Improvement of the method of express bioindication by yeast fungi of environmental pollution with mercury using mechanical activation and electrophysical action

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Abstract. The main results of determining the presence of mercury in components of the environment by the developed method of express bioindication by yeast fungi based on the improvement of the existing, operating design of the installation are presented in the article. It was found that mercury salts contribute to the rapid release of carbon dioxide at any concentrations presented in the article if the culture medium is stirred, and an increase in the rate of CO₂ release by yeast fungi with constant electrophysical treatment of water, VFMS has been established. It has been established that when the culture medium is stirred with different concentrations of yeast fungi, with constant electrophysical treatment of water, mercury salts increase the rate of carbon dioxide evolution.

1. Introduction
Over the past few decades, volume of oil and gas production and processing has increased significantly [1, 2]. At the same time, stocks of the highest quality raw materials have significantly decreased. The development includes deposits enriched in heavy metals (mercury), which affect the environmental situation at the mining sites. Currently, there are no available, proven methods for controlling the amount of mercury in environmental components that meet such requirements as: high rate of determination of the presence of mercury in the total composition; the accuracy of the quantification; cheapness and ease of use of the method of determination, the use of which will improve measures to ensure the identification and control of sources of chemical hazard [3].

For this purpose, a method was previously developed for express bioindication by yeast fungi of the species *Saccharomyces cerevisiae* of environmental pollution with mercury, based on measuring the increase in the rate of fermentation of carbohydrate-containing compositions (culture media) by yeast when the concentration of a toxicant - a heavy metal (mercury cation) changes [1]. On the basis of the developed method, an assessment was made of the possibility of controlling this process by
engineering methods of exposure, in particular, reducing the toxic effect of mercury by the electrophysical effect of the MAG device on yeast fermentation.

2. Materials and methods

For experimental studies, 36% sugar solution (4±0.02) g and water (25 ml) at a temperature of (22±2)°C is used as a nutrient medium. Then yeast is inoculated into the nutrient medium, (2±0.02) g in each flask with an interval of 1-2 minutes, then the mixture is stirred with a glass rod for 25-30 seconds, and the flasks are connected to the developed installation. Measurements during the experiment are carried out with the same interval. When analyzing the results of previous experiments, it was found that the process will go faster and more clearly, provided that there is constant stirring of the culture liquid in the flask. The magnetic stirrer PE-6100 was chosen as the device for carrying out this process.

The experiment of series 1-1 was carried out in parallel on two elements of the installation and in two passes. The studies were carried out with the addition of the concentrations of the mercury salt HgCl$_2$: 69.4 mg/l (1:10) - I, 6.94 mg/l (1:100) - II, 0.694 mg/l (1:1000) - III, 0.0694 mg/l (1:10000) - IV, but already, setting the flasks with the culture liquid on magnetic stirrers. Namely, in the first run, the culture was seeded in nutrient media with HgCl$_2$ salt concentrations of 69.4 mg/l and 6.94 mg/l (curves I and II), respectively, in the first and second flasks, and in the second run, the culture was seeded in nutrient media with HgCl$_2$ salt concentrations of 0.694 mg/l and 0.0694 mg/l (curves III and IV). And also, at this stage, it was decided to add a little more boiled water to the nutrient medium, that is, not 25 ml, but 35 ml.

3. Results

Since the experiment was carried out in two stages, the calculation of mean values and errors was carried out for two stages, both in this experiment and in subsequent ones, because the possibility of their being carried out on different days is not excluded, that is, the results should not be compared. The results of experiment 1-1 are shown in Table 1 and Figure 1.

| Installation item numbers | No added HgCl$_2$ | Duration, min | After adding HgCl$_2$ |
|---------------------------|------------------|---------------|-----------------------|
|                           |                  | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
| I                         |                  | 10.17 | 9.68 | 5.08 | 5.56 | 6.06 | 6.20 | 6.28 |
| II                        |                  | 10.52 | 9.84 | 3.15 | 3.21 | 3.55 | 4.25 | 4.75 |
| Average $V_{avg}$         |                  | 10.35 | 9.76 | 4.11 | 4.39 | 4.80 | 5.23 | 5.52 |
| Error ($V_{avg} - V_{100}/V_{avg}$, %) |    | 1.74 | 0.82 | 23.6 | 26.88 | 26.04 | 17.51 | 13.77 |
| III                       |                  | 12.63 | 10.34 | 3.79 | 4.43 | 4.69 | 5.08 | 5.15 |
| IV                        |                  | 11.53 | 10.0 | 4.82 | 5.08 | 5.68 | 6.42 | 6.57 |
| Average $V_{avg}$         |                  | 12.08 | 10.17 | 4.35 | 4.75 | 5.16 | 5.75 | 5.86 |
| Error ($V_{avg} - V_{100}/V_{avg}$, %) |    | 4.55 | 1.67 | 10.57 | 6.95 | 9.69 | 11.65 | 12.12 |
It should be noted that when HgCl$_2$ salt is added to the flask after a sharp drop in the rate of CO$_2$ evolution, it slowly increases, which means that mercury chloride has a beneficial effect on the development of yeast and does not show its toxic properties. Also, possible reasons that caused an increase in the rate of CO$_2$ release may be: either the rapid reproduction of yeast fungi in the culture medium, or their rapid use of sugar as an element of the nutrient medium.

