Express bioindication of environmental pollution with mercury using yeast fungi

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Abstract. The issues of external influence on biological objects have interested mankind since ancient times. It can significantly affect vital activity of yeast cells. Their use for bioindication and quantitative determination of the rate of gas emission depending on composition of culture liquid makes it possible to use this process for bioindication of environment. In the article, on the basis of a developed machine for measuring the rate of carbon dioxide emission by yeast fungi, which makes it possible to use it in laboratory and field conditions, the values of the effect of mercury on the process of fermentation of culture medium by yeast are given. A method for express bioindication by yeast fungi of environmental pollution with mercury has been worked out. It serves as the basis for development of guidelines for identifying and controlling sources of chemical hazards during emergency response. The experiments have shown that the maximum effect on bioactivity of yeast is exerted by the HgCl₂ salt with different concentrations, which leads to a decrease in the rate of CO₂ emission and deterioration of yeast vital functions.

1. Introduction
Vapors of mercury and its compounds are highly toxic substances belonging to thiol poisons [1, 2]. The spillage of mercury leads to an increase in the evaporation area, its saturation with air vapor, which negatively affects ecological situation [3]. If ingested, they can lead to serious consequences.

The main research task is the development of a method for rapid bioindication by yeast fungi of environmental pollution with mercury in connection with expected increase in environmental pollution with mercury due to the lack of reliable systems and institutions for mercury disposal. An installation of expert bioindication has been developed and a method for studying the rate of the process of fermentation of carbohydrate-containing compositions (culture media) by yeast is proposed.

The main object of research is yeast fungus (for the process of sugar fermentation by yeast) from powdered yeast (TU 9182-001-48975583-2000). Powdered yeast was cosen due to the long shelf life of powdered yeast, which is 6 months. Thus, better reproducibility of results is ensured than, for example,
when using "fresh" yeast, which is stored for no more than 10 days. Boiled and tap water was used as a liquid phase, due to the fact that it is more accessible and inexpensive for conducting both laboratory experiments and for practical use, and boiled water is of interest because of its chemical "purity" [4, 5]. The nutrient medium is an aqueous solution of sugar (GOST-21-94) with a concentration of 36% of the mass.

2. Methods and materials

Mercury salt, hydrochloric acid GOST TU 2624-009-48438881-07, was chosen as toxicants of the culture liquid.

The nutrient medium is a 36% solution of the following composition: 

\[(4 \pm 0.02) \text{ g of sugar per 25 ml of water brought into temperature equilibrium (22} \pm 2) ^\circ \text{C with room atmosphere. After preparation of the nutrient medium, yeast is inoculated at (2} \pm 0.02) \text{ g in each flask with an interval of 1-2 minutes, then the mixture is stirred with a glass rod for 25-30 seconds, and the flasks are connected to the developed installation (figure 1). Measurements during the experiment are carried out with the same interval.}\]

![Figure 1. Laboratory setup for measuring the output of carbon dioxide during fermentation (one element out of four is shown, fasteners are not conventionally shown). 1 – burette with two-way stopcock, 2 – flask with bottom discharge, 3 – conical flask, 4 – Hofmann clamp, 5 – conical plug.](image)

To simplify the research process, the installation was modified by abandoning a flask with bottom discharge, a burette with a two-way valve and a conical flask, as well as replacing gas burette and hydrostat with a foam flow meter, thus, instead of 4 gas burettes, 4 foam flow meters were installed ("soap dishes ").

The research process in a laboratory machine consists of several stages.

1. Preparation of a laboratory machine:

   - Before starting the experiment, four weighed portions of sugar and yeast are prepare (I, II, III, IV);
   - The plastic jars connected to each foam flow meter are filled with soapy water (detergent to water ratio 1: 5).
   - A weighed portion of sugar is poured into each flask, 25 ml of boiled water is added, as a result of which a colloidal solution of sugar is obtained, a nutrient medium for yeast.
   - A pre-taken weighed portion of yeast is sowed into nutrient medium. Received mixture is stirred with a glass rod for 25-30 seconds.
   - Immediately after that, the flask is connected to the machine through a thin section with a branch pipe, the time of beginning of fermentation process is recorded, the time for the first and subsequent measurements is noted and proceeds to the preparation of the next section.
   - If necessary, required amount of toxicant can be injected into the flask using a syringe with a needle through a glass stopper with a cut, closed with a rubber tube.
2. Fermentation process:

- After the flask of this section is connected to the machine, carbon dioxide released during fermentation in the flasks passes through the connecting pipes, entering the flow meter.
- The time a soap ring passes 1 cm$^3$ of the flow meter volume every 5 minutes is measured. Thus, we determine the rate of carbon dioxide evolution. In total, the experiment lasts 40 minutes.

