dyachok et al., 2018; Dyachok et al., 2019a). Therefore, it is important to study the influence of these anions concentrations on the dynamics of wastewater treatment.

The aim of this work is to determine critical or maximum permissible concentrations of anions HSO_3^- , NO_3^- and H_2PO_4^- in wastewater where the cultivation of the *Chlorella* type microalgae is possible.

2. Theoretical part

Using the strain of the *Chlorella* type microalgae a high degree of biological purification from mineral pollutants is achieved. The biomass, accumulated in this way, can be used as an organic “green” fertilizer or converted by biomethanization to methane gas (Zolotaryova et al., 2008). The use of this microalgae strain does not require reorganization or major construction of new treatment plants.

During the experimental research, culture of green microalgae – *Chlorella* was the object of observation. For this purpose, the culture of microalgae – *Chlorella* was placed into wastewater containing anions HSO_3^- , NO_3^- and H_2PO_4^- . Nutrients – carbon dioxide and mineral nutrients elements microalgae cells obtain directly from the environment by absorbing them with their entire surface. Since anions like HSO_3^- , NO_3^- and H_2PO_4^- are absorbed by microalgae, their effect on the growth of chlorophyllsynthesizing microalgae was studied at different concentration values of the corresponding anions.

To study the effect of anions HSO_3^- on the increase of chlorophyllsynthesizing microalgae, the values of anion HSO_3^- concentration were investigated and shown in Fig. 1. To study the effect of anion NO_3^- and H_2PO_4^- on the increase of chlorophyllsynthesizing microalgae, the values of anion concentration are shown in Fig. 3 and Fig. 5. Accordingly, the control solution, which did not contain the corresponding anions in three variants of the research, it should be also noted that the conditions of the experiment involved the presence of natural light and the temperature of 30 ± 5 °C.

The increase in the biomass of chlorophyllsynthesizing microalgae under these conditions was determined by the photocolimetric method using a blue light filter according to the law of Bouguer-Lambert-Beer (Poltorak et al., 1972). Since the optical absorption of light at a given wavelength is proportional to the

concentration of microalgae, the obtained experimental data on the accumulation of microalgae biomass depending on the time within the studied concentrations of anions HSO_3^- , NO_3^- and H_2PO_4^- are proportional to the values of optical densities. The optical density value of the researched and control solutions was measured in relation to the reference solution. In our case, it was water.

3. Presentation of the main material and discussion of the results

During the experimental data processing, dependencies were obtained which illustrate the change in microalgae concentration over time at different values of anion HSO_3^- in solution with its single introduction (Fig. 1). The obtained data indicate that presence of the anion HSO_3^- in the wastewater significantly affects the growth of microalgae cells biomass in comparison with the control. Under the conditions of an increase in anion HSO_3^- concentrations in wastewater, the growth of microalgae biomass decreases. In the control sample, however, its stable growth is observed. Therefore, it is appropriate to assume that the anion HSO_3^- presence starting with concentrations of 0.001 mg/ml and more, under the conditions of the experiment, there is a suppression of the wastewater treatment process with chlorophyllsynthesizing microalgae of the *Chlorella* type.

A more detailed data analysis in Fig. 1 allows us to argue that the change in the number of microalgae cells per time unit under the experimental conditions is determined by the number of born and died cells. Quantitatively, this process can be described by a known equation (3), which is in the coordinates $\ln \frac{C}{C_0} = f(t)$, allows you to determine the growth coefficient – μ (Dyachok et al., 2017).

The growth coefficient may be $\mu > 0$, under the conditions of a certain value activity of the inhibitor concentration (anion HSO_3^-) photosynthesis can gain a negative value $\mu < 0$, as well as being equal to zero. Experimental research data (Fig. 1), in coordinates $\ln \frac{C}{C_0} = f(t)$, [Dyachok et al. 2019b] are graphically presented by the lines in Fig. 2.

At anion HSO_3^- concentration values in wastewater 0,001 mg/ml; 0,002 mg/ml; 0,003 mg/ml; 0,004 mg/ml, the growth rates are less than zero $\mu < 0$. The numerical values of the coefficients are respectively: $\mu_4 = - 0.0563 \text{ d}^{-1}$; $\mu_5 = - 0.0543 \text{ d}^{-1}$; $\mu_6 = - 0.0537 \text{ d}^{-1}$; $\mu_7 = - 0.0406 \text{ d}^{-1}$. Thus, the inhibitory properties of the anion HSO_3^- at given concentration values are obvious.