Based on the data obtained during the research, it was concluded that it is impossible to compare the results of experiments obtained on different days due to the possible influence of changes in environmental conditions on the vital activity of yeast during fermentation. It was decided to produce further results for comparison and discussion at the facility strictly in parallel.

In a series of experiments I-1, HgCl$_2$ salt was replaced with sodium chloride NaCl. Moreover, it was taken the same amount of Na$^+$ ions as the previously used amount of Hg$^{2+}$ ions, that is, it is necessary to dissolve 0.267 g of NaCl in 0.5 liter of water or 0.534 g per 1 L and, in the same way, distribute into four concentrations, like the mercury salt HgCl$_2$. This will correspond to the same mole quantity of cations of these salts in the nutrient medium in different experiments.

**Figure 1.** Results of measuring the rate of CO$_2$ release by yeast inoculated into a nutrient medium containing HgCl$_2$ (the culture medium is stirred).

**Figure 2.** Results of measuring the rate of CO$_2$ release by yeast inoculated into a nutrient medium containing NaCl salt (stirring in the flask is performed with magnetic stirrers).
The experiment was carried out using the same magnetic stirrers, that is, with constant stirring, and also in two passes: in the first case, the culture was inoculated into nutrient media with NaCl salt concentrations of 14.8 mg/l and 1.48 mg/l (curves I and II), respectively, in the first and second flasks, and in the second case, the culture was inoculated into nutrient media with NaCl salt concentrations of 0.148 mg/l and 0.0148 mg/l (curves III and IV).

The results of experiment 1-2 are shown in the form of curves in Figure 2.

According to the results of the data in Figure 2, it was found that NaCl salt with a concentration of 14.8 mg/l has the worst effect on yeast development, in contrast to HgCl₂ with the same concentration, and gives a slight decrease in the rate of CO₂ release until the 25th minute, but after that, an increase of speed occurs, which means there is an improvement in the vital activity of yeast [4]. But the experimental error remains high enough for the first concentrations - 18.29% and already becomes low for III and IV concentrations - 1.83%, which is possibly explained by the influence of various changes in temperature or pressure in the laboratory on the fermentation process.

Then the experiment of the second series 2-1 was carried out with Hg(NO₃)₂ salt, in which, at the first run, the culture was inoculated into nutrient media with Hg(NO₃)₂ salt concentrations of 85 mg/l and 8.5 mg/l, respectively, in the first and in the second flask, and in the second run, the culture was inoculated into nutrient media with Hg(NO₃)₂ salt concentrations of 0.85 mg/l and 0.085 mg/l. Moreover, by weight, we took 1.53 g of Hg(NO₃)₂ salt per 0.5 L of water, so that the amount of mercury cation in nitrate in a mole ratio was the same as the amount of mercury cation in chloride.

The results of experiment 2-1 are shown in Figure 3.

![Figure 3](image)

Figure 3. Results of measuring the rate of CO₂ release by yeast inoculated into a nutrient medium containing Hg(NO₃)₂ salt.

Based on the analysis of the data in Figure 3, it was found that mercury nitrate Hg(NO₃)₂ added to the culture liquid with concentrations of 85 and 8.5 mg/l (curves I and II, respectively) in solution does not exhibit toxic properties and contributes to a high rate of carbon dioxide evolution compared to curves III and IV (concentration 0.85 and 0.085 mg/l, respectively), which reflect a decrease in the rate of CO₂ evolution.
The error in the first run turned out to be higher than the desired norm (5%) and amounted to 8.17%, the reason again may be not the best conditions for yeast growth. Although in the second run, the error was 0.57%, which confirms the reliability of the results.

Further, in a series of experiments 2-2, Hg (NO₃)₂ salt was replaced by sodium nitrate NaNO₃. Moreover, it was taken the same the amount of Na⁺ ions as the previously used amount of Hg²⁺ ions, that is, it is necessary to dissolve 0.41 g of NaNO₃ in 0.5 L of water or 0.82 g per 1 L. And this will correspond to the same mole quantity of cations in the nutrient medium in different experiments.

The experiment was also carried out in two runs: in the first run, the culture was inoculated into nutrient media with NaNO₃ salt concentrations of 22.7 mg/l and 2.27 mg/l, respectively, in the first and second flasks, and in the second run, the culture was inoculated into nutrient media with NaNO₃ salt concentrations of 0.227 mg/l and 0.0227 mg/l.

