

БІОЛОГІЧНІ ДОСЛІДЖЕННЯ

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THE USE OF ALGAE'S PHOTOSYNTHETIC ACTIVITY IN TOXICITY ASSESSMENT WITH THE PURPOSE OF CREATING PORTABLE DEVICES

The choice of test organisms and test reactions to be used in biotesting devices for wastewater toxicity, including portable ones, is caused by such factors as ease of cultivation and keeping test organisms in the laboratory, relatively high sensitivity to toxic substances, possibility of instrumental recording of physiological indicators used as test reactions on toxicity, a short time from the beginning of the toxicant action to the appearance of changes in the test reaction. Based on these criteria, we can assume that algae are a fairly convenient test organism for instrumental methods of biotesting since they have the a great deal advantages.

Purpose. To find the best options for assessing the photosynthetic activity of algae.

Methods. The polarography method.

Results. The authors have analyzed dependence of the main characteristics of the sensor on the structure of the diffusion layer and temperature and have found out that the optimal choice of the structure can be made depending on the biological object and experimental conditions. The research has shown that test reactions characterizing physiological state of algae are very diverse. This facilitates their choice for the purposes of instrumental toxicity biotesting, including biotesting toxicity of wastewater with portable instruments. Analysis of the experimental data has shown that it is possible to achieve the difference between concentrations of dissolved oxygen in a liquid culture before and after the exposure of the algae of 1-8 mg / l in a sufficiently short period of time by adjusting the density of algal cultures and light intensity. This indicates the fact that in principle it is possible to quantify photosynthetic activity of algae at short time intervals when exposed to light.

Conclusions. The most promising method for assessing the photosynthetic activity of algae is the polarography method, which makes it possible to develop a portable instrument for wastewater toxicity biotesting.

KEYWORDS: algae, photosynthetic activity, biotesting, wastewater, portable device

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ВИКОРИСТАННЯ ФОТОСИНТЕТИЧНОЇ АКТИВНОСТІ ВОДОРОСТЕЙ ЗАДЛЯ ОЦІНКИ ТОКСИЧНОСТІ З МЕТОЮ СТВОРЕННЯ ПОРТАТИВНОГО ПРИСТРОЮ

Створення ефективних стаціонарних пристроїв для біотестування токсичності є значним досягненням в області контролю якості стічних вод. Однак, поряд із стаціонарними пристроями, виникає нагальна потреба у створенні переносних або польових варіантів пристроїв (приладів) для інструментального

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контролю токсичності. Переносні пристрої такого типу могли б знайти широке застосування на тих підприємствах, де установка стаціонарних пристроїв недоцільна з технічних, експлуатаційних або економічних причин.

Мета. Знайти оптимальні варіанти для оцінки фотосинтетичної активності водоростей.

Методи. Метод полярографії.

Результати. Проаналізовано залежність основних характеристик датчика від будови дифузійного шару та температури і з'ясовано, що оптимальний вибір структури може бути зроблений залежно від біологічного об'єкта та умов експерименту. Дослідження показало, що тестові реакції, що характеризують фізіологічний стан водоростей, дуже різноманітні. Це полегшує їх вибір для цілей інструментального біотестування токсичності, включаючи біотестування токсичності стічних вод за допомогою переносних приладів. Аналіз експериментальних даних показав, що можна досягти різниці між концентраціями розчиненого кисню в рідкій культурі до та після впливу водоростей у концентрації 1-8 мг/л за досить короткий проміжок часу, регулюючи щільність культур водоростей та інтенсивності світла. Це вказує на той факт, що в принципі можливо кількісно оцінити фотосинтетичну активність водоростей через короткі проміжки часу при впливі світла.

Висновки. Найбільш перспективним методом оцінки фотосинтетичної активності водоростей є метод полярографії, який дає можливість розробити портативний прилад для біотестування токсичності стічних вод.

КЛЮЧОВІ СЛОВА: водорості, фотосинтетична активність, біотестування, стічні води, портативний пристрій

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ИСПОЛЬЗОВАНИЕ ФОТОСИНТЕТИЧЕСКОЙ АКТИВНОСТИ ВОДОРΟΣЛЕЙ ДЛЯ ОЦЕНКИ ТОКСИЧНОСТИ С ЦЕЛЮ СОЗДАНИЯ ПОРТАТИВНОГО УСТРОЙСТВА

Создание эффективных стационарных устройств для биотестирования токсичности является значительным достижением в области контроля качества сточных вод. Однако, наряду со стационарными устройствами, возникает насущная необходимость в создании переносных или полевых вариантов устройств (приборов) для инструментального контроля токсичности. Переносные устройства такого типа могли бы найти широкое применение на тех предприятиях, где установка стационарных устройств нецелесообразна по техническим, эксплуатационным или экономическим причинам.

Цель. Найти оптимальные варианты для оценки фотосинтетической активности водорослей.

Методы. Метод полярографии.

