UDC 57.084.1
DOI: 10.15587/1729-4061.2017.107201

BIOTESTING OF PLASMA-CHEMICALLY ACTIVATED WATER WITH THE USE OF HYDROBIONTS

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Досліджено вплив плазмохімічно активованої води на функціональний стан Riccia fluitans, Lémna mínor L., Paramecium caudatum, Artemia salina і Cyprinus carpio. Визначено позитивну дію плазмохімічно активованої води на біологічні об'єкти. Встановлено основні показники для кожного методу біотестування. Отримані результати доповнюють відомості щодо безпечності використання плазмохімічно активованої води як компонента харчового ланцюга

Ключові слова: плазмохімічно активована вода, біотестування, гідробіонти, газорозрядна візуалізація

Исследовано влияние плазмохимически активированной воды на функциональное состояние Riccia fluitans, Lémna mínor L., Paramecium caudatum, Artemia salina и Cyprinus carpio. Определено позитивное действие плазмохимически активированной воды на биологические объекты. Установлены основные показатели для каждого метода биотестирования. Полученные результаты дополняют сведения о безопасности использования плазмохимически активированной воды как компонента пищевой цепи

Ключовые слова: плазмохимически активированная вода, биотестирование, гидробионты, газоразрядная визуализация

1. Introduction

Water plays an important physiological role for living organisms and acts as the main activator of microbiological, biochemical and physicochemical processes in food and feed production technologies. Regulation of water properties can influence the course of the technological process [1]. The use of physicochemical methods of water treatment is quite important because due to such preliminary preparation of water it is possible to change its characteristics depending on the needs of production [2, 3]. Among the well-known multi-purpose methods of water treatment, attention is drawn to the use of contact non-equilibrium low-temperature plasma, or nonthermal plasma (NTP) [4–6]. Since water is the main component of living organisms, a substantial change in its properties requires a comprehensive assessment of the reaction of test objects to evaluate the multifaceted action of plasma-chemically activated water on systems of living organisms.

Recently, methods of direct assessment of toxicity of the aquatic environment with the help of bioindicators have become of increasing importance. The urgency of using biological objects for biotesting and phytotesting is manifested, first of all, in the specific ability of the organizational system of such objects to reflect a wide range of factors of different nature [7]. One of the methods for evaluating the biological reflection of non-traditional methods of processing food raw materials is gas dispersion visualization. The peculiarity of this method is the use of high-energy chemistry to regulate water characteristics. The use of the gas-discharge visualization method has versatile directions: plant growing, medicine, and the pharmacological branch [8]. Meanwhile, a combination of different express methods allows for more reliable results. The feasibility of conducting research in this case has objective justification, which acquires scientific and practical significance. For example, a study of the physiological state of a biotesting facility involves the implementation of an entire chain of sequential tests. Using several express methods makes it possible to get results in a shorter time.

2. Literature review and problem statement

Unconventional methods of processing raw materials in food and feed production technologies are gaining increasing popularity among the population due to their ability to neutralize negative factors of influence on the formation of the quality of the final product during the technological process [9]. Furthermore, the benefits of creating eco-products are evident from the point of view of a consumer oriented towards healthy eating. The production of such foods eliminates the use of artificial improvers that are potentially hazardous elements for humans [10, 11]. At the same time, the parameter of economic efficiency of the process should be maintained provided the minimum environmental burden is guaranteed.

The use of nonthermal plasma for treatment of water and aqueous solutions has the potential to produce benefits in the agro-industrial complex and the production of food and feed products. Due to plasma-chemical treatment, water acquires high permeability through the formation of a fine-cluster structure and the emergence of additional possibilities for interaction with structural components [12]. Depending on the needs of the production process, it is possible to maintain the required parameters of the active acidity of the medium [13]. A separate property of water exposed to nonthermal plasma is the presence of its active oxygen in the form of peroxide and super-permeable compounds, providing its antiseptic properties. Water prepared in this way can suppress pathogenic and opportunistic microorganisms [14]. Also known is the fact that plasma-chemically activated water has a positive effect on bread-making pressed yeast [15]. Under the condition of cultivating a microbiological culture with water subjected to nonthermal plasma, there is an increase in the number of viable cells of *Saccharomyces cerevisiae* 10–15 times.

