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DECONTAMINATION OF BIOLOGICAL AGENTS IN WASTEWATER THROUGH SYNERGETIC OXIDATIVE METHODS AS AN EFFECTIVE APPROACH FOR SAFEGUARDING PUBLIC HEALTH AND AQUATIC ECOSYSTEMS

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Abstract. We employed a highly concentrated model of wastewater to conduct our research, accurately mimicking the composition of wastewater generated by milk processing enterprises. Wastewater contained milk protein, carbohydrates, and whey sugars. Domestic wastewater, which served as a source of indicator fecal microorganisms, was added to them. The water purification and disinfection scheme involved treating a model wastewater using the biosorption method with an immobilized biocenosis in a laboratory installation, followed by further purification and disinfection in a decontamination tank. In comparison, we assessed the degree of microorganism elimination using distinct methods such as ozonation, hydrogen peroxide treatment, and combination of O_3/H_2O_2. It has been demonstrated that model wastewater, purified and disinfected using the AOPs method, also contains dissolved oxygen, which is non-toxic to aquatic microbiota.

Keywords: disinfection, wastewater, AOPs, hydrogen peroxide, ozone, biosorption treatment, toxicity.

1. Introduction

The UN State of the World's Water Report (World Water Development Report 2018) forecasts a growing water crisis, substantial declines in water resources, and increased freshwater pollution by 2050. The Water Strategy of Ukraine for the period up to 2025 identifies the natural deficit and depletion of water resources, as well as the significant flow of chemical and biological pollutants into water bodies as the main ecological problems. According to the Ministry of Health of Ukraine, more than 23 % of the studied water samples from surface water bodies do not meet regulatory requirements for sanitary, chemical, and microbiological indicators. Drinking water from surface water bodies is potentially dangerous in terms of viruses, as the current technology for its treatment does not guarantee the removal of these pathogens. Contamination of natural water with biological agents mainly occurs due to the discharge of insufficiently treated wastewater into water bodies[1, 2]. Today, many countries around the world are actively searching for the most rational and highly effective methods and technologies for wastewater treatment that meet the criteria for obtaining high-quality purified water while ensuring complete safety for human health and the environment[3].

In particular, the research of such purification methods as adsorption[4], ion exchange[5], reverse osmosis[6], reagent method[7], as well as biological aerobic and anaerobic methods[8, 9] have recently gained significant development. To conserve water resources and use water more efficiently, treated wastewater is often reused for household needs. According to Ukrainian legislation, such water must be disinfected and meet the criteria for safe use regarding microbiological and parasitological indicators. It should not contain pathogenic microorganisms, virulent viruses, or viable helminth eggs[10, 11]. In addition, the discharge of treated wastewater into surface water bodies is regulated, including the content of E. coli bacteria and coliphages[12].

Water decontamination is conducted at the final stage of water purification and aims to prevent diseases that can be transmitted through water. Traditional wastewater disinfection schemes are mainly based on the use of active chlorine, hypochlorite, or chlorine dioxide[13]. However, the use of these compounds poses a risk to

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humans. Moreover, there is a possibility of the formation of by-products that are toxic to aquatic organisms in water bodies where disinfected water is discharged.

Residues of free chlorine (more than 5 mg/L) in surface water bodies worsen their organoleptic properties. Chlorine-containing compounds, along with waste products of aquatic organisms and residues of industrial chemicals, can form substances hazardous to human health-mutagenic, carcinogenic, and highly toxic.

Besides, over the years, when chlorine, ozone, and UV radiation are used individually, there is an increase in the resistance of microorganisms to their effects. This factor necessitates higher doses and concentrations of reagents, which could be economically impractical and ecologically hazardous.

Therefore, to ensure public access to high-quality drinking water and preserve the diversity of aquatic ecosystems, research focused on developing and implementing environmentally friendly and safe water disinfection methods – alternatives to chlorination – is extremely relevant today. Recently, there has been a growing emphasis on researching, developing, and implementing innovative technologies for wastewater treatment and disinfection based on oxidation methods, contributing significantly to the improvement of water quality. Oxidation methods or advanced oxidation processes – Advanced Oxidation Processes (AOPs), based on the combination of natural oxidants, such as ozone (O_3), UV radiation (UV), hydrogen peroxide (H_2O_2), titanium dioxide (TiO_2), iron compounds (Fe^{3+}), *etc*. The interaction of these potent oxidizers creates a synergistic effect, contributing to highly efficient water purification. AOPs processes occur during the oxidation reaction of organic substances facilitated by powerful hydroxyl radicals (OH^-) or oxygen radicals (superoxide, hydroperoxide, and singlet oxygen). This process results in the removal of persistent organic pollutants, toxic and hazardous compounds from wastewater, and enhances biodegradation. As a result, the efficiency of disinfection against pathogenic microorganisms increases[13, 14–19].