Результаты. Авторы проанализировали зависимость основных характеристик датчика от строения диффузионного слоя и температуры и выяснили, что оптимальный выбор структуры может быть сделан в зависимости от биологического объекта и условий эксперимента. Исследование показало, что тестовые реакции, характеризующие физиологическое состояние водорослей, очень разнообразны. Это облегчает их выбор для целей инструментального биотестирования токсичности, включая биотестирование токсичности сточных вод с помощью переносных приборов. Анализ экспериментальных данных показал, что можно достичь разницы между концентрациями растворенного кислорода в жидкой культуре до и после воздействия водорослей в концентрации 1-8 мг/л за достаточно короткий промежуток времени, регулируя плотность культур водорослей и интенсивности света. Это указывает на тот факт, что в принципе возможно количественно оценить фотосинтетическую активность водорослей через короткие промежутки времени при воздействии света.

Выводы. Наиболее перспективным методом оценки фотосинтетической активности водорослей является метод полярографии, который дает возможность разработать портативный прибор для биотестирования токсичности сточных вод.

КЛЮЧЕВЫЕ СЛОВА: водоросли, фотосинтетическая активность, биотестирование, сточные воды, переносное устройство

Introduction

Today the number of substances potentially polluting water bodies reaches 10 thousand and may increase in the future. It can be assumed with certainty that it is impossible to

fully solve the problem of water quality with the help of analytical tools, since in most cases analytical controls are designed to measure one or, at best, several parameters, characterizing

water quality. However, it is basically possible to control the presence of many substances in water quite accurately with the help of analytical tools. In practice, such control faces a number of difficulties, primarily of an economic nature.

In this regard, it is necessary to develop new approaches to the selection of water quality indicators and creation of fundamentally new technical means to monitor these indicators. One of the ways to improve the efficiency of natural and wastewater control is the development and use in practice of biological control methods and technical means to implement these methods. In contrast to the physical-chemical parameters, biological indicators generally characterize biological properties of water and in this case are integral indicators of biological effect of the substances in wastewater [1].

Analysis of Recent Studies Results

The choice of test organisms and test reactions to be used in biotesting devices for wastewater toxicity, including portable ones, is caused by such factors as ease of cultivation and keeping test organisms in the laboratory, relatively high sensitivity to toxic substances, possibility of instrumental recording of physiological indicators used as test reactions on toxicity, a short time from the beginning of the toxicant action to the appearance of changes in the test reaction. Based on these criteria, we can assume that algae are a fairly convenient test organism for instrumental methods of bioindication since they have the following advantages:

- numerous types of unicellular algae are easily cultivated in laboratory conditions on liquid mineral environment;

- physiological state of algae can be characterized by a fairly wide range of indicators, many of which are easily controlled by instrumental methods;

- single-celled algae have a short development cycle, which makes it possible to predict not only the short-term effects of the toxicants' action but also their action in subsequent generations of algae.

These factors allow to extensively use algae in toxicological studies in assessing the chronic and acute toxicity of wastewater and some chemicals. The authors [2] used the blue-

At present intensive studies are being conducted to develop effective methods for biological control of water quality, primarily industrial wastewater, as well as technical means to introduce these methods, suitable not only for research in laboratory practice, but also at industrial plants. As a rule, these devices are stationary and are designed for continuous monitoring of acute toxicity of wastewater. However, along with stationary devices, there is an urgent need to create portable or field versions of devices for instrumental monitoring of toxicity. Portable devices of this type could be widely used at the enterprises where the installation of stationary devices is impractical for technical, operational or economic reasons. In addition, these devices could be used by the authorities regulating utilization and protection of water resources for periodical monitoring of industrial wastewater toxicity.

green algae *Anabaena spiroides* to assess the toxicity of a number of heavy metals. The growth rate, intensity of photosynthesis and ion exchange between algae cells and the medium were chosen as test reactions. It was found that the most sensitive indicator of toxicity was the intensity of ion exchange. The threshold concentrations of metals causing metabolic imbalances of potassium and sodium were about 2 times lower than the accepted MPC. Such test reactions as the growth rate and photosynthesis of algae were less sensitive to the action of heavy metals. In the work [3], the effect of mercury and lead on the division of *Chlorella vulgaris* cells was investigated. It has been found out that concentration of metals, suppressing algae cell division by 50%, is 0.06 for cadmium, 0.18 for copper, 1.03 for mercury, 5.1 for zinc, and 1.0 mg / l for lead. The authors of the work [4] used blue-green algae to assess the toxicity of oil and oil products. As a result of the study it has been determined that photosynthesis of algae is completely inhibited when the concentration of petroleum products in the medium is from 0.1 to 1.0 g / l.

A number of researchers [5-7] used algae of various systematic groups to determine the toxicity of substances from the class of herbicides and insecticides, and heavy metals. In particular, [5] investigated the effect of

many herbicide-related substances on the *Chlorella* proto-cocca alga. It was determined that all the substances they studied had a toxic effect on algae in a concentration of from $1 \cdot 10^{-5}$ to $2 \cdot 10^{-7}$ M. The fact, that propanide causes a decrease in photosynthesis in the *Anabaena variabilis* in a concentration of 0.0025 mg / l has been defined in the work [6].

