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DETOXIFICATION ANALYSIS OF AQUATIC ENVIRONMENT WITH BIOLOGICAL TESTING METHOD

The article is devoted to the problem of waste water clearing from heavy metals. For analyses of water detoxification it was examined a method of microalgae *E. gracilis* movement testing at light and dark conditions. It was shown that cells *E. gracilis* were the most active in organic-mineral media at light and more sensitive to heavy metals in dark. Velocity of cells motion and their life ability were decreased with increasing of heavy metals Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cr^{6+} concentration from 10^{-7} to 10^{-2} M and time of testing in water medium. Biological testing of cells movement makes it possible to appreciate in for 15–30 min a level of waste water detoxification at different stages of technological water cleaning.

Introduction. Toxicity is one of the key indicators of ecological safety of aquatic environments and efficiency of treatment facilities. Such hardly decomposed organic substances as oil, pesticides, cleaning and disinfecting agents and some inorganic contaminants such as heavy metals, nitrates, phosphates, etc. can be found among the hazardous pollutants in urban wastewater. Their content in the wastewater is normalized and monitored daily at treatment plants [1].

The most dangerous and most common wastewater contaminants are heavy metals which have toxic effects on activated sludge microorganisms. The content of some heavy metals entering the city sewage treatment plant varies from 1 to 10 maximum concentration limit (MCL), and in the case of volley emissions can reach 100 MCL or more [1, 2].

To monitor the content of heavy metals in sewage chemical, physicochemical and physical methods of analysis are commonly used. However, they only indirectly allow to judge the toxicity of wastewater by the calculated total toxicity index (IT):

$$\mathrm{IT} = \sum(C_i / \mathrm{MCL}_i), \qquad (1)$$

where C_i, MCL_i are current and maximum allowable concentrations of separate heavy metals [3].

Analysis of wastewater on the elemental composition does not give adequate assessments of its safety and do not take into account the effects of antagonism, additivity, synergistic action of substances.

Increasing number of pollutants entering the aquatic environment in the result of human activities, as well as their interaction with formation of new compounds, sometimes more toxic than the analyzed compound require a large number of individual methods of chemical analysis for each substance.

The complexity and duration of the chemical analysis and the high cost of equipment by using physical methods of control reduce their economic efficiency and require of bio-toxicity testing of the aqueous medium.

For screening of wastewater toxicity the lower forms of living organisms are used. Such microorganisms as bacteria, microalgae, protozoa are the most attractive [3].

The level of aquatic toxicity is conducted on its impact on the integral functions of cell cultures, their reproduction, respiration, heat production, motility and others.

One of the highly sensitive bioindicator characterizing viability of mobile forms of microorganisms is their motility. It reflects the behavioral responses of microorganisms for the presence of toxic substances and is associated with cell bioenergetics.

Microalgae *E. gracilis* is a single-celled organism, widely spread in fresh water and engaged in the process of aquatic environment self-purification, as well as exhibiting a high sensibility to its contamination.

The main property of these organisms is their ability to mixotrophic nutrition. In the light, they behave itself as autotrophic organisms using photosynthesis. In the dark, *E. gracilis* cells convert to heterotrophic type of nutrition and behave itself like the protozoa [4].

In the context of bio-testing of water environment the change in the relative velocity of cells movement can serve as one of indicators of water safeness.

Main part. The aim of this work was to check up the possibility of assessing the degree of detoxification of wastewater in urban wastewater treatment plants by bio-testing motility of test culture *E. gracilis*.

As the objects of study, the samples of wastewater of Minsk treatment plant (MTP-1, MTP-2) were used. They were selected at the entrance to the shop of mechanical cleaning, after gratings, sand traps, in primary settling tanks, in the sections of the aeration tank, secondary sedimentation tanks and in the places of discharge of treated wastewater into the river Svisloch.

Samples of wastewater were collected in accordance with GOST R 51592–2000 “Water. Gen-

eral requirements for sampling", PND F 12.15.1–08 "Guidance on sampling for the analysis of wastewater".

Selected samples of wastewater were filtered through the paper filter and used for biological testing of their toxicity on the changing of motion activity of microorganisms.

Microalgae *E. gracilis* from the collection of the department of biotechnology and bio-ecology of BSTU were used as a test culture.

Such salts of heavy metals as $FeSO_4$, $CuSO_4$, $ZnSO_4$, $CoC1_2$, $NiC1_2$, $CdC1_2$, K_2CrO_4 were emploied to test the presence of toxic substances in the model aquatic environment. Concentration of heavy metal ions in the samples was varied in the range 10^{-8}–10^{-1} M.