The results of experiment 2-2 are shown in Figure 4.

![Figure 4](image)

The analysis of Figure 4 shows that sodium nitrate NaNO₃ added to the culture liquid contributes to an increase in the rate of carbon dioxide evolution after 20 minutes, that is, it has a positive effect on the growth and development of yeast within the specified concentration range. The experimental error in both runs also turned out to be below the desired limit of 5% and amounted to 1.91% in the first case and 0.93% in the second, which gives the right to call the results reliable.

The study of the influence of electrophysical effect on the fermentation process was carried out in order to study the influence of the variable frequency-modulated signal (VFMS) of the "MAG" device on the bioactivity of microorganisms in the culture medium. Moreover, VFMS processing was carried out in two stages: at the first stage, the fermentation process in one flask was directly processed, the second was without processing by the "MAG" device; at the second stage, the same was done to confirm the result. It was necessary to find out what effect (negative or positive) VFMS has on the vital activity of microorganisms.

Water treatment by VFMS was carried out in one version - continuously operating [5]. For continuous treatment of water (i.e., the aqueous phase in a colloidal solution of sugar with a volume of 35 ml with a microbiological medium), VFMS was applied to a strip of foil sealed with abundant,
biologically inert lubricant in a thin section of the flask connected to the installation. In this case, one end of the strip, 15x180mm in size, is immersed in the liquid in the flask to a depth of (5±1) mm; the other is taken out of the thin section and connected to the clamping contact of the “MAG” device. The experiment was carried out by wrapping both flasks (a flask with treated water and a flask without treatment) in metal foil 130x400 mm in size.

The experiment was carried out with the isolation of the flask from external magnetic influence by wrapping in foil, simultaneously with the direct processing of the fermentation process by electrophysical influence. VFMS connection in the experiment occurs at 20 minutes.

The results of the experiment are shown in Table 2 and Figure 5.

**Table 2. Results of measurements of the rate of carbon dioxide release in the study of the effect of electrophysical treatment**

| Installation item numbers | Duration, min |
|---------------------------|---------------|
|                           | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
| I (treated)               | 9.80 | 7.72 | 7.84 | 7.98 | 8.45 | 8.82 | 10.50 |
| II (untreated)            | 8.70 | 7.55 | 8.50 | 8.89 | 8.96 | 9.09 | 10.76 |
| Average $V_{avg}$         | 9.25 | 7.64 | 8.17 | 8.44 | 8.71 | 8.96 | 10.63 |
| Error ($V_{avg}$ - $V$)/$V_{avg}$, % | 5.95 | 1.18 | 4.04 | 5.45 | 2.99 | 1.56 | 1.22 |

**Figure 5.** Results of measuring the rate of CO$_2$ release by yeast, seeded in a nutrient medium under the influence of electrophysical effects.

Comparing curves I and II in Figure 5, it can be concluded that the electrophysical treatment of the culture liquid when wrapped in a metal plate (foil) leads to a temporary increase in the rate of CO$_2$ evolution (the experimental error is only 1.22%), and after 20 minutes of the rate carbon dioxide emissions become almost the same.

It follows from this that the effect of VFMS takes place and has a positive effect on the bioactivity of microorganisms in the culture medium. But, insulating the flasks in foil from external conditions (light, temperature, pressure), possibly, adversely affects microorganisms (yeast), but can be used to purposefully control the fermentation processes.

At the second stage, based on the data in Figure 5, the result did not change; the effect of VFMS takes place and has a positive effect on the growth and development of yeast.

Thus, in order to refine the operating procedure, optimize the technical details of the installation, the measurement process, a series of experiments was carried out to study the following factors affecting the vital activity of the yeast *Saccharomyces cerevisiae* and the possibility of their use for bioindication: the effect of various concentrations of heavy metal, anionic background (mercury
nitrate) with mechanical activation (stirring) of the culture medium and without it; electrophysical impact as a possible engineering method of process control.

It was also found that salts NaNO₃ and NaCl contribute to the rapid release of carbon dioxide at any given concentration from 0.0148 to 14.8 mg/l for NaCl and from 0.0227 to 22.7 mg/l for NaNO₃. These also include the mercury salt HgCl₂ with concentrations from 0.0694 to 69.4 mg/l, added to the flasks where the culture medium is being stirred.

Continuous treatment of water with VFMS, which is subsequently used to prepare the culture medium, shows an increase in the rate of evolved CO₂ by 1.22%. Thus, the use of the “MAG” device contributes to a more significant effect based on the “magnetic memory” of water.

4. Conclusion

The studies carried out reveal new possibilities for determining the presence of mercury in the components of the environment, are relevant in assessing the problems of anthropogenic impact on nature, in the search for methods to control environmental pollution. The researchers can be useful in the disinfection of domestic and industrial wastewater [6, 7], in the monitoring of environmental indicators to support decision-making at various national and regional levels [8-12].

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