Studies [16, 17] confirm the effectiveness of plasma-chemically activated water in various food technologies, in particular, for the production of baking products. Such a technological approach makes it possible to obtain a high-quality product with high consumer qualities and high microbiological stability without the use of artificial enhancers.

The reaction of living organisms to the investigated factor of influence can reflect the efficiency of using components of the food chain in view of the biochemical effect and changes in the characteristics of biotested objects [18].

At present, there are virtually no experimental studies on the impact of such water on biological objects in view of the possibility of occurrence of immediate and distant consequences.

Determination of the toxicity of a test sample is based on the ability of the test objects to react to the presence of substances that can negatively affect their livelihoods in the aquatic environment. In Ukraine today, the harmonization of biotechnological methods, national legislation and standards with modern international requirements is underway. The emphasis is on the fact that the control over the manufacturing of products must occur consistently throughout the food chain [19]. Already existing methods are somewhat inferior to modern ones since the latter are more informative, implemented in shorter terms and capable of producing a resource-saving effect on the background for obtaining high quality products. Literary sources contain information on toxicity studies using *Decapoda* and *Paramecium caudatum*. In this case, the main informative parameters are the functional system of viability of organisms, the biochemical and physiological status, etc. [2, 20]. In studies under review [20, 21], gas-discharge visualization (GDV) demonstrates the possibility of obtaining the probable results of testing objects of various nature in an express mode. Today, one of the interesting and informative methods of diagnostics is the interpretation of GDV-grams (photos) of the studied object. This may be a different structure of water, a living organism, etc. [21]. It should be noted that in the agro-industrial sector this practice is not widely used in European countries and is practically absent in Ukraine. For example, there is relatively little experience in using the method of interpreting GDV-grams in determining the biopotential of products in stockbreeding [22].

It is known that phytotesting is an effective method for determining the level of eco-safety of environmental factors and studying the toxicity of substances [18]. For example, the quality of drinking water is formed in a certain way under the influence of these factors. However, information on implementing scientific and practical experiments while using phytoindicators to determine the toxicity of water exposed to nonthermal plasma is absent. Therefore, in this aspect, the chosen topic of research is promising as it has scientific and practical value in the fields of agriculture and food industry.

3. The aim and objectives of the study

The aim of the study is to determine the influence of water exposed to nonthermal plasma on biological objects.

To achieve the aim, the following objectives are set to be solved:

– to carry out biotesting on infusoria for cultivating which the water is exposed to the action of a nonthermal plasma;

– to determine the effect of plasma-chemically activated water on phytoplankton and zooplankton;

– to study changes in the biological state of the Cyprinus carpio in the conditions of the influence produced by the treated water;

– to analyze the water subjected to the action of nonthermal plasma by the method of gas-discharge visualization.

4. Materials and methods of biotesting of water exposed to nonthermal plasma

4. 1. Characteristics of plasma-chemically activated water and equipment used for water preparation

Water treatment with nonthermal plasma was carried out at the laboratory of plasma-chemical technologies of the Ukrainian State Chemical Technology University (Ukraine) on a laboratory installation of a discrete type with a reactor volume of 0.1 dm^3 (Fig. 1). For the test reference groups of biological objects, drinking water of the main of the city of Dnipro (Ukraine) was used.

In the course of the research, the water used was water taken from the main without additional treatment and water exposed to the NTP treatment (Table 1).

The concentration of peroxide compounds in the water was determined using the test strips Peroxid-Test (Germany). The active acidity of the water and the redox potential were determined by the pH meter EZODO MP-103 (Taiwan).