From the literature data we can draw the following conclusions:

- algae are widely used as test organisms in assessing the toxicity of chemicals and wastewater;

- algae are most sensitive to chemical compounds belonging to the class of herbicides and insecticides, as well as to heavy metals. This determines the possible scope of algae as test organisms in assessing the toxicity of wastewater categories containing these substances.

Methods for assessing the physiological condition of algae mainly used in the physiol-

ogy and biochemistry of plants, and which are not yet widely used in aquatic toxicology, are of considerable interest to scientists.

A number of researchers have claimed that the electrophysiological properties of plant cell membrane structures, including the outer membrane, the cell membrane, can serve as a very sensitive indicator of the functional state of the plant organism. Studies in this area have shown that in the presence of substances from the class of herbicides, heavy metals and a number of other compounds in incubation media, electrophysiological characteristics of membranes, such as electrical potential, conductivity, capacity, etc., change [8-11].

These examples show that test reactions characterizing the physiological condition of algae are very diverse, which facilitates their selection for the purposes of instrumental toxicity biotesting, including biotesting of sewage toxicity, using portable instruments.

Methodology Description

From the analysis of the literature data given in the previous section, we can conclude that when assessing the toxicity of wastewater, the following indicators of the physiological state of algae are most often used as test reactions:

- growth intensity (reproduction);
- electrophysiological characteristics of plant cell membranes;
- intensity of the photoinduced afterglow (delayed fluorescence);
- intensity of photosynthesis (photosynthetic activity);
- ion exchange.

Tests on changing pH of the incubation medium and the nature of algae movement are much less frequently used. It should be noted that the test reaction or toxicity test used in instrumental biotesting, must meet a number of specific requirements, which may be optional in classical aquatic toxicology. The main ones are as follows:

- possibility of instrumental registration of the reaction in continuous or discrete modes;
- possibility of quantitative registration;
- rather high sensitivity to toxicants;
- unambiguity, that is, the selected test reaction must unambiguously characterize the physiological states of the test object;
- low inertia, that is, the time from the beginning of the toxicant action to the appear-

ance of changes in the tested reaction should be minimal.

The test reaction used in portable biotesting devices, apart from the listed features, should be rather simple in terms of its measurement technique under production and field conditions.

All devices used to register a particular algal test reaction are intended for scientific, not industrial, purposes. We consider the registration methods of one or another test reaction of algae only from the point of view of its possible use in portable devices, as well as creating portable devices for measuring these reactions.

The growth intensity (reproduction). This test reaction, as noted above, is used in assessing chronic toxicity of wastewater. In classical aquatic toxicology the growth rate is determined by method of direct counting of cells under a microscope. However, this indicator can be measured using existing instruments, for example, a photocolorimeter. Portable models of photocolorimeters are mastered by industry and are produced both in our country and abroad. However, given the fact that algae have a relatively long generative cycle, this indicator cannot be used as a test for rapid toxicity analysis.

Electrophysiological characteristics of plant cell membranes. Technical aspects of measuring the electrophysiological characteristics of plant cell membranes, especially *chara*

algae, are now quite well developed for the practice of scientific research. They can be measured by contact methods introducing microelectrodes into the cell, as well as by contactless ones. Measuring tools required for these purposes are general-purpose electrical measuring instruments, that is, micro- and millimeters and voltmeters. Such devices are manufactured by our industry, including those in portable versions. Despite this, creation of a portable instrument for assessing wastewater toxicity by measuring the electrophysiological characteristics of algae cells is currently impractical because of complex methodological process of measuring these characteristics in production practice.

Intensity of photoinduced afterglow (delayed fluorescence). This test reaction is highly sensitive to the action of many toxicants. Afterglow is registered with the help of detectors of weak light fluxes. Since the spectral composition of the photoinduced afterglow lies mainly in the red part of the spectrum, it is most appropriate to use spectrofluorimeters to register it. Based on the technical characteristics of spectrofluorimeters produced by the industry, as well as on the analysis of the structural diagrams of these devices, it can be assumed that development of a portable instrument for assessing wastewater toxicity by measuring the long afterglow of algae is a difficult technical problem.