Subsequently, we studied the effect of anions NO_3^- and H_2PO_4^- on the dynamics of microalgae

growth. Based on the results of the experimental data and the calculated values, graphical dependences of the change in the concentration of microalgae cells on the time at the corresponding concentrations of anions NO_3^- and H_2PO_4^- in wastewater under the conditions of their single introduction Fig. 3 and Fig. 5 were obtained. Analyzing the data (Figs. 3, 5), it should be noted that the growth of microalgae cell biomass over time also significantly depends on the anions NO_3^- and H_2PO_4^- concentration compared to the control where anions NO_3^- and H_2PO_4^- are absent. With the increase of anions NO_3^- and H_2PO_4^- concentration, microalgae cell growth is increased compared to the control. Such an increase in the values of the microalgae concentrations, only to a certain

value of the concentration of the available anions NO_3^- and H_2PO_4^- . As can be seen in Fig. 3, at the anion NO_3^- concentration, 68 mg/ml (4th sample) on the second day behaves in the same way as others, it means that the adaptation phase takes place up to two days, with a slight increase starting on the third day, which is higher on the 5th day than in the control sample, but from the sixth day there is a decline and the next five days there is a decrease in growth. At higher upper values of anion NO_3^- concentrations, there is a dying of microalgae compared to the control.

The values of the growth coefficients - μ were found similarly, by the same methodology and they were: 0.075 d^{-1} ; 0.076 d^{-1} ; 0.077 d^{-1} .

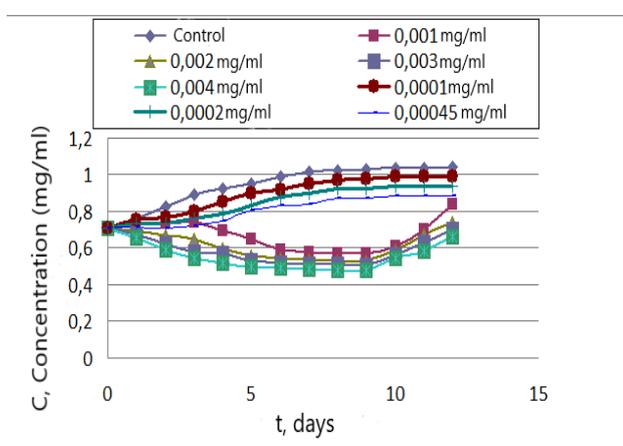


Fig. 1. The change in the concentration of microalgae cells over time at the corresponding values of anion HSO_3^- concentrations

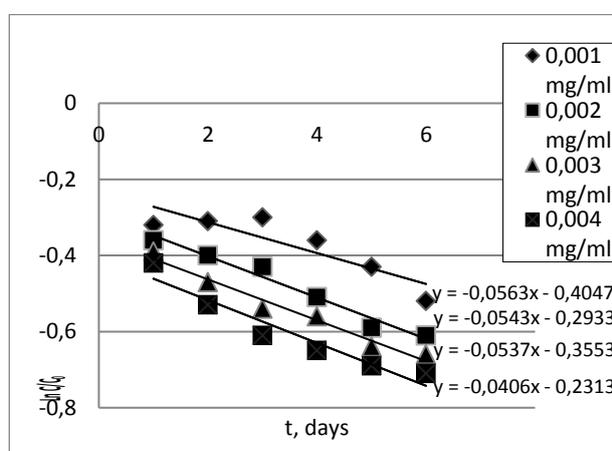


Fig. 2. The dependence of the logarithm change of the microalgae cells concentration on time at the corresponding anion HSO_3^- concentrations

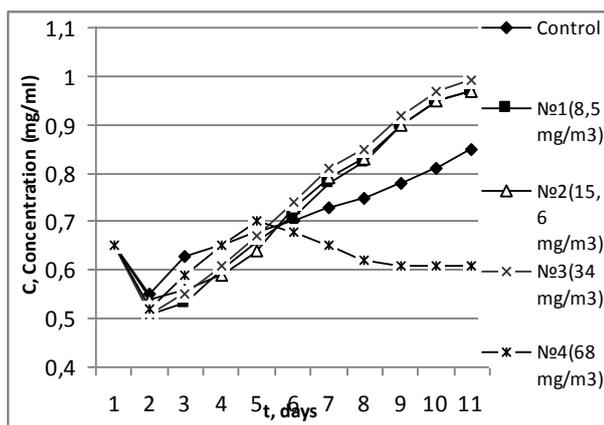


Fig. 3. The dependence of the change in the concentration of microalgae cells over time at the corresponding anion NO_3^- concentrations

