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BIOCHEMICAL ASPECTS OF WATER DECONTAMINATION WITH OZONE

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Abstract: *An article deals with the mechanism of ozone effect on microorganisms. It is known that ozone can destruct various bacteria. Kinetics of bacteria inactivation under the influence of ozone depends on ozone content in the ozone-air mixture, dissolved ozone concentration, amount of ozone absorbed by bacteria, time of the microorganisms' exposure and their type. It is suggested that in the biochemical sense, ozone acts through the catalytic oxidation of bacterial proteins and their destruction to amino acids. An influence of the oxidized products of bacteria decay on the kinetics of the residuary bacteria dying in the process of water ozonization is evaluated. It is proved that the water ozonization promotes better population health conditions.*

Keywords: *ozone, fluorescence, bovine serum albumin, conformations changes, microorganisms*

1. Introduction

New effective technologies of the municipal wastewater decontamination have been developed in the beginning of 20^{th} century. These technologies involve infiltration fields, jet filters with sand and broken stone filling and forced ventilation air-tanks. The latter equipment is used as the key stage at the municipal wastewater treatment plants. Simple wastewater decontamination was considered as the main target of the treatment technologies before. However, better understanding of complexity of the clean water problem has been developed later and pushed more efforts towards higher quality of the water treatment and protection of all kinds of the natural water and water bodies.

Modern approaches to the high quality wastewater treatment assume almost complete decomposition of the organic pollution agents. According to the actual legislation, the content of the residual organic compounds in water should not exceed 10 mg/l [1].

Bacterial contamination of water with municipal, meat-and-dairy, other food processing, tannery, sugar, wood-pulp, pharmaceutical, microbiological and other industrial wastewater is considered as one of the most dangerous types of the biological pollution. Dangerous bacterial contamination is also generated