The AOPs processes, which include such combinations as O_3/UV, O_3/H_2O_2, O_3/H_2O_2/UV, O_3/Fe^{2+}, H_2O_2/UV, O_3/metal oxide catalyst, O_3/activated carbon, O_3/ultrasound, O_3/Fenton, and photocatalytic ozonation, provide effective detoxification of heavy industrial wastewater, including the efficient disinfection of pathogens[13]. The use of hydrogen peroxide as a disinfectant in wastewater treatment promotes environmentally friendly processes without the formation of toxic by-products. Atomic oxygen, formed during the decomposition of hydrogen peroxide, possesses strong oxidizing power and bactericidal properties. The active radical OH- is non-selective against microorganisms and other organic contaminants in wastewater[20, 21]. However, H_2O_2 exhibits a bactericidal effect at high concentrations. This can contribute to its increased presence in wastewater discharges, reaching established and extremely dangerous concentrations (0.01 mg/dm^3) for water bodies used for fishing. However, when hydrogen peroxide interacts with other oxidants during AOPs reactions, the redox properties of H_2O_2 increase significantly, even with minimized doses.

Recently, ozone technologies have been widely employed for the purification of wastewater and drinking water. Ozone, which is dissolved in water, has a high oxidation-reduction potential, which leads to the deep decomposition of organic pollutants and the destruction of bacterial cells[13]. Inactivation is achieved through the molecular form of ozone (direct oxidation) or through the hydroxyl radical (indirect oxidation), which forms through reactions (1), (2)[22]:

$$O_3+2H^+ +2e^- \rightarrow O_2+H_2O \quad (1)$$

$$O_3+H_2O+ e^- \rightarrow O_2+2OH^-. \quad (2)$$

However, the direct reaction of ozone oxidation is slow and requires larger doses than indirect reactions. A significant enhancement in the oxidizing capacity of O_3 occurs during AOPs reactions, for example, in combination with UV irradiation (O_3/UV) or in the presence of hydrogen peroxide (O_3/H_2O_2). When ozone interacts with hydrogen peroxide (O_3/H_2O_2), hydroxyl radicals and active oxygen are formed according to the reaction (3):

$$H_2O_2 + 2O_3 \rightarrow 2OH— + 3O_2. \quad (3)$$

Synergistic oxidation processes occur due to the formation of reactive OH-radicals, which significantly accelerate all reactions, even at low concentrations of O_3. The redox potential (E^θ) of H_2O_2 is 0.68 V; E^θ O_3 is equal to 2.07 V, and E^θ of the OH-radical is 2.9 V. At the same time, non-specific oxidation reactions of almost all complex organic substances occur, including the elimination of microorganisms and viruses[13, 20]. Sommer *et al.*[23] investigated the biocidal efficiency of the AOPs process in the case of the combined application of O_3/H_2O_2 during the treatment of groundwater contaminated with a set of test microorganisms: *Escherichia coli* (vegetative cells), *Bacillus subtilis* (spore cells), and viruses (bacteriophages) (F+ RNA). A significant effect of decontamination was established for *Escherichia coli* (*E. coli*) and viruses, with a reduction in their content by approximately 6-log. The content of *Bacillus subtilis* spores decreased by 0.4-log.

The results corresponded to the effect of ozone treatment, with a residual content of 0.4 mg/L after 4 min of contact (at 20 °C). A significant bactericidal effect was observed when using a combination of oxidants O_3/H_2O_2/UV irradiation in water disinfection practices. At the same time, the disinfection of *E. coli* culture occurred in a shorter period compared to the use of each agent separately. The synergistic effect of action was achieved at the H_2O_2 concentration of (1–2) g/dm^3 and ozone concentration of 1.6 mg/dm^3 with UV irradiation. The

highest value of the synergism coefficient was observed at the combined effect of H_2O_2 (2 g/dm^3) and UV radiation at a dose of 7 mJ/cm^2, as well as with the introduction of ozone and simultaneous irradiation of the suspension with a UV radiation dose of 4.82 mJ/cm^2. However, hydrogen peroxide concentrations are considered to be too high[24].

Combining ozone with other oxidants enables the inactivation of a broad spectrum of microorganisms and viruses, achieving a population viability reduction of more than 3 logs. This includes the disinfection of *Staphylococcus aureus*, which occurs in 2 min, as well as *Pseudomonas fluorescens* and *Listeria monocytogenes*, which take about 8 min.

It was established that the AOPs reaction, activated by hydrogen peroxide and persulfate under the influence of photocatalysis with the Fe (III) complex, allows the inactivation of *Enterococcus faecalis* – fecal microorganisms in wastewater, which typically exhibit significant resistance to disinfection.