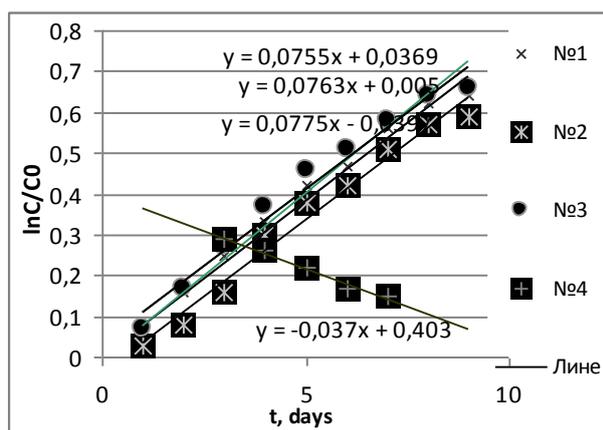


Fig. 4. Dependence of change in logarithm of suspension concentration of microalgae cell on time (at corresponding anion NO_3^- concentrations)

Concerning anion H_2PO_4^- , a similar pattern is observed only with other concentrations. As we can see (Fig. 5), the fifth sample, where the concentration

is 0.1 mg/m^3 , negative growth of microalgae biomass is observed. This indicates that this concentration of anion H_2PO_4^- has a detrimental effect on the

microalgae growth. At the same time, at smaller values of anion $H_2PO_4^-$ concentrations, there is an increase in the biomass of microalgae cells compared to the control. The main parameter is the value of the growth coefficients – μ which was determined

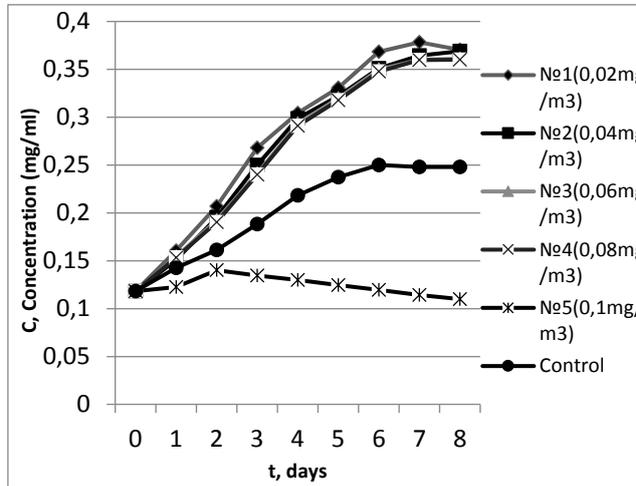


Fig. 5. The dependence of the change in the concentration of microalgae cells over time at the corresponding anion $H_2PO_4^-$ concentrations

For the analytical calculation of the optimum values of anions concentrations in wastewater to be purified using chlorophyll synthesizing microalgae, a mathematical model of microalgae biomass growth depending on the concentration of the corresponding anions was created:

$$\begin{cases} \frac{dC}{dx} = \mu_1 C - \mu_2 C \\ \frac{dC}{dx} = \mu_1 C; \\ x = 0, C = C_0; \end{cases} \quad (1)$$

where, x is concentration of anions HSO_3^- , NO_3^- and $H_2PO_4^-$; μ_1 , μ_2 are growth coefficients at favorable and unfavorable values of anions HSO_3^- , NO_3^- and $H_2PO_4^-$

$$x_{max} = \frac{\ln \mu_2 - \ln \mu_1}{(\mu_1 + \mu_2)} = \frac{\ln(-0,037) - \ln 0,076}{(0,076 - 0,037)} = 18,46 \text{ mg/m}^3; \quad (3)$$

Similarly, using equation (2), we calculate the optimal value of anion $H_2PO_4^-$ concentration in the cultivation medium:

$$x_{max} = \frac{\ln \mu_2 - \ln \mu_1}{(\mu_1 + \mu_2)} = \frac{\ln(-0,0426) - \ln 0,2425}{(0,2425 - 0,0426)} = 0,061 \text{ mg/m}^3; \quad (4)$$

According to the equations 3 and 4, the concentration of anions NO_3^- and $H_2PO_4^-$ is determined at which the maximum value of the

microalgae biomass concentration in the culture medium is reached. To check the adequacy of the mathematical model and its solution, we build graphs