Collectible culture of cells *E. gracilis* was plated in mineral nutrient Lozino-Lozinski (LL), as well as in organic media: nutrient broth (NB), the wort broth (WB), a mixed organo-mineral (OM), medium (LL:NB). It was grown for 3 days in Erlenmeyer flasks in the light at 1000 lux of illumination and in the dark at (20 ± 2)°C. The content of the cells was determined by using a Goryaev's camera and a light microscope "Biolam" [5].

For biological testing of water toxicity, cells in the logarithmic growth stage were used. Analyzing the samples toxicity, 1 ml of 3-day culture of the tested *E. gracilis* cells was added to 9 ml of the tested aqua medium, samples were incubated for 15 min at room temperature in the dark or light, and the velocity of cells movement was measured under a microscope [6].

The relative motility (B) of the cell test culture in the presence of toxicants was characterized by value:

$$B = v_i / v_k, \quad (2)$$

where v_k, v_i are the average velocity of cell's swimming in an aqueous medium in the absence and presence of toxic substances, respectively.

$$v_{i,k} = l / t_{i,k}, \quad (3)$$

where l – length (travel path), mm; $t_{i,k}$ – travel time between the fixed markers, c.

The aquatic toxicity degree (T, %) was determined according to a formula:

$$T = (v_k - v_i) / v_k \cdot 100. \quad (4)$$

Averaging of readings of the cells motion activity was performed by $n = 10$ measurements. The obtained data were statistically processed via Microsoft Excel software.

At the start of the research the nutrient media suitable for growing *E. gracilis* test culture in the dark and in the light was selected. The cells culture medium after 3-day cultivation under illumination and in the dark was used as a criterion for the selection.

Fig. 1 presents data on the changes in the content of *E. gracilis* cells when cultured in nutrient media in the light and in the dark

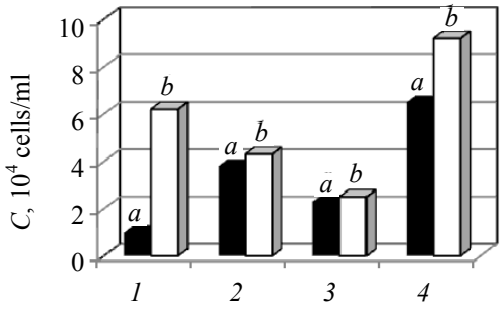

Fig. 1. *E. gracilis* cell concentration after 3-day cultivation in the light (*a*) and in the dark (*b*):
1 – mineral nutrient Lozino-Lozinski; *2* – NB;
3 – WB; *4* – mixed organo-mineral medium

According to the Fig. 1 the mineral medium LL is good for growing *E. gracilis* cells in the light, but is poorly suited to their cultivation in the dark. Organic medium NB and WB provided approximately the same growth activity of cells in the dark and in the light, but less than the LL environment under illumination. The maximum cell growth activity of microalgae was marked for mixed OM medium in the light.

These results indicate that the OM medium is most valuable for *E. gracilis* cell multiplication both in the light and in the dark, and it was selected for further study.

At the second stage of the work, kinetics and concentration influence of individual ions of heavy metals on *E. gracilis* cell motility were studied.

Fig. 2 shows changes in the relative *E. gracilis* cell motility versus time in the presence of Cu^{2+}.

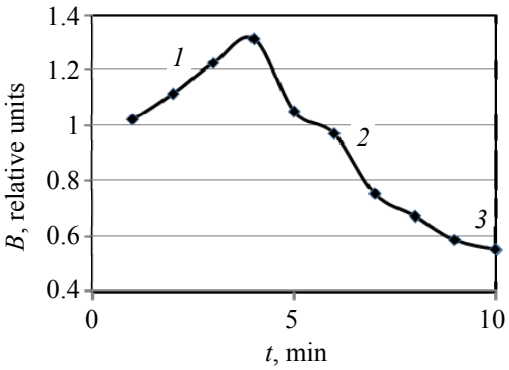

Fig. 2. Relative *E. gracilis* cell motility changes versus time in presence of Cu^{2+} ($C = 1 \cdot 10^{-3}$ M; 20 ± 2°C):
1 – activation area; *2* – inhibition area;
3 – stationary area

The motility of microorganisms in presence of other heavy metals in model conditions as well as

in the case of the analysis of wastewater was similarly changed.

This indicates the stress nature of the heavy metals action on cells and occurrence of transient processes.

The obtained data shows that for achieving a steady-state of B value changes in the result of stress response is necessary not less than 10 min. This time was chosen for cell motility testing for all analyzed samples.

Speed and nature of motility changes of cells also depend on the concentration of the toxicants impact.

Fig. 3 shows the results of variation of the relative motility of *E. gracilis* cells depending on the logarithm of Cr^{6+} concentration in the light and in the dark.