Ion exchange. This test reaction is not used in classical aquatic toxicology but a number of studies suggest that a disruption of ion exchange, especially exchange of potassium and sodium ions between cells and the environment is a sensitive indicator of the physiological state of the cell [2]. At present, simple and fairly sensitive methods for determining ion exchange using ion-selective electrodes have been developed. We can measure ion activity using electrical measuring tools for general use (millimeters, millivoltmeters) or special instruments, such as ionomers. Given the simplicity of the instrumental measurement of ion exchange and high sensitivity of this test, it can be assumed that this method of toxicity bioindication can be used both in stationary and in portable devices. However, more in-depth studies are needed for the final decision on the application of this test in instrumental biotesting, such as the relationship between ion

exchange and other indicators of the physiological state of algae, specificity of various substances and influence of side factors on ion exchange.

pH of the medium. A number of researchers [12,13] used a change in pH in an algae culture under the action of toxicants as a test reaction. Change in pH indirectly characterizes the intensity of algae photosynthesis. The use of pH as test reactions in portable devices is impractical because the pH of the medium can change not only due to the photosynthesis of algae, but also due to bacteria respiration, always present in wastewater. At a certain ratio of algae and bacteria the process of emission and absorption of CO₂ can be in equilibrium, and pH of the medium will not change. In this case, pH will not reflect the actual physiological state of the test object, that is, the algae. Thus, we cannot obtain reliable data on the toxicity of the investigated wastewater.

Movement of algae. The use of this indicator as a test reaction is reported in the work [14]. As a test object a blue-green alga *Phormidium* was taken. This work describes an automated device for determining the nature of algae movement by photocolometric method.

Methods used in cytophotometry are of special interest for instrumental biotesting of toxicity. Using cytometric methods of analysis, it is possible to determine such indicators of the physiological state of microorganisms as the ratio of living and dead cells, growth intensity, concentration of individual biochemical compounds, enzyme activity [15,16]. However, modern cytophotometers and cytofluorimeters are very complex and expensive devices and their use is limited mainly to the field of scientific research in physiology, biochemistry, microbiology and cytology. The use of such devices to assess the toxicity of wastewater in production conditions is not advisable for economic and technical reasons.

Intensity of photosynthesis (photosynthetic activity). The intensity of photosynthesis is the most common test for toxicity when using algae as test objects. Instrumental recording and automating the measurement process, rapidity, ease of measurement are important for instrumental methods in assessing wastewater toxicity. The refore, it is necessary to consider in more detail the currently used

methods for determining the intensity of algae photosynthesis, to briefly assess each of them in terms of possible use in portable devices for biotesting wastewater. All methods for determining photosynthesis are based on measuring the rate of oxygen evolution or carbon dioxide absorption in an incubation medium before and after a certain exposure of an algae culture to light. Concentration of these gases is determined by manometric, chemical, radiocarbon and polarography methods.

In manometric determination of photosynthesis, a certain volume of algae culture is placed in an airtight vessel connected with pressure gauges. Since in the process of photosynthesis, algae emit oxygen, the pressure of gases in a confined space increases. From the difference in pressure recorded by pressure gauges before and after algae exposure to light, we can calculate the intensity of photosynthesis. There are many types of devices for assessing the rate of gas exchange of algae by the manometric method, however, all of them are cumbersome and it is not advisable to create a portable device for bioindication of wastewater toxicity on their basis.

The main standardized method for determining the rate of photosynthesis is the iodometric method. This method refers to chemical determination methods and is based on the reaction of dissolved oxygen in liquids with manganese hydroxide and the subsequent iodometric determination of oxidized manganese compounds. This method cannot be used in portable devices as it includes a large number of manual operations, is laborious and time-consuming in execution.

The radiocarbon method for determining the rate of photosynthesis is mainly used in the laboratory practice of physiological research. It is based on the use of $C^{14}O_2$ as a carbon source. Since algae use $C^{14}O_2$ in the process of photosynthesis, by measuring their biomass radioactivity before and after the introduction of this compound into the medium, a specific rate of photosynthesis can be calculated. The method is not suitable for use in portable devices, due to the difficulties associated with obtaining a radioactive carbon isotope.

The most promising method for determining the intensity of algae photosynthesis from the point of view of its use in portable toxicity bioindication devices can be consid-

ered the polarography method. The main advantages of this method are simplicity of technical implementation, high sensitivity, low inertia, insensitivity to adverse chemical environmental factors, ability to quantify and record, ease of performing the measurement process, can be used in analyzing small volume samples. All this have led to the fact that the polarography method for the oxygen determination in biological fluids almost supplanted all other methods.

To study physiological and biochemical aspects of photosynthesis of plants, including algae, a number of laboratory installations have been designed, including flow-type installations. Although structurally these installations are different from each other, their diagrams are almost identical. As a rule, they include incubation chambers, dissolved oxygen sensors, measuring and recording systems, thermal and lighting systems control. We can draw the following conclusions from the above analysis:

1. algae can be used as test objects in wastewater biotesting devices;
2. the most appropriate of the test reactions characterizing the physiological state of algae to be used in biotesting devices are the following - the intensity of the long afterglow, ion exchange and photosynthetic activity of algae;
3. there are technical prerequisites for the creation of portable bioindication devices for wastewater toxicity, using various test reactions of algae on phytotoxic substances;
4. the most acceptable test reactions from the point of view of the available technical capabilities to create portable instruments for instrumental measurement of these reactions are algae photosynthetic activity and ion exchange.