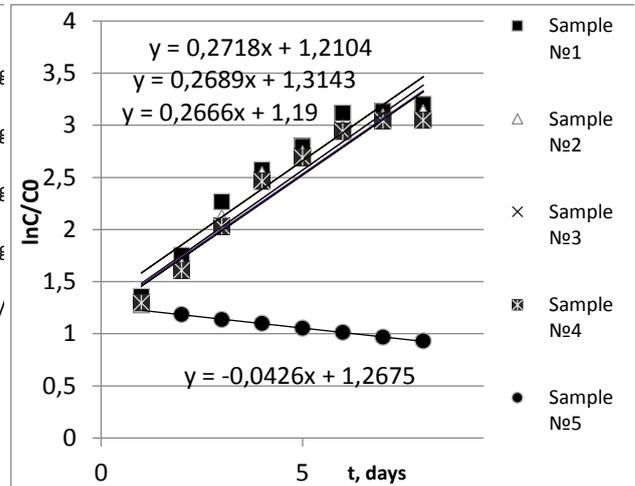


Fig. 6. Dependence of change in logarithm of suspension concentration of microalgae cells on time (at corresponding anion $H_2PO_4^-$ concentrations)

concentration; C is the concentration of microalgae in the cultivation medium.

Its solutions have several analytical expressions. One of them allows you to calculate the critical values of the corresponding anion concentrations according to the known values of the growth coefficients μ ;

$$x_{max} = \frac{\ln \mu_2 - \ln \mu_1}{(\mu_1 + \mu_2)}; \quad (2)$$

Using the data of mathematical processing of the experimental study results of the microalgae biomass growth, the corresponding values of the growth coefficients were calculated μ_1 , μ_2 . After substituting the obtained values for the anions NO_3^- and $H_2PO_4^-$ in equation (2) we calculate the optimum value of anion NO_3^- concentration in the cultivation medium:

of the dependence of anions NO_3^- and H_2PO_4^- concentration on the growth coefficient μ .

From (Figs. 7 and 8) it is seen that the maximum increase in the concentration of microalgae is achieved at the concentration of anion $\text{NO}_3^- \approx 18\text{mg}/\text{M}^3$ and anion $\text{H}_2\text{PO}_4^- \approx 0,06\text{mg}/\text{M}^3$

concentration. This indicates that the mathematical model accurately describes the course of the study process, and the obtained solutions will allow us to predict equipment for the implementation of wastewater treatment technology under the presence of anions NO_3^- та H_2PO_4^- (Fig. 9).

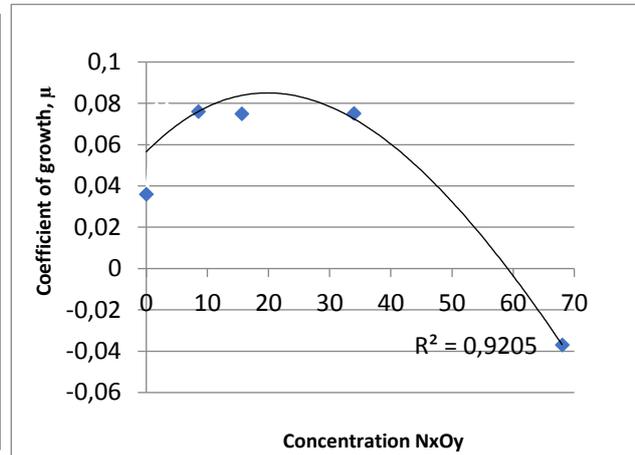
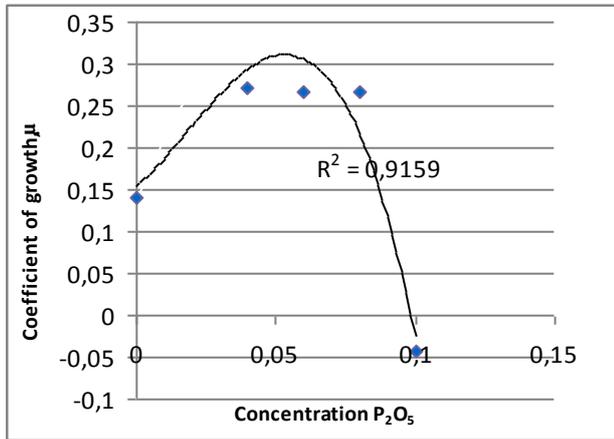