To assess reproducibility of the results during parallel experiments and to determine magnitude of error, the experiment was carried out on all four elements of the machine. To do this, in the experiment marked as 1-1 (a series of experiments 1 - devoted to assessing reproducibility), I, II, III, IV - the culture was seeded in a nutrient medium based on boiled water, respectively, on I, II, III and IV- th element of the laboratory machine.

3. Results

The results of experiment 1-1 are shown in tables 1-3 and in figure 2.

**Table 1.** Results of measurements of carbon dioxide emission rate in the study of reproducibility of the results.

| Machine block numbers | Duration, min |
|-----------------------|---------------|
|                       | 20  | 25  | 30  | 35  | 40  |
| I                     | 3.37| 3.57| 3.4 | 3.33| 3.53|
| II                    | 4.13| 3.97| 3.9 | 3.43| 3.68|
| III                   | 2.7 | 3.16| 2.87| 2.56| 2.95|
| IV                    | 3.32| 4.0 | 3.33| 3.03| 3.49|
| Average value $V_{av}$| 3.38| 3.67| 3.39| 3.08| 3.41|
| Error ($V_{av}$ - $V$), cm$^3$ | 0.38 | 0.32 | 0.31 | 0.30 | 0.23 |
| Error ($V_{av}$ - $V$)·100/ $V_{av}$, % | 11.24 | 8.72 | 9.14 | 9.74 | 6.82 |

**Table 2.** Assessment of inaccuracy between I and II machine blocks.

| Machine block numbers | Duration, min |
|-----------------------|---------------|
|                       | 0          | 5          | 0          | 5          | 0          |
| I                     | 3.37       | 3.57       | 3.4       | 3.33      | 3.53      |
| II                    | 4.13       | 3.97       | 3.9       | 3.43      | 3.68      |
| Average value $V_{av}$| 3.75       | 3.76       | 3.65      | 3.38      | 3.61      |
| Error ($V_{av}$ - $V$), cm$^3$ | 0.38 | 0.21 | 0.25 | 0.05 | 0.075 |
| Error ($V_{av}$ - $V$)·100/ $V_{av}$, % | 10.13 | 5.59 | 6.85 | 1.48 | 2.08 |

**Table 3.** Assessment of inaccuracy between I and IV machine blocks.

| Machine block numbers | Duration, min |
|-----------------------|---------------|
|                       | 20  | 25  | 30  | 35  | 40  |
| I                     | 3.37| 3.57| 3.4 | 3.33| 3.53|
| IV                    | 3.32| 4.0 | 3.33| 3.03| 3.49|
| Average value $V_{av}$| 3.35| 3.78| 3.34| 3.18| 3.51|
| Error ($V_{av}$ - $V$), cm$^3$/ min. | 0.025 | 0.23 | 0.01 | 0.15 | 0.02 |
| Error ($V_{av}$ - $V$)·100/ $V_{av}$, % | 0.75  | 6.08 | 0.30 | 4.72 | 0.57 |
Figure 2. Results of measuring the rate of CO$_2$ emission by yeast inoculated into a nutrient medium based on boiled water. X - speed, cm$^3$/min, Y - duration, min I II III IV

As a result, a stable slow decrease in CO$_2$ emission rate was obtained by yeast inoculated into a nutrient medium based on boiled water in a period of time from 25 to 35 minutes in elements I, II, III and IV of the machine.

In the analysis of this and subsequent experiments, error will be determined by 40 minutes of the experiment. The experimental error was found to be 6.82%, which exceeds the desired limit of 5%. The reason for the increased error is a small number of experiments carried out and their novelty.

When comparing curves, I and II, the experimental error is only 2.08%. Curve IV data are somewhat lower. The error is only 0.57%.