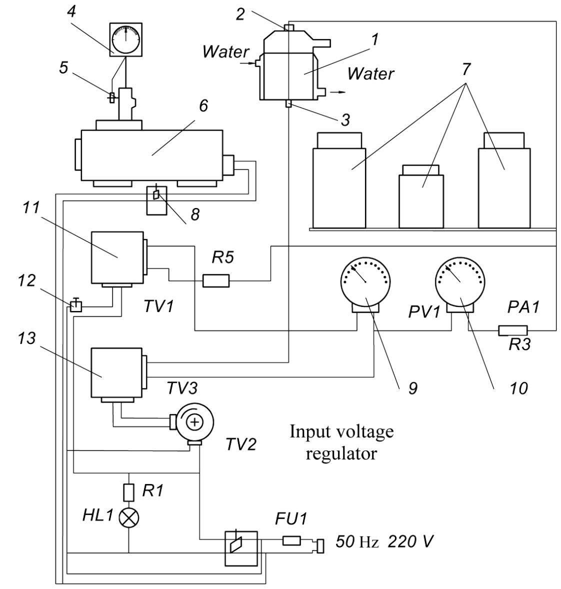


Fig. 1. The circuit diagram of the installation for plasma-chemical treatment of water: 1 – reactor; 2, 3 – electrodes; 4 – vacuum gauge; 5 – crane; 6 – pump; 7 – filtering elements; 8 – switch; 9 – voltmeter; 10 – ammeter; 11 – firearm transformer; 12 – switch; 13 – voltage transformer

Table 1

Characteristics of the main and plasma-chemically activated water

Sample No.	Duration of NTP action on water, min	Concentration of peroxide compounds, mg/l	pH of water	Oxidation-reducing (redox) potential, mV
1	–	–	7.2	235
2	10	100	9.7	135
3	30	500	10.1	101

4. 2. Investigated test objects

Phytoplankton, zooplankton and hydrobionts were selected as test objects. The species compositions of these objects depended on the individual properties of influence on the hydrochemical composition of water. This provides an opportunity to comprehensively evaluate the properties of plasma-chemically activated water at the structural organization level of various organisms.

During the phytoplankton testing, the elements under consideration were the level of development, pigmentation, as well as the biomass of riccia (*Riccia fluitans*) and duckweed (*Lémna mínor L.*). The functional states of infusoria (*Paramecium caudatum*) and artemia (*Artemia salina*) were analyzed in view of changes in their vital activity. The chemotaxis of the organisms was determined by visual observation systematically according to the methods described in [18, 23].

Other biological objects in the experimental part were newly hatched scaly carps *Cyprinus carpio*. The selection of the experimental and reference groups was carried out using the pair-analog method. The monitored parameters were the rate of carp survival and the speed of development as well as the results of visual observation. All the findings were statistically processed in MS Excel.

4. 3. Methods of cultivating hydrobionts and determining the indicators of their physiological activity

Mother cultures of phytoplankton were cultivated in aquarium water; then water was added, having been treated with nonthermal plasma, to substitute 70 % of the total volume of the aquarium water. During the experiment, 10 leaves of *L. minnor L.* and *R. fluitans* (working culture) were systematically counted for each breeding culture. The samples were placed in 3 Petri cups and exposed to 6–8 hour daily illumination. On days 3, 6, 9 and 12, the number of duckweed leaves in each cup was counted. At the end of the cultivation, measurements were made, and the maximum length of the roots and the level of pigmentation were registered. The phytotoxic effect (PTE) for all water samples was calculated according to the method described in [18].

In studying the influence of plasma-activated water on the state of phytoplankton, two cultures (duckweed and riccia) were placed in chemical tubes with prepared water in accordance with the experiment. Every day, they were controlled for the level of pigmentation and the development of their root systems.

Cultivation of the infusoria *Paramecium caudatum* was carried out in aquariums and in a Weiss incubation apparatus. The biotesting was performed with the use of a test kit, aerators, a laboratory microscope, and slide glass. The investigated water, treated with nonthermal plasma, was added to each sample to replace 70 % of the total amount of the aquarium water. The trajectory of the infusion of the cells and their redistribution in the cuvettes with plasma-chemically activated water were investigated by chemotoxic reaction.

In general, using single-celled algae and infusoria as test objects, control was performed on the rate of their survival, changes in the number of cells in the culture, cell division coefficient, growth rate, and pigmentation level. The water toxicity index was investigated on the basis of determining the percentage of the infusoria that retained vital functions and moved to the upper zone of the cuvette (Fig. 2). Visual inspection was performed using the microscope, cuvettes, and slide glass.