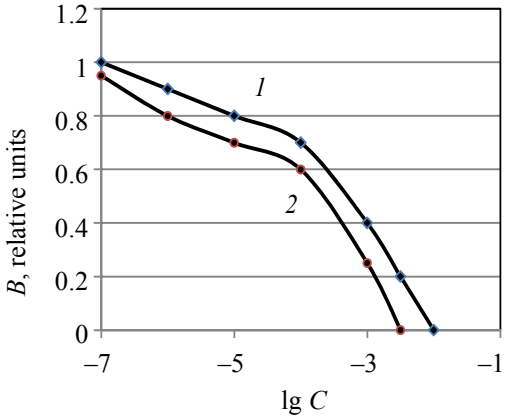

Fig. 3. Variation of the relative motility of *E. gracilis* cells depending on lg*C* ions Cr^{6+}, 20 ± 2°C: *1* – in the light; *2* – in the dark

It is seen from Fig. 3 that there is a two-stage cells mobility behavior in the presence of ions Cr^{6+} as in the dark as in the light. Similar dependences have been observed for other heavy metals.

Some decreasing of cells motion activity was observed in the result of increasing the concentration of heavy metal ions in the range from 10^{-7} to 10^{-4} M. The changes were reversible. It was indicated by the restoration of motility of microorganisms when they were transferred into pure nutritive medium.

At concentrations of 10^{-4}–10^{-1} M heavy metal ions of cobalt, nickel, cadmium, chromium have a toxic influence on microalgae *E. gracilis*.

With increasing concentrations of heavy metals above 10^{-4} M the character of the cells movement varied from straight to the rotational and up to a full stop.

The microalgae *E. gracilis* cells died at concentrations of investigated heavy metals 10^{-2} M and higher.

As it can be seen from Fig. 3, microalgae *E. gracilis* exhibited higher sensitivity to heavy metal ions in the dark rather than in the light. Differences in cell motility were increased with increasing concentration of the heavy metals- in an aqueous medium.

At the heart of the damaging effect of heavy metals on bioenergetics of microalgae *E. gracilis* in the dark is the impact on transport and respiratory systems of cells. In the light heavy metals also have an additional influence on the photosynthetic system of cells.

Higher resistance to heavy metals for *E. gracilis* cells cultured in the light, compared to cells grown in the dark may be associated with an increase in their power capabilities for the repair of damages caused by the heavy metals.

The obtained data may also indicate that the photosynthetic apparatus of cells of microalgae *E. gracilis* has a high resistance to heavy metal ions as compared to respiratory and transport system of cell activity, which requires additional verification.

Changes in the toxicity level of MTP-1 and MTP-2 sewage at different phases of their treatment were studied in the next stage of work. The results obtained in the process of analyzing of sewage detoxification while the cleaning process at MTP-1 and MTP-2 are given in the table.

Biological testing of wastewater toxicity using *E. gracilis* cells at different stages of cleaning in MTP-1 and MTP-2

Samples	Toxicity level, %	
	MTP-1	MTP-2
Sand traps	45.3 ± 3.0	40.2 ± 2.3
Primary settlers	43.0 ± 3.3	36.0 ± 2.8
Aerotanks	29.0 ± 1.9	21.0 ± 1.6
Secondary settlers	10.5 ± 0.8	5.6 ± 0.4

Knowledge of the B value makes it possible to evaluate toxicity level (T) of aquatic environments by measuring a motion activity of *E. gracilis* cells in accordance with (3) and (4).

When $T \leq 10\%$, water may be considered as nontoxic; when $10\% \leq T \leq 20\%$ – as slightly toxic; at $20\% \leq T \leq 50\%$ – as moderately toxic; $50\% < T$ – as highly toxic.

The table shows that at the input of MTP-1 and MTP-2 wastewater refers to the average level of toxicity and it was less toxic at MTP-2 rather than at MTP-1.

Grilles and sand traps slightly reduced the toxicity level of wastewater. More significant decrease in the level of water toxicity was observed

after the passage of primary settlers. Maximum sewage detoxification occurred in the sections of aerotanks of MTP-1 and MTP-2.

Detoxifying ability of activated sludge of aerotanks was 75% at the MTP-1 and 84% at MTP-2, indicating the main role of the activated sludge aeration tanks in the wastewater detoxification process in urban sewage treatment plants.

The toxicity level of water at the point of discharge into the river Svisloch according to the method of cells motility testing was (9.5 ± 0.6)%. It characterizes the discharged water as non-toxic and means that they correspond to the demands of normative and technical documentation.

Conclusion. The obtained results prove that the motion activity of *E. gracilis* cells in biological testing allows determining the level of acute chemical toxicity of aquatic environment for 15–30 min and evaluating the effectiveness of wastewater detoxification at different cleaning stages in urban wastewater treatment plants.

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