The mechanism of bioagents decontamination under the influence of hydroxyl or oxygen radicals is associated with the damage of the cell membrane, destruction of proteins, and oxidation of lipids, enzymes, and genetic material (DNA or RNA). This leads to the elimination of pathogens and the effective disinfection of wastewater[25, 26]. The food industry, including dairies and meat processing plants, has recently experienced rapid growth. These industries consume significant amounts of water and generate highly polluted wastewater[27]. Such wastewater is a complex polydisperse system containing residues of animal proteins, fats, carbohydrates, and mineral salts, which is an excellent environment for the development of microorganisms, including pathogens. Therefore, high-quality wastewater treatment and disinfection before discharge into a water body are crucial tasks.

The purpose of this study is to evaluate the effectiveness of the wastewater decontamination process using the synergistic oxidation reactions resulting from the interaction of ozone with hydrogen peroxide (the AOPs method) and to determine the impact on the microbiota of potential residues from the decomposition of reagents in the treated water.

2. Experimental

The object of the study was disinfection methods of model wastewater using such natural oxidants as hydrogen peroxide (H_2O_2), ozone (O_3), and their combination (AOPs). For the study, a highly concentrated model wastewater was used, which corresponded in composition to the wastewater generated at dairy processing plants and contained milk protein, carbohydrates, and whey sugars. Municipal wastewater, which is the main source of fecal microorganisms, was added to the solution. The model wastewater was then treated using the biosorption method, followed by disinfection using the oxidation method (O_3/H_2O_2). The complete cycle of treatment and disinfection was conducted using a laboratory setup, which comprised two settling tanks, a disk-type bioreactor with an immobilized biofilm, and a decontamination chamber.

The scheme of treatment with disinfection according to the AOPs method is given in Fig. 1.

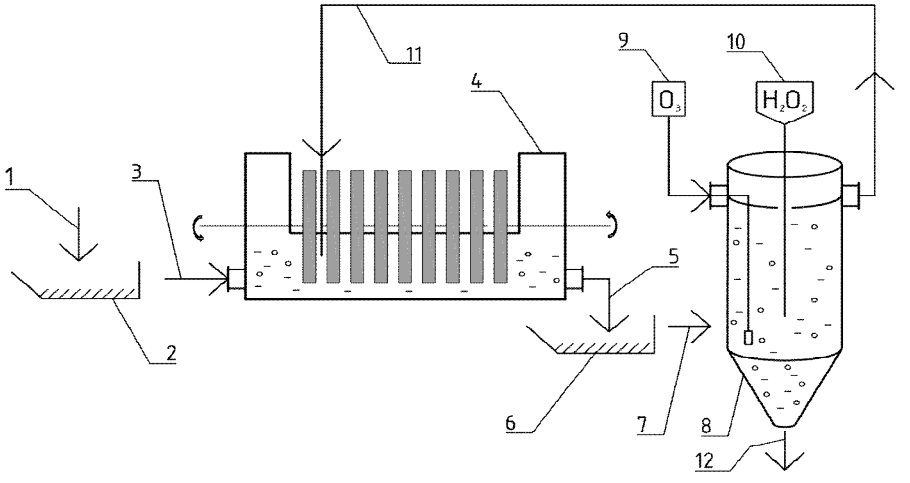

Fig. 1. Scheme of wastewater treatment with disinfection according to the AOPs method (O_3/H_2O_2):
1 – source water, which is directed for purification and disinfection; 2 – primary settling tank;
3 – settled source water; 4 – experimental biosorption installation (hereinafter – bioinstallation);
5 – purified water; 6 – secondary settling tank; 7 – purified water, which is supplied for disinfection;
8 – decontamination tank; 9 – ozonator; 10 – H_2O_2 dispenser;
11 – return of excess ozone; 12 – purified and disinfected water

Wastewater (1), after preliminary removal of ether-soluble substances and gravity removal of suspended solids (2), directed (3) to the bioinstallation (4) – a horizontal disk-type reactor with a working volume of 6.5 dm^3. The material of the movable disks had a cellular structure, which contributed not only to the effective immobilization of the biofilm but also to the creation of microbiocenoses of various types: on the outer surface of the disks, a biocenosis was formed mainly by aerobic chemoorganoheterotrophic and chemolithoautotrophic microorganisms, while inside the disks, a biocenosis was formed, the metabolism of which was directed towards redox processes in anaerobic and anoxic conditions.

This allows to effectively treat wastewater with a multicomponent composition. The total concentration of immobilized and suspended biocenosis was approximately 14–16 g/dm^3. Purified wastewater (5) from the bioreactor was diverted to a secondary settling tank (6), where excess biomass settled, and then fed (7) to a decontamination chamber (8) of 1.2 m high, where water was disinfected and treated with ozone (O_3) (item 9), hydrogen peroxide (H_2O_2) (item 10) and their combination and then supplied (7) to the 1.2 m high decontamination chamber (8), where water was disinfected and treated with ozone (O_3) (9), hydrogen peroxide (H_2O_2) (10) and their combination (O_3/H_2O_2).