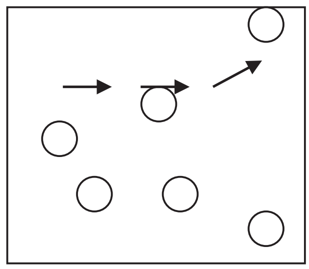


Fig. 2. The method of the schematic registering of infusoria in the study of the influence of plasma-chemically activated water

When cultivating artemia, miniaquariums with a prepared ecosystem, aeration, and a hydrochemical regime were used. Nauplii of the brine shrimp, artemia, were placed right before the beginning of the experiment in the aquariums with diluted plasma-chemically activated water in the amount of 90 % to the aquarium water. In the reference groups, main water was used without additional preparation. The state of artemia after hatching was evaluated visually and controlled by the level of their functional activity by microscopy.

The newly hatched scaly carps Cyprinus carpio were cultivated in the main diluted water and in a medium of water previously exposed to nonthermal plasma, which was added in the amount of 70 % of the volume of the aquarium water. The effect of the plasmochimic water on the scaly carp was estimated by the survival rate, which was registered for weeks 1, 2 and 3 of the experiment, changes in the overall ethology in the aquariums, and the growth rate. The peculiarity of the feeding of the experimental fish groups was the use of duckweed as a feed cultivated in water, previously exposed to nonthermal plasma.

4. 4. The method of determining the bioenergetic potential of plasma-chemically activated water by gas-discharge visualization

The electrical discharge of the luminescence on the surface of the objects in a variable electric field of high frequency was detected using the equipment of the GDV Scientific Laboratory. The level of radiation was determined for drops of water without further processing and preliminarily treated with nonthermal plasma. The resulting GDV-grams (photos processed by the GDV Scientific software) were interpreted in terms of the intensity and the colorless scale, and a statistical analysis was carried out for the data obtained.

5. Results of studying the influence of plasma-chemically activated water on the bio-indicator organisms

The nature of the motion and the resistance of the biotest objects depended on the structural features of the water and the chemical composition of the environment. *Paramecium caudatum* is widely used to assess the chronic toxicity of medicines and nutritional supplements [7]. As can be seen from Fig. 3, in all of the studied groups the degree of survival of infusoria was 87–89.5 %.

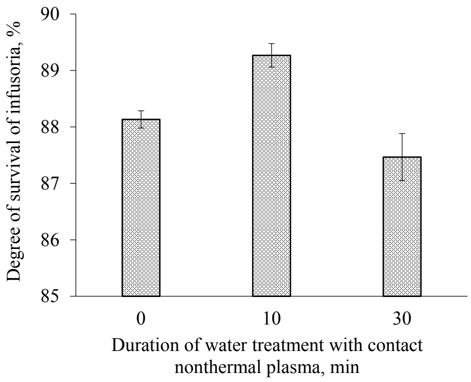
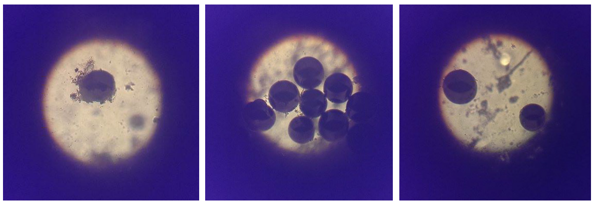


Fig. 3. The toxicity of *Paramecium caudatum*

At the same time, in experimental group 1, where single-celled organisms were cultivated in a medium of water exposed to nonthermal plasma for 10 minutes, the test substance corresponded to the "non-toxic" class. Similarly, for the experimental group of organisms serving as test objects for plasma-chemically activated water with a concentration of peroxide compounds of 500 mg/l, this figure was somewhat lower, but it also belonged to the "non-toxic" class.