To test the method of algae photosynthetic activity measuring by the printing method, a laboratory device layout was developed and manufactured. The device includes a thermoluminostat, a PL-700ALS oximeter with a culture chamber. A distinctive feature of this setup is the fact that it can work with dense cultures of algae and small sample volumes. Samples of *Scenedesmus quadricauda* u *Chlorella vulgaris* were used as test organisms. These types of algae are easily cultivated under laboratory conditions; they are rather sensitive to toxicants and are widely used as test objects

in classical aquatic toxicology. Algae were grown in Tamiya and Uspensky No. 1 environments in 5-liter glass containers at illumination of 2500-3000 lx, temperature 21-26 ° C and constant stirring. For the experiments, 7–10-day cultures in the exponential phase of

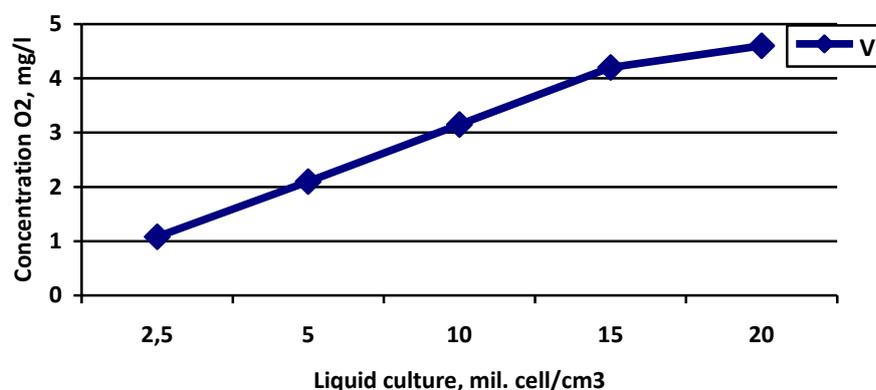
development were used. Immediately before carrying out the analysis, the cultures were compacted by condensing them on membrane filters. Assessment of the cultures density was carried out by direct counting of cells in the Goryaev chamber.

Results of the Research

One of the goals of our work was to find the best options for assessing the photosynthetic activity of algae, using the polarography method. In general, the definition of photosynthesis is reduced to measuring the concentration of dissolved oxygen in a liquid culture of algae before and after exposing samples to light. The value of this difference should always be much greater than the maximum sensitivity of the used method, in this case polarography. In the event the specified difference is less than the sensitivity of the method used, the results of the determination will be unreliable. As our task was not to determine sensitivity of the polarography method, in order to obtain reliable results it was important to study under what conditions the algae emit maximum amount of oxygen during the selected time of their exposure to light. There are several ways to ensure that the difference between the concentrations of dissolved oxygen in the samples before and after their exposure is large enough. The first way is to use a high density culture. As is well known, ceteris paribus, the amount of oxygen released during photosynthesis depends on the number of algae cells per unit volume of the liquid medium, and on density of the culture. In classical aquatic toxicol-

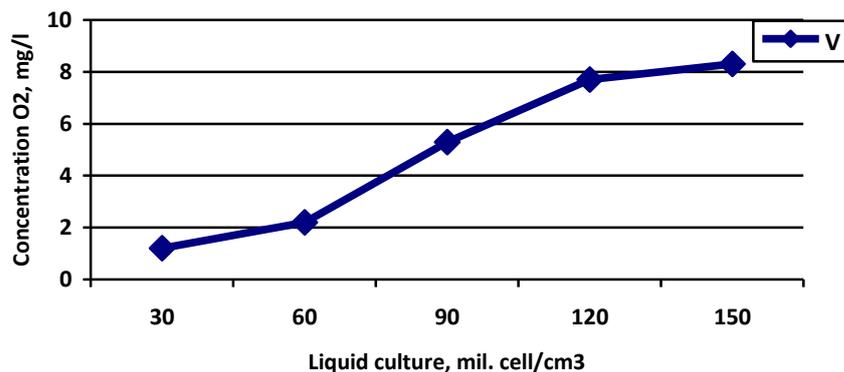
ogy cultures with density $1 \cdot 10^5 - 5 \cdot 10^5$ cells / cm^3 are used. At the same time, it is desirable to minimize the contact time. Such density of cultures may not be sufficient to obtain reliable results while assessing concentrations differences of dissolved oxygen in the control and experimental samples. Experiments were conducted to study the rate of increase in dissolved oxygen concentration in liquid culture of algae *Sc. quadricauda* and *Chl. Vulgaris* of varying density. The results of these experiments are presented in figures 1; 2; 3.

As can be seen from figures 1 and 2, the rate of concentration increase of dissolved oxygen in cultures is proportional to their density in a wide range. For *Sc. quadricauda* this interval is in the range of $2.5 - 15 \cdot 10^6$ cells / cm^3 , for *Chl. Vulgaris* – about $30 - 120 \cdot 10^6$ cells / cm^3 . With increasing density of above-specified limits, the rate of oxygen increase in the medium slows down. Slowing down of oxygen evolution rate at a culture density of $20 \cdot 10^6$ cells / cm^3 for *Sc. quadricauda* and $120 \cdot 10^6$ cells / cm^3 for *Chl. Vulgaris* can be explained by influence of factors limiting photosynthesis; first of all, by the exhaustion of dissolved carbon dioxide in the medium.



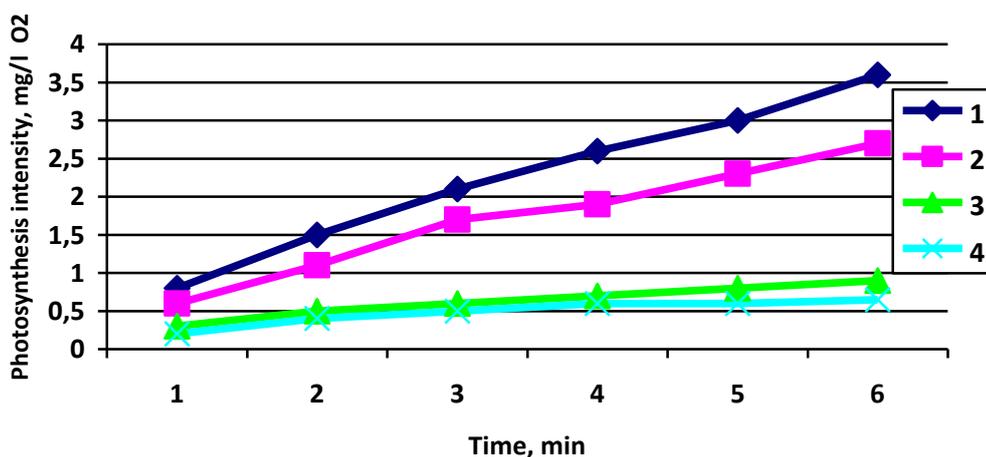
Light intensity – 8000 lx. Algae exposure time in the light – 7 min

Fig. 1 – The rate of concentration increase of dissolved oxygen in liquid culture *Sc. quadricauda* of varying density



Light intensity – 8000 lx. Algae exposure time in the light – 7 min

Fig. 2 – The rate of concentration increase of dissolved oxygen in the liquid culture *Chl. Vulgaris* of varying density



- 1 – *Sc. quadricauda*, culture density - $15 \cdot 10^6$ cells / cm³;
- 2 – *Sc. quadricauda*, culture density - $10 \cdot 10^6$ cells / cm³;
- 3 – *Chl. Vulgaris*, culture density - $15 \cdot 10^6$ cells / cm³;
- 4 – *Chl. Vulgaris*, culture density - $10 \cdot 10^6$ cells/cm³.

Fig. 3 – Comparative characteristic of photosynthesis intensity of cultures *Sc. quadricauda* and *Chl. Vulgaris* with the same density. Light intensity - 8000 lx

Analyzing the results given in Figure 3, we can conclude that the specific photosynthetic activity of *Sc. quadricauda* is several times higher than that of *Chl. Vulgaris*. Based on the fact that the photosynthesis activity of these algae was determined under identical conditions, it can be concluded that the differences are explained only by species peculiarities of these algae.

Analysis of oxygen release rate by algae cultures of varying density indicates that the

increase in oxygen concentration in the culture *Sc. quadricauda*, depending on its density is 1–4 mg / l in 7 minutes of algae exposure to light 8000 lux (Fig. 1). For *Chl. Vulgaris* this increase is 0.4 - 8.4 mg / l (Fig. 2). It can be assumed that with an increase in exposure time to light, the increase in dissolved oxygen concentration will be higher. Such a significant increase in cultures during the exposure of algae to light suggests that the sensitivity limits of the polarography method for determining

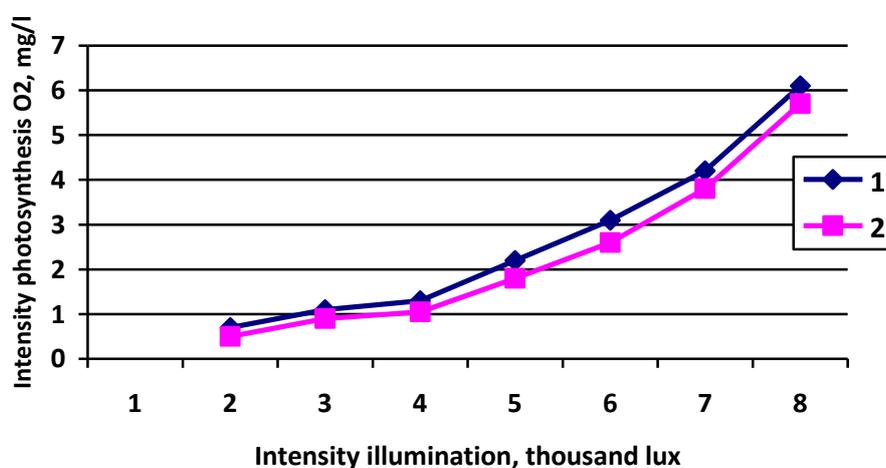
oxygen in a liquid will be sufficient to obtain reliable results when measuring the photosynthesis rate of algae of these species with a culture density of 5 to 150 million cells / cm³.