Ozone was produced directly from the air using a "glow" discharge in a special device – an ozonizer. Ozone was introduced into the water through ejectors operating under elevated back pressure. The ozone mixture entered the water through filter plates. The ozone dose in the mixture was 5 mgO_3/dm^3; the ozone consumption was 1 dm^3/min with the concentration of 7 mgO_3/dm^3. Hydrogen peroxide was added through a dispenser, drop by drop, with a concentration of 3.5 mg/dm^3 of liquid. The O_3/H_2O_2 ratio was 1/0.5. The doses and concentrations of ozone and hydrogen peroxide for experimental studies were selected based on the type of pollutants and the initial concentration of microbial contamination, taking into account previous experiments. If excess ozone is generated, it can be redirected to the bioreactor for water saturation, contributing to the enhancement of oxidation processes (11).

The characteristics of the purification and disinfection processes were determined under the contact conditions of the experiment.

Experiments were carried out at a temperature of (25±1) ^{0}C. According to microbiological methods, the control of the disinfection process was monitored by the following indicators: indicators of fecal contamination – Bacteria of the Escherichia coli Group (BEC), enterococci and coliphages, Total Viable Count (TVC). The concentration of bacteria per unit volume of water was measured in the number of colony-forming units (CFU); and the concentration of coliphages – in the number of plaque-forming units (PFU). Microbiological indicators were measured according to the procedure described by several authors[28–30]. Hydrochemical indicators (COD, pH, dissolved oxygen) were measured according to the certified methods.

The properties of purified and disinfected wastewater in terms of aquatic microbiocenosis were tested using the biochemical method of determining the dehydrogenase activity (DHA). DHA of activated sludge microbiocenosis was determined according to a modified method used at biological treatment plants.

The essence of the method is that a colorless water solution of 2,3,5-triphenyltetrazolium chloride (TTC) is transformed into triphenylformazan (TF), which has a red color, under the influence of dehydrogenases of bacterial cells. The higher concentration of dehydrogenase enzymes in the solution results in a more vibrant color of the studied water sample, indicating active metabolic processes within the cells. Conversely, when the external environment contains toxicants, DHA is inhibited, leading to reduced TF formation and a change in the color intensity of the sample, often resulting in discoloration. The intensity of the solution color was recorded using spectrophotometry. A nutrient substrate (1 % glucose solution and 0.5 % TTC reagent solution) was added to each water sample under study. After keeping the samples at a temperature of 40 ºC for 40 min and extracting TF with ethyl alcohol, the light transmittance of the samples was determined on a spectrophotometer at a wavelength of 490 nm. The amount of formed TF corresponded to the dehydrogenase activity of activated sludge (AS). DHA was calculated according to the formula (4):

$$Cx = (41.224 \cdot D + 0.0362) \cdot n/V \quad (4)$$

Cx is DHA, mg/L; D is an optical density of formazan, nm; n is a degree of dilution (1:2); and V is a volume of the analyzed sample (1 mL).

Statistical processing of experimental data was performed using Microsoft Excel.

3. Results and Discussion

3.1. The Results from Experimental Studies on the Decontamination Process of Model Effluent Using Various Methods

The bacteria of the Escherichia coli group (*E. coli*), which entered the bioinstallation with untreated model wastewater, were partially integrated into the immobilized biocenosis but some remained suspended. However, they

also played a role in the decomposition and transformation of organic substances. During the oxidation of water components, *E. coli* multiplied, but their growth was partially restricted by the nutrient substrate, which decreased over time. Nevertheless, their concentration remained very high in treated wastewater. Table 1 shows the results of the model wastewater treatment and the dynamics of BEC.

Table 1. Dynamics of *E. coli* during model wastewater treatment in the biodisc reactor

Treatment period, h	Model wastewater treatment in biodisc reactor		
	pH	COD, mgO/dm^3	Amount of *E. coli*, CFU /dm^3
Model wastewater received for treatment	5.3	1050.0	$(8.9 \pm 1.8)\cdot10^6$
1	6.18	790.0	$(8.2 \pm 2.4)\cdot10^6$
3	7.54	530.0	$(2.4 \pm 0.4)\cdot10^6$
4	7.93	390.0	$(8.2 \pm 1.5)\cdot10^5$
5	7.94	160.0	$(5.8 \pm 1.2)\cdot10^5$
6	8.04	90.0	$(2.8 \pm 1.4)\cdot10^5$
7	8.20	90.0	$(3.2 \pm 1.2)\cdot10^5$