Artemia salina is a highly sensitive hydrobiont that is used in aquaculture as a natural feed for fish. These organisms are particularly susceptible to changes in the aquatic environment during hatching of nauplii. The hydrochemical regime parameters adjust their hatching and vital activity. The cultivation of artemia nauplii in the plasma of chemically activated water showed a change in the index of their hatching, the level of ability to maintain vitality, and the mobility in the first days of life after hatching. In particular, a significantly higher level of hatching was observed in the cultivation of *Artemia salina* in water previously exposed to nonthermal plasma (Fig. 4). In this case, the use of plasma-chemically activated water with a concentration of peroxide compounds in the amount of 500 mg/l, the amount of viable organisms increased by 60–70 % compared with the use of the main water without pre-treatment.



a *b* *c*

Fig. 4. The functional status of *Artemia salina* nauplii, cultivated in water, exposed to nonthermal plasma for, min: *a* – 0, *b* – 10 and *c* – 30

The phytoplankton of *Riccia fluitans* and *Lémna mínor L.* is the direct constituent of the ecosystem. Since the natural habitat of the phytoplankton is water, its hydrochemical state is extremely important. In the cultivation of duckweed and riccia in the environment of the plasma-chemically activated water, differences were found in the trends in the development of the phytoplankton, depending on the duration of the water treatment. The results of measuring the biometric characteristics of the duckweed (Fig. 5) showed that the root length (L_{max}) in the reference group was the smallest in comparison with the registered length in the experimental groups.

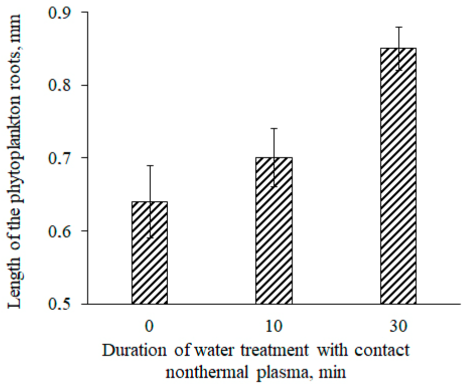


Fig. 5. The influence of plasma-chemically activated water on *Lemna minor L.*

The results of the plasma-chemically activated water on the duckweed in experimental group 2 were probable in comparison with the other groups. At the same time, in experimental group 1 only the tendency to increase the studied indicator was noted. The intensity of developing the root system of the test objects increased by 7–12 and 28–39 % compared with the reference group in the case of using the water that had been exposed to the plasma for 10 and 30 minutes, respectively. There was a decrease in the intensity of the phytoplankton color in the experimental groups. The value of the phytotoxic effect in the experimental groups did not exceed 20 %, while for the reference this figure was 30 %.

The scaly carp *Cyprinus carpio* by its nature belongs to the difficultly organized hydrobionts that have trophic ductility. This fish feeds on various forms of zooplankton and is well adapted to the consumption of feed. The physiological state and development of these hydrobionts directly depend on the environmental conditions of cultivation, in particular the aquatic ecosystem. Feeding the scaly carp on artemia, duckweed and riccia, previously cultivated in plasma-chemically activated water, resulted in an accelerated growth of the fish. The feeding of the hydrobionts with the natural feed treated with plasmochimic water also had a positive effect on the survival rate (Fig. 6). The rate of growth in the ontogenesis in experimental group 2 was marked as the highest among all the groups. In the first week of research, the value by 2 % exceeded the indicator of the reference group. In experimental group 1, the difference in the rate of the fish growth was only 1.5 % relative to the reference group.

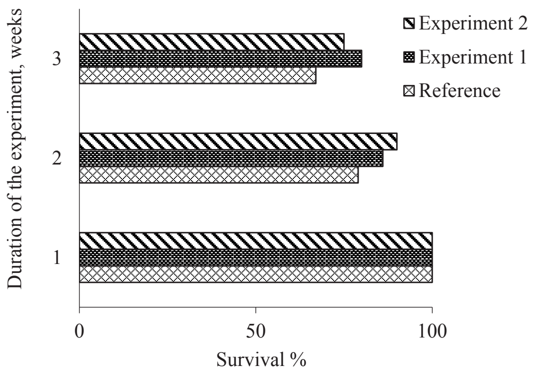


Fig. 6. Survival of *Cyprinus carpio* depending on the cultivated medium and the characteristics of the feed, n=6

When comparing the *Cyprinus carpio* body weight at the end of the third week, it was found that in experimental groups 1 and 2 individuals had bigger weights compared with the reference group by 3.5 % and 6.3 %, respectively. For all of the investigated samples, the number of the carp species remaining viable had declined by an average of 33 % compared to the first week of the research. However, in experimental groups 1 and 2, the fish had by 13 % and 8 % higher values than the reference after two and three weeks of the experiment, respectively.