To assess the effect of concentrations of toxicant impurities on the process of sugar fermentation by yeast, experiments were carried out on 4 elements of the machine for:

- Determination of influence of concentrations of heavy metal of mercury with different anionic composition on vital activity of yeast.
- Selection of the optimal concentration of heavy metal for further research.

The second experiment, marked as 2-1 (a series of experiments 2 is devoted to assessing the effect of toxicants), in contrast to the first, was carried out when various concentrations of mercury hydrochloric acid were added to nutrient medium, mercuric chloride HgCl$_2$, respectively on elements I, II, III and IV laboratory machine. For this, a weighed portion of 1.25 g of HgCl$_2$ was dissolved in 0.5 L of water, and then 1 ml of this salt solution and diluted with 9 ml of water, obtaining a concentration of 69.4 mg/l (1:10). Obtained solution with a volume of 1 ml was diluted with 9 ml of water, thus obtaining a solution with a concentration of 6.94 mg/l (1: 100) and also two more concentrations 0.694 mg/l (1: 1000) and 0.0694 mg/l (1: 10000), which were then added to four flasks. All further salt concentrations were obtained in a similar way.

The results of Experiment 2-1 are shown in table 4 and figure 2.

Table 4. Results of measurements of carbon dioxide emission rate in the study of effect of toxicants.

| Machine elements numbers | 20  | 25  | 30  | 35  | 40  |
|--------------------------|-----|-----|-----|-----|-----|
| I                        | 2.23| 2.38| 2.97| 3.0 | 2.57|
| II                       | 2.69| 2.35| 2.24| 2.20| 2.01|
According to figure 3 and table 4, it can be seen that starting from 25 minutes, the rate of gas evolution on the II, III and IV elements of the machine steadily decreases slowly, that is, mercury begins to exhibit toxic properties, and on the I element, on the contrary, an increase in the rate is observed within time from 20 to 35 minutes.

Many mercury compounds are toxic. But in turn, toxicity of mercury compounds for living organisms is very different. These differences are due to the ability of many organisms to adapt to different concentrations of heavy metals in solution, as well as to its anionic composition. Acute toxic effect on microorganisms is manifested depending on sensitivity. In addition, physicochemical conditions of environment can significantly affect bioavailability of mercury compounds.

That is, the HgCl$_2$ salt with a concentration of 69.4 mg/l has a positive effect on vital activity of yeast for some time, as evidenced by an increase in the rate of CO$_2$ emission, but after 35 minutes the rate begins to decrease.

When comparing curves I and II, the experimental error is 12.23%, but this is much higher than in the first experiment. The error value when comparing curves I and IV is also higher than for the first time, it is 5.76%. Most likely, the difference in the error of experiments 1-1 and 1-2 by 30% is due to leaks in the machine hoses, as well as the presence of a toxicant in nutrient medium, or changes in climatic conditions (temperature, pressure) in the laboratory room in which the test was carried out were simply not taken into account.

4. Conclusion

Thus, a device for measuring the rate of carbon dioxide emission by yeast fungi has been developed, which makes it possible to use it not only in laboratory but also in field conditions [6].

A method for express bioindication of environmental pollution with mercury by yeast fungi, which requires 20-40 minutes to determine the presence of mercury has been developed. It can subsequently become the basis for the development of guidelines for identifying and controlling sources of chemical hazards during emergency response. A promising area of application of the developed method is ability to monitor state of areas adjacent to...
major highways, when deciding on the placement of agricultural land and other objects on them, where human activity may be subject to the risk of toxicological effects [7]. Waste recycling that could potentially contain mercury also needs to provide a safe environment for personnel and an appropriate risk assessment [8]. Mercury is not on the last place in anthropogenic transformation of water bodies [9].

It has been specified that presence of a mercury cation with different concentrations in the nutrient medium has a different effect on emission of carbon dioxide by yeast. As follows from these experiments: the HgCl$_2$ salt with concentrations of 6.94 mg/l, 0.694 mg/l and 0.0694 mg/l had maximum inhibitory effect on yeast bioactivity. At these concentrations, there was a decrease in the rate of CO$_2$ emission, which means that vital activity of yeast deteriorated.

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