Table 2. Disinfection of treated model wastewater

Treating time, min	Disinfection with hydrogen peroxide (H_2O_2)			Disinfection with ozone (O_3)			Disinfection with AOPs method (O_3/H_2O_2)		
	pH	COD, mgO/dm^3	Index of *E. coli*, CFU /dm^3	pH	COD, mgO/dm^3	Index of *E. coli*, CFU /dm^3	pH	COD, mgO/dm^3	Index of *E. coli*, CFU /dm^3
0	8.04	90.0	$(3.2 \pm 1.2)\cdot10^5$	8.04	90.0	$(3.2 \pm 1.2)\cdot10^5$	5.30	90.0	$(3.2 \pm 1.2)\cdot10^5$
15	8.00	90.5	$(8.5 \pm 1.2)\cdot10^4$	7.75	85.5	$(1.5 \pm 1.3)\cdot10^4$	5.89	65.5	$(1.0 \pm 0.2)\cdot10^3$
30	8.10	85.0	$(1.5 \pm 1.3)\cdot10^4$	7.10	85.0	$(1.2 \pm 1.4)\cdot10^3$	6.50	65.0	566 ± 15
60	7.52	85.0	$(1.2 \pm 0.4)\cdot10^4$	7.20	80.0	$(1.2 \pm 0.4)\cdot10^2$	6.90	65.0	80 ± 24

Note: According to regulatory requirements,[12] treated wastewater should not contain more than 1000 CFU/dm^3 of BEC.

It can be observed from Table 1, that within 6 h under contact conditions in the bioreactor, the efficiency of model wastewater treatment for organic pollutants is 91 %. During this period, there was a slight decrease in *E. coli*, approximately by a factor of 10 (1-log). Such water is considered to be hazardous and, if intended for discharge into a surface water body, requires disinfection. Therefore, wastewater treated for 7 h in the bioreactor was additionally disinfected under contact conditions by the addition of hydrogen peroxide, ozonation, and through the method of combining ozonation with hydrogen peroxide (O_3/H_2O_2). The parameters of the treated wastewater in the biosorption plant served as the starting points for the decontamination process. The results of the experiment are given in Table 2.

Table 2 shows that the *E. coli* index in wastewater, under the influence of H_2O_2, decreased on 1-log in 15 min of treatment to the amount of $(8.5 \pm 1.2)\cdot10^4$, and further decontamination was practically not observed. During interaction with O_3, the *E. coli* index on 1-log in 15 min of treatment, and on 2-log in 30 min and almost reached the normative values $(1.2 \pm 1.4)\cdot10^3$. The disinfection of water by the AOPs method over the same period, compared to the disinfection by H_2O_2 and O_3 separately, was much faster. Thus, after 15 min of treatment by the combined method, the amount of *E. coli* decreased on 2-log and reached a value corresponding to the normative values for discharging such wastewater into a surface water body. One can see that during additional treatment of wastewater, post-oxidation of its organic components was observed. During water treatment with the combined method, after 15 min the amount of COD also reached acceptable values and wastewater can be discharged into a surface water body. While using the AOPs method a pH shift to the alkaline side was observed.

To establish the effect of strong oxidation reactions (O_3/H_2O_2) on biosorption treatment, an experiment was conducted with pretreatment of model wastewater using the AOPs method. The wastewater treatment scheme was changed in such a way that the decontamination tank (Fig. 1, pos. 8) was installed at the beginning of the treatment process before the bioreactor. After the untreated water came into contact with O_3 in the

presence of H_2O_2, it was directed to a biological reactor, where the model wastewater underwent biological treatment. The parameters of the model wastewater in the process of such treatment are shown in Table 3.

After wastewater treatment in the decontamination chamber, a slight decrease in COD (by 14 %) was observed; wastewater saturation with oxygen increased from 3.74 mg/dm^3 to 5.08 mg/dm^3, which is a positive factor for further biological treatment. In the first hours of treatment in the bioreactor, a rapid decrease in COD was observed, which can be explained by the fact that the pretreatment of the influent wastewater by the AOPs method (O_3/H_2O_2) allowed oxidizing the complex polymer chains of protein molecules and milk carbohydrates to simpler compounds, which became an easily accessible substrate for immobilized microbiocenosis and free-floating bacteria. As the result of the intensive constructive metabolism of microorganisms, on the one hand, the rate of destruction of pollutants increased, but, on the other hand, the biomass of the immobilized biocenosis and the number of bacteria in general, including BEC, increased rapidly. Under the experimental contact conditions, this resulted in a decrease in the efficiency of wastewater treatment and secondary pollution of wastewater due to excess biomass.

To establish the possibility of model wastewater disinfection by the AOPs method in the process of its treatment by the biosorption method, the following experiment was performed. Highly concentrated model wastewater (COD – 1400 mgO/dm^3) with a contamination index according to the *E. coli* – $5.6\cdot10^6$ CFU/dm^3, *Coliphages* – $8.8\cdot10^4$, PFU/dm^3, *Fecal enterococci* – $5.2\cdot10^4$ CFU/dm^3 and TVC – $2.6\cdot10^7$ CFU/cm^3 were treated in an experimental biosorption unit for 6 h (Table 4).