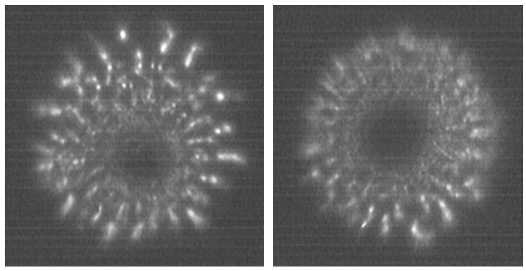
The use of gas-discharge diagnostics in studying the bio-potential of plasma-chemically activated water allowed for a more objective analysis of its characteristics (Table 2, Fig. 7).

Table 2

Results of determining the water biopotential

Features	Duration of water treatment with nonthermal plasma, min		
	0	10	30
Right projection, entropy	3.8±0.027	3.8±0.039	3.6±0.028
Left projection, entropy	4.0±0.066	4.1±0.086	3.3±0.036
Right projection, area	17,123±41.1	17,869±41.3	18,250±35.1
Left projection, area	16,164±65.1	16,354±65.7	19,987±54.9

When processing the results, it was taken into account that the gas discharge that occurred during the analysis of the sample, affected the state of the research object, causing secondary emission and thermal processes [24]. The receiver of the radiation converted the spatial distribution of illumination into the image (Fig. 7), the analysis of which led to the formation of a set of parameters. Among the many parameters that were handled by the software GDV Capture (Canada), the main criteria selected for the digital value were entropy and radiation area in two projections. The features of the right and left projections of the reference and experimental samples showed a probable difference between them in the case of analyzing the sample of water exposed to plasma for 30 minutes (Table 2). The difference in the results of the study between the main water without additional treatment and the water treated with the plasma for 10 minutes was within the measurement error. As can be seen from the data in Table 2, the plasma-chemically activated water with the increased exposure duration of up to 30 minutes had lower entropy compared with the reference sample.



a *b*

Fig. 7. GDV-grams of the biopotential: *a* — main water; *b* — plasma-chemically activated water

The observed intensity of the water radiation on the GDV-gram shows that after the treatment with the nonthermal plasma, the shape of the "crown of glow" and its area decreased. The plasma-chemically activated water was characterized by higher radiation rays and a wider spectrum, indicating an increase in the biopotential compared to the reference.

6. Discussion of the results of biotesting of plasma-chemically activated water in view of changes in the physiological activity of the test-objects

The results of the systematic control and visual observation of the development of two representatives of the phytoplankton – riccia and duckweed – have shown that such water activates metabolic processes in the plants and induces changes in their pigmentation. The obtained results give grounds for the expediency of using the phytoplankton in studying the properties of plasma-chemically activated water. The difference between the influence of water without treatment and plasma-chemically activated water is found to indicate a positive activating effect of water exposed to the nonthermal plasma on the intensity of the phytoplankton development. In the published literature, information on experimental research is not available in full with the use of phytotesting with such water. Therefore, it is expedient, taking into account the positive results, to expand the range of the studied indicators and to continue research in this direction.

The biotesting of the zooplankton points to the possibility of using water subjected to nonthermal plasma for the incubation of artemia nauplii as an adaptogen and as a way to increase the percent of hatching nauplii. Similar experimental studies with the use of plasma-chemically activated water as ecosystems had not been conducted. Therefore, this direction has a scientific and practical relevance and provides grounds for further continuing experimental research. It is obvious that the increase in the level of nauplii hatching during the cultivation of zooplankton has an industrial value [25] as a factor in the intensification of production processes.

When cultivating the scaly carp in the reference group and using the main water without pre-treatment, the lowest percentage of survival was found. At the same time, the general functional state of the carp organism, which stayed in the water exposed to nonthermal plasma, was recorded as higher than in the reference group.

In aquaculture, studies are conducted on the effects of a significant number of factors, solutions, and additives on hydrobionts. There are results of scientists about the positive effects of such substances of different nature; however, the use of the method proposed in this work for the cultivation of the newly hatched carp was not noted in the scientific publications of other authors.