Fig. 7. Dependence of the microalgae growth coefficient μ on the concentration of anions H_2PO_4^-

Fig. 8. Dependence of the microalgae growth coefficient μ on the concentration of anions NO_3^-

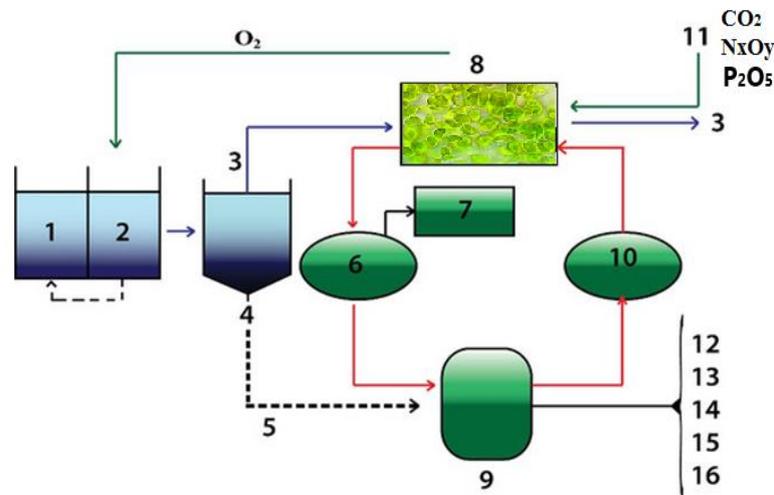


Fig. 9. Combined wastewater treatment scheme using Chlorella type microalgae.

- 1 – anoxide zone; 2 – oxide zone; 3 – wastewater after the process with activated sludge;
- 4 –secondary sedimentation tank; 5 – precipitate secondary sedimentation tank;
- 6 – microalgae biomass; 7 – tank; 8 – cultivation of the Chlorella type microalgae;
- 9 – hydrothermal liquefaction or anaerobic digestion; 10 – biogenes and CO_2 ;
- 11 – CO_2 , P_2O_5 and N_xO_y from other systems; 12 – biochar; 13 – protein/nutritional supplements;
- 14 – fertilizers; 15 – soil amendment; 16 – biofuels

Fig. 9 presents a scheme of wastewater treatment using the Chlorella type microalgae. The scheme consists of a biofilm reactor with a movable layer of nozzle for air bubbling 1, and an open pond for the cultivation of microalgae as a biological reactor 2. Wastewater after the process with activated sludge 3, fall into the container 8,

where the cultivation of the Chlorella type microalgae happens. The wastewater sludge enters the secondary sedimentation tank 4 and from there the precipitate of the second sludge 5 enters the hydrothermal liquefaction or anaerobic digestion 9. Since microalgae cultivation requires CO_2 , there is an opportunity to use its emission at the

enterprises of other industries (for example, power plants), reducing the emission into the atmosphere. Therefore, CO₂, P₂O₅ and NxOy from other systems 11 fall into the tank 8 for the cultivation of microalgae. From tank 8, the biomass of microalgae enters tank 6, from which it enters tank 9 for hydrothermal liquefaction or anaerobic digestion, or tank 7 where organic “green” fertilizer is made from it. From tank 9 biogenes and CO₂ enter tank 10, and from it again into tank 8 of microalgae cultivation. Everything else after hydrothermal liquefaction or anaerobic digestion 9 is also converted into fertilizers 14, protein or nutritional supplements 13, soil amendment 15, and biofuels 16 (Khlorella..., 2020).

4. Conclusion

Chlorella microalgae are the most appropriate option for wastewater treatment. They are also a promising substrate for biofuel production since obtaining biomass creates the potential for energy production.

Based on the results of the experimental research, mathematical models have been created that allow predicting the maximum values of anions NO₃⁻ and H₂PO₄⁻ concentrations and at which effective wastewater treatment with chlorophyll synthesizing microalgae is possible.

The result of wastewater treatment by this method is the change of alkaline acid balance from acidic to neutral after the experiment.

The scheme of wastewater treatment using chlorophyll synthesizing microalgae of the Chlorella type is proposed. Limit values of concentrations of anions HSO₃⁻, NO₃⁻ and H₂PO₄⁻ are set for the successful operation of this treatment plant.

The use of the Chlorella type microalgae for wastewater treatment creates new opportunities for environmental safety improvement by designing and building a reliable system.

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