Table 3. Parameters of model wastewater treatment at the biodisc reactor with its pretreatment by the AOPs method (O_3/H_2O_2)

Parameter	Untreated model wastewater	Pretreatment with O_3/H_2O_2	Wastewater treatment in bioreactor after pretreatment with O_3/H_2O_2				
			1 h	2 h	3 h	4 h	5 h
O_2, mg/dm^3	3.74	5.08	3.02	3.52	4.16	5.31	5.21
pH	5.25	5.25	6.40	6.66	6.83	6.85	6.88
COD, mgO/dm^3	2180	1865	1230	805	368	155	180
TVC, CFU /cm^3	$9.5\cdot10^7$	$1.5\cdot10^4$	–	–	–	–	$2.9\cdot10^8$
BEC, CFU /dm^3	$4.6\cdot10^6$	$2.6\cdot10^4$	–	–	–	–	$3.8\cdot10^6$

Table 4. Parameters of model wastewater treatment in the laboratory biosorption unit

Parameters	Input data	Treatment period, h					
		1	2	3	4	5	6
COD, mgO/dm^3	1400	816	516	280	218	168	122
TVC, CFU /cm^3	$2.6\cdot10^7$	–	–	$1.2\cdot10^5$	–	–	$4.6\cdot10^4$
BEC, CFU /dm^3	$5.6\cdot10^6$	–	–	$3.6\cdot10^5$	–	–	$6.4\cdot10^4$
Coliphages, PFU/dm^3	$9.8\cdot10^5$	–	–	$6.2\cdot10^4$	–	–	$1.8\cdot10^4$
Enterococci, CFU/dm^3	$5.2\cdot10^4$	–	–	$8.2\cdot10^3$	–	–	$3.2\cdot10^3$

After three hours of treatment, a part of the wastewater was selected for further treatment in the decontamination chamber using the AOPs (O_3/H_2O_2) method, and treatment continued for another three hours in the bioreactor. As can be seen from Table 4, during the first 3 h of treatment, COD decreased to 280 mgO/dm^3, and after 6 hours – to 122 mgO/dm^3. But the concentration of bacteria and viruses after 3 h decreased slightly – on approximately (1–2)-log. After 6 h TBC decreased on 3-log, E. coli – on 2-log, coliphages and enterococci – on 1-log. The concentration of *E. coli* and coliphages did not meet the permissible standards for the discharge of treated wastewater into a surface water body. Wastewater with such characteristics requires mandatory disinfection.

The characteristics of the wastewater treated in the bioreactor for 3 h served as the initial parameters for further processing in the decontamination chamber (Fig. 1, pos. 8). Hydrogen peroxide was injected *via* dispenser, and ozonation was carried out with an ozone-air mixture produced in the ozonator. The results of the research are given in Table 5.

After additional wastewater treatment in the decontamination chamber, a significant decrease in the content of all types of bacteria was observed already after 10 min of decontamination. Within 20 min of treatment, the indices of BEC (860 CFU/dm^3) and coliphages (25 PFU /dm^3) corresponded to the normative values for treated wastewater that can be discharged into

a surface water body. Wastewater with a *coli-index* of no more than 1000 per 1 dm^3 and coliphages of no more than 1000 per 1 dm^3 after disinfection is considered epidemically safe[31]. It was noted that significant disinfection of coliphages occurs, presumably due to the rapid oxidation of the lipoprotein component of the virus membrane. The structure of the viruses is easily exposed to strong oxidizing agents O_3 and H_2O_2[32]. A significant decrease in the concentration of enterococci by (2–3)-log, which are considered the most resistant to chlorine oxidizers, was also observed. After 10 min of treatment, the content of BCCP decreased by 100 times, and after 40 min – on 4-log.

Along with decontamination (disinfection), wastewater was additionally treated for the COD parameter, which was reduced to 75 mgO/dm^3 after 20 min of treatment. This value also complies with the regulatory limits for discharging treated wastewater into surface water bodies[31].

If excess ozone is generated in the water, it is either released into the air, where it quickly decomposes, or it can be returned to the bioreactor to saturate the liquid, promoting oxidative processes during biosorption purification. (Fig. 1, pos. 11). Due to the decomposition of organic substances in household wastewater, which are known to cause unpleasant odors, we observed an environmental deodorizing effect.

Table 5. Additional treatment and disinfection of model effluent using AOPs method

Parameter	Before treatment	Period of treatment, min			
		10	20	30	40
O_2, mg/dm^3	3.22	6.21	5.26	5.28	6.04
COD, mgO/dm^3	280	105	75	45	42
TVC, CFU $/cm^3$	$1.2 \cdot 10^5$	1200	722	132	112
BEC, CFU $/dm^3$	$3.6 \cdot 10^5$	7800	860	290	125
Coliphages, PFU/dm^3	$6.2 \cdot 10^4$	240	25	is not defined	is not defined
Enterococci, CFU/dm^3	$8.2 \cdot 10^3$	615	256	121	is not defined

3.2. Examining the Impact of Water Decontaminated Using the AOPs (O_3/H_2O_2) Method on the Vital Activity of Aquatic Microbiocenosis

Ozone, which is a strong oxidizing agent, can remain in purified and disinfected water. Therefore, studies were conducted to assess the impact of potential reagent residues in such water on the viability of aquatic microbiocenosis.