As can be seen from this figure, the intensity of photosynthesis is proportional to the intensity of illumination in the range of 4–8 thousand lux. An increase in illumination intensity up to 10 thousand lux sharply worsens measurements. Illumination intensity of is less than 4 thousand lux leads to a significant decrease in algae photosynthesis intensity. Increase in dissolved oxygen concentration in 6 minutes of exposure of cultures to light in this case is 1 mg / l.

Experiments on the study of photosynthesis and respiration of high-density algae cultures were conducted on a test device, and some dynamic characteristics of the installation itself were also investigated.

We have also conducted experiments to study the relationship between the intensity of algae photosynthesis and the illumination intensity of the measuring cuvettes. The results of the experiments are presented in Fig.4.

Dynamic characteristics of the installation are largely determined by the “inherent” dynamic characteristics of the dissolved oxygen sensor.



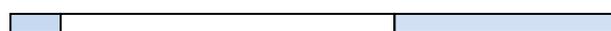
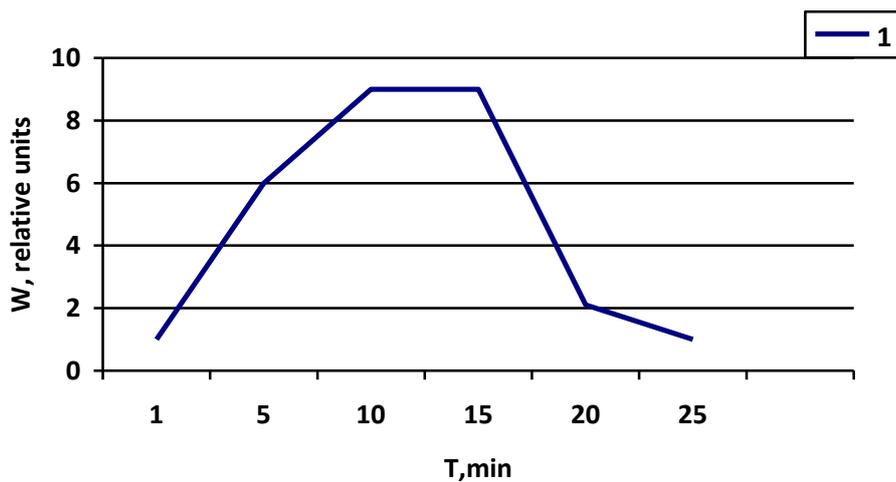
1 – *Chl. Vulgaris*, culture density – $60 \cdot 10^6$ cells / cm³;
 2 – *Sc. quadricauda*, culture density – $8,1 \cdot 10^6$ cells / cm³.
 Algae exposure time in the light – 7 min.

Fig. 4 –The dependence of the photosynthesis intensity of algae on the illumination of the measuring cuvettes

Fig. 5 shows a general view of the characteristic kinetic curve of photosynthesis and respiration of algae for these measurements. In their form, the curves coincide with those previously described in the literature. After turning on the light, rapid emission of oxygen begins, reaches a maximum, slightly decreases and then stabilizes. When you turn off the light, there is a sharp decrease in oxygen concentration down to zero. This is due to the absorption of oxygen by algae during their dark respiration. Experimental studies have shown that the shape of the kinetic curve depends on both external factors, such as illumi-

nation intensity and the spectral composition of the light, and on the physiological state of the test object.

Thus, summarizing the results of experimental data, it can be concluded that, by adjusting the density of algal cultures and light intensity, it is possible to achieve the condition when difference between the dissolved oxygen concentrations in the liquid culture before and after the algae exposure to light will be 1-8 ml/l for a sufficiently short period of time. It means that it is possible in principle to quantify photosynthetic activity of algae at short time intervals when exposed to light.



 – the lights are turned off

 – the light is on

Fig. 5 – Dependence of the release or absorption of oxygen in the illuminated and dark culture

Conclusions

A review of current methods and devices for wastewater biotesting indicates that at present there are technical prerequisites for creation of portable devices for bioindication of toxicity, using unicellular algae as test objects and an indicator of their photosynthetic activity as a test reaction.

The most promising method for assessing the photosynthetic activity of algae is the polarography method, which allows developing a portable device for bioindication of wastewater toxicity.

Having analyzed construction of structural diagrams of portable devices for assessing the toxicity of liquids, we have found out that its main specificity is the combination of biological systems with technical ones.