The revealed influence of the plasma-chemically activated water on the livelihoods of the phytoplankton, zooplankton, and complex-organized hydrobionts – fish – is directly related to the change of the water biopotential. The objects of the GDV-diagnostics may be biological fluid, a plant or animal organism, or a biologically active additive as a component of a food product, etc. Thus, the authors of [2] noted significant differences in a droplet of water after filtration and a change in the aggregate state from solid to liquid compared with main water. In [20, 24], it was established that gas-discharge visualization registered an inversely proportional relationship between the physiological state of the organism and the index of entropy as a result of the conducted testing.

The results of the conducted studies indicate a change in the position of one structural element (water molecule) under the influence of factors of physical and chemical nature, which in this case is nonthermal plasma. It must be assumed that this occurs due to a change in the orientation of molecules that are localized one next to another, which obviously serves as an additional confirmation of the formation of a fine-cluster water structure [5]. The heterogeneity of the surface and volume of the investigated object as well as the processes of emitting charged particles affect and can correct certain parameters of the electromagnetic field, due to which the characteristics of the discharge current and optical radiation change. Thus, the GDV-diagnostics allowed determining an increase in the bioenergy potential of the test facility by the express method, which, in turn, makes it possible to justify the emergence of activating functions of the water exposed to the nonthermal plasma with respect to biological objects.

The proposed integrated approach to the study and the results obtained during the experiments indicate that the flow of hydrographic substances to the organism in plasma-chemically activated water does not inhibit their vital activity. The positive dynamics of changes in the studied indicators show that treated water can act as an activator of catalytic processes. It is likely that the effect of such water on the body of test objects may have adaptive, hepatoprotective and immunomodulatory effects. However, to confirm the presence of such properties, it is necessary to expand research in the direction of determining the transformation of biological objects at the cellular level. It is obvious that after water treatment with nonthermal plasma such water can have a positive effect on the course of adaptive-compensatory mechanisms in living organisms, which in turn affects the indicators of the overall functional and physiological-biochemical state. Therefore, subsequent research should be directed to studying the influence of these factors on the morphofunctional parameters and mechanisms of the flow of metabolic processes in the organisms of hydrobionts.

Further research would be feasible with regard to the possibility of expanding the spectrum of studying the impact of such water on biological objects. This approach should provide a scientific basis for the implementation of the proposed method of water treatment in the food chain. This will enable the improvement of technological processes for the production of food and feed.

7. Conclusions

1. When cultivating infusoria in a medium with water exposed to nonthermal plasma, it was found that in all of the groups the degree of infusoria survival was 87–89.5 %. The value of this parameter was somewhat different between the experimental and reference groups, but it referred to the "non-toxic" class.

2. With the use of plasma-chemically activated water with a concentration of peroxide compounds in the amount of 500 mg/l, the amount of viable zooplankton organisms increased by 60–70 % compared with the use of main water without pre-treatment. The positive influence of the plasma-chemically activated water is manifested in the hatching of zooplankton representatives. In the cultivation of duckweed and riccia in the environment of plasma-chemically activated water, differences in the trends of the phytoplankton development were determined depending on the duration of the water treatment. The intensity of the development of the root system of the phytoplankton increased by 7–12 % and 28–39 % compared with the reference group in the case of using water exposed to the plasma for 10 and 30 minutes, respectively.

3. When the feed of the scaly carp was supplemented with artemia, duckweed and riccia, previously cultivated in plasma-chemically activated water, it resulted in an accelerated growth of the fish. Positive changes in experimental groups 1 and 2 were found; the fish had higher rates of growth – by 3.5 and 6.3 %, respectively, relative to the reference group.

4. The use of the GDV-diagnostic method has made it possible to establish that water subjected to nonthermal plasma has higher radiation rays. The analysis of the GDV-grams has shown that the treatment of water with nonthermal plasma increases the biopotential.

Acknowledgements

The research was conducted with the support of the grant of Ukraine for young scientists under the state budget topic No. 0116U007412. The authors also express their gratitude to the postgraduate student V. Yu. Sokolov for the help in conducting the laboratory research.

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