Under the influence of various chemicals, the enzymatic processes of microorganisms can be activated or inhibited. Hydrogen peroxide and ozone, which are dissolved in water, under certain concentrations can saturate the aquatic environment with dissolved oxygen and stimulate redox processes during cellular metabolism. However, under certain concentrations, they can be toxic to microbiocenosis and inhibit the enzymatic system. Therefore, the impact of disinfected water on the viability of microbiocenosis was determined through the activity of dehydrogenase enzymes (DHA), which serve as biocatalysts in the metabolism of microorganisms and sensitively react to the influence of toxic substances. The degree of its impact on the microbiocenosis was determined by comparing the DHA of activated sludge under optimal conditions of existence with the DHA of activated sludge after contact with purified and disinfected water.

The test variant (control sample) was the microbiocenosis of activated sludge (AS) taken from the regenerator of a municipal wastewater treatment plant (WWTP). Samples that were studied: untreated wastewater, treated wastewater in the bioreactor, wastewater from decontamination chambers (AOPs) during decontamination, and wastewater from the secondary sedimentation tank after decontamination. Activated sludge samples were mixed sequentially with studied wastewater samples. AS control samples were mixed with a physiological solution. The procedure for determining the DHA was carried out according to the methodology. The results corresponded to the amount of triphenylformazan (TF) formed in AS samples. After recalculation in units of biomass volume (Eq. 4), the value of DHA of AS samples was obtained (Table 6).

The toxicity level of the examined water samples was evaluated in comparison with the control sample of activated sludge (Fig. 2).

The data in Table 5 and Fig. 2 show that the control sample exhibited “zero” toxicity. The water sample from the decontamination chamber, where the model wastewater was disinfected under the combined action of strong oxidants (O_3/H_2O_2), inhibited the enzymatic activity of activated sludge and had the highest toxicity of 81.2 % (Fig. 2, pos. 4). Moreover, under the influence of untreated model wastewater, the dehydrogenase activity (DHA) of activated sludge microorganisms decreased,

resulting in toxicity of 35.3 % (Fig. 2, pos. 2). This likely occurred due to the high concentrations of organic substances in the untreated wastewater.

During wastewater treatment in the bioreactor, the negative impact of wastewater decreased, and the activity of enzymes increased, leading to a decrease in toxicity, which corresponded to 14.5 % (Fig. 2, pos. 3). In the secondary settling tank, the water exhibited no toxic characteristics; on the contrary, the DHA of the activated sludge increased during contact with it, attributed to the saturation of the wastewater with molecular oxygen (Fig. 2, pos. 5). Thus, it was established that wastewater disinfected by the AOPs method (O_3/H_2O_2) is not toxic to microbiocenosis; on the contrary, the activity of enzymatic reactions of microorganisms increased under its influence.

Table 6. Results of DHA measurement of activated sludge (AS) according to the optical density of the formazan solution

Test examples	Optical density TF (average)	DHA, mg/dm^3 (average)
Activated sludge from municipal WWTP + physiological solution (control sample)	2.54	105.64
Activated sludge + untreated water	1.65	68.42
Activated sludge+ wastewater, treated in bioreactor	2.18	89.89
Activated sludge + treated wastewater during decontamination (H_2O_2/O_3)	0.48	19.88
Activated sludge + treated and decontaminated wastewater from a secondary settling tank	2.72	112.42