The main element of the biological system is a primary biological transducer, perceiving the effects of toxic substances and converting biological signals into a form that is acceptable for registration with technical devices. The task of creating a primary biological transducer and the choice of an informative biological signal can be solved by using single-celled algae placed in a sealed chamber and measuring gas exchange in the chamber. The authors have analyzed dependence of the main characteristics of the sensor on the structure of the diffusion layer and temperature and have found out that the optimal choice of the structure can be made depending on the biological object and experimental conditions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, data fabrication and double publication have been completely observed by the authors.

References

1. Krainiukov, O. M. & Yakusheva, A. V. (2020). Research of the possibility of using *Ceriodaphnia affinis* Lilljeborg (Crustacea) in a short-term test while setting ecological quality standards in Ukraine. *Visnyk of V. N.*

- Karazin Kharkiv National University, Series "Geology. Geography. Ecology", (51), 199-206.
<https://doi.org/10.26565/2410-7360-2019-51-14> (in Ukrainian).
2. Kostyaev, V., Yagodka, S. & Sokolov, V. (1980). Anabaena spiroides sensitivity to zinc and cobalt. *Gidrobiol. magazine*, 16(2), 89-92 (in Russian).
 3. Raso, J. & Rachlin, I. (1977). The effect of cadmium, copper, mercuru, zincum and lead on cell division growth and chlorophyll a content a content of the chlorophyte. *Chl. vulgaris. Bull. Torreu. Bot. Club.*, (3), 226-233.
 4. Kravchenko, N. & Gapochka, L. (1977). Effect of petrochemical products on some blue-green algae. *Ed. AN Tur. SSR. Ser. "Biol. science"*, (2), 52-56 (in Russian).
 5. Matorin, D., Venediktov, P. & Makevnina, M. (1977). Application of the method of registration of the afterglow of green algae to determine the contamination of soil and water with phytotoxic substances. *Scientific reports High School "Biol. science"*, (12), 122-125 (in Russian).
 6. Pokrovskaya, N. (1977). The action of propanide and Yalan on the photosynthetic process of the Alaba Anabaena spiroides. *Prince Cultivir and approx. microalgae*. Tashkent, 22-23 (in Russian).
 7. Shokodko, T. & Merezhko, A. (1978). Changes in the activity of the photosynthetic apparatus of aquatic plants under the action of DDT. *Gidrobiol. Magazine*, (5), 86-89 (in Russian).
 8. Belghith, T., Athmouni, K., Bellassoued, K., El Feki, A. & Ayadi, H. (2015). Physiological and biochemical response of *Dunaliella salina* to cadmium pollution. *Journal of Applied Phycology*, 28(2), 991-999. <https://doi.org/10.1007/s10811-015-0630-5>
 9. Folgar, S., Torres, E., Pérez-Rama, M., Cid, A., Herrero, C. & Abalde, J. (2009). *Dunaliella salina* as marine microalga highly tolerant to but a poor remover of cadmium. *Journal of Hazardous Materials*, 165(1-3), 486-493. <https://doi.org/10.1016/j.jhazmat.2008.10.010>
 10. García-ríos, V., Freile-pelegrín, Y., Robledo, D., Mendoza-cózatl, D., Moreno-sánchez, R. & Goldbouchot, G. (2007). Cell wall composition affects Cd²⁺ accumulation and intracellular thiol peptides in marine red algae. *Aquatic Toxicology*, 81(1), 65-72. <https://doi.org/10.1016/j.aquatox.2006.11.001>
 11. Nikookar, K., Moradshahi, A. & Hosseini, L (2005). Physiological responses of *Dunaliella salina* and *Dunaliella tertiolecta* to copper toxicity. *Biomolecular Engineering*, 22(4), 141-146. <https://doi.org/10.1016/j.bioeng.2005.07.001>
 12. Goryunova, S. (2002). Photo-induced pH change as an integral method for controlling natural and waste waters. *Proceedings of the International Conference: New technologies in the protection of biodiversity in aquatic ecosystems*, Moscow, 2002, May 27-29 (p. 97). Moscow: Moscow State University (in Russian).
 13. Plekhanov, S., Pimentel, F., Goryunova, S. & Chemeris, Yu. (2004). Early diagnosis of the toxic effect of heavy metals on green microalgae according to their photosynthetic characteristics. *Proceedings of the International Conference: Aquatic Ecosystems and Organisms-6*, Moscow, 2004, May 18-19 (pp. 87-88). Moscow: MAX Press (in Russian).
 14. Beneche, G. (1977). Automatisierung der auswertur einer algenheteste hemmung der kreichenbewegung einer blaualge (*Phornidium sp.*) durch deigat. *Z. Waasser und Abwasser-Forsch.*, (6), 195-197.
 15. Einsele, A., Riatroph, D. & Humphereu, A. (1979). Substrate uptacemechanisms for yeast cells. A new approach utilizing a fluorometer. *Eur. J. Appl. Microbiol. and Biotechnol.*, (4), 335-339.
 16. Healeu, F. & Headzel, L. (1979). Fluorometric measurement of alkaline phosphatase activity in algae. *Freshwater Biol.*, (5), 429-439.

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