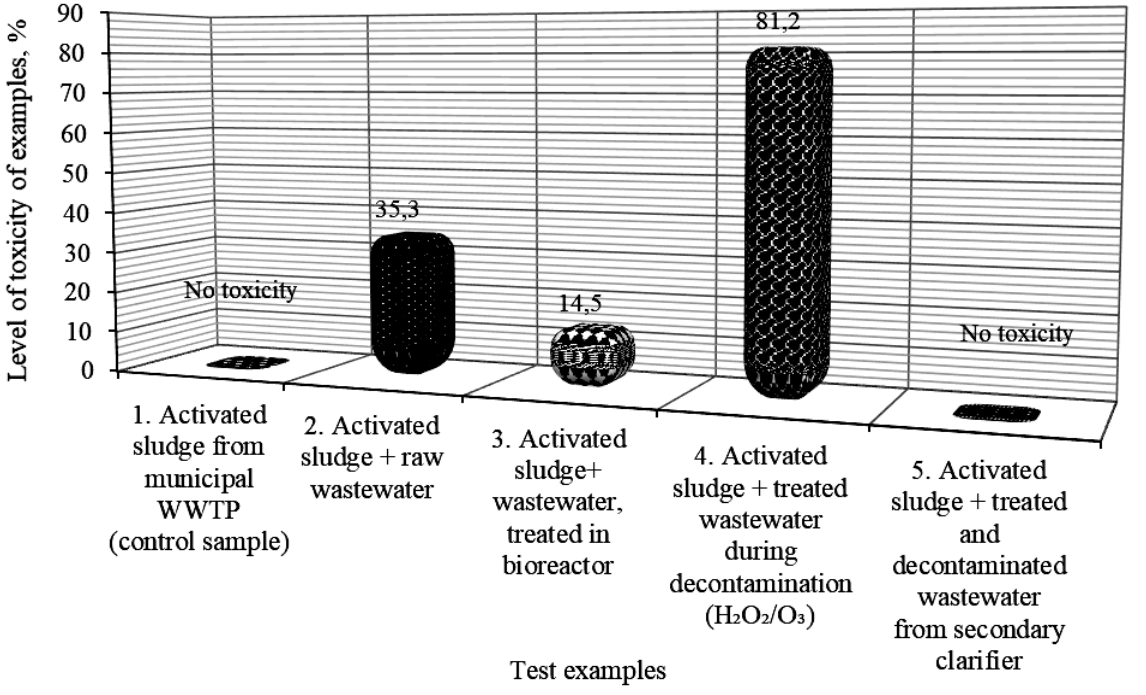

Fig. 2. Level of toxicity of the wastewater samples under study

4. Conclusions

The combination of natural oxidizing agents, such as ozone and hydrogen peroxide (referred to as the AOPs reaction), produced a potent bactericidal effect during the decontamination of model wastewater. The composition of the model wastewater corresponded to wastewater from dairy processing plants. As a result of such disinfection, there was an effective elimination of bacteria and viruses (coliphages), including the indicator group: no less than 3-log population of *E. coli*, 4-log – coliphages. The content of fecal enterococci resistant to disinfection by traditional methods decreased by 100 times after 10 min of treatment, and after 40 min they completely disappeared. It was found that during treated wastewater disinfection, organic pollutants were oxidized, resulting in COD not

exceeding the permissible values for discharging treated wastewater into a surface water body. It was confirmed that the residues of reagents after water disinfection are not toxic to aquatic microbiocenosis, and the remnants of free ozone stimulate metabolic processes in cells, increasing the activity of their enzymatic systems. It was found that the processes of pollutants oxidation and water disinfection can be accelerated by about half if, after 3 h of biosorption treatment, water is treated in a decontamination unit using the AOPs oxidation method. After 20 min of decontamination, BEC index reached 860 CFU/dm^3, and the coliphage index – 25 PFU/dm^3, *i. e.* the decontaminated wastewater, according to the sanitary microbiological indicators that are controlled, corresponded to the permissible normative values for discharge into a surface water body. The advantages of using synergistic oxidative methods, in particular, combined O_3/H_2O_2 for wastewater disinfection, are noted: significant efficiency of the process – disinfection of indicator microflora reaches regulatory concentrations; high rate of destruction of populations of water-polluting microorganisms – 15–20 min; additional oxygen saturation of purified and disinfected water; environmental friendliness of the process, since by-products formed in wastewater after disinfection do not have a negative impact on the microbiocenosis of the aquatic environment.

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ДЕКОНТАМІНАЦІЯ БІОЛОГІЧНИХ АГЕНТІВ У СТІЧНИХ ВОДАХ ЗА ДОПОМОГОЮ СИНЕРГЕТИЧНИХ ОКСИДНИХ МЕТОДІВ ЯК ЕФЕКТИВНИЙ СПОСІБ ЗАХИСТУ ЗДОРОВ'Я НАСЕЛЕННЯ І ВОДНИХ ЕКОСИСТЕМ

Анотація. *Для виконання досліджень використовували висококонцентровані модельні стічні води, які за складом відповідали стічним водам, що утворюються на виробництвах молокопереробних підприємств, і містили молочний білок, вуглеводи й цукри сироватки. У них додавали господарсько-побутові стічні води, які були джерелом індикаторних фекальних мікроорганізмів. Схема очищення і знезараження води складалася з оброблення модельного стоку біосорбційним методом за допомогою іммобілізованого біоценозу на лабораторній установці з подальшим доочищенням і знезараженням у резервуарі деконтамінації. Порівняно ступінь елімінації мікроорганізмів окремо методами озонування, оброблення пероксидом водню і комбінуванням O_3/H_2O_2. Показано, що очищені та знезаражені за методом AOPs модельні стічні води додатково містять розчинений кисень, який не є токсичним відносно водного мікробіоценозу.*

Ключові слова: *знезараження, стічні води, пероксид водню, озон, біосорбційне очищення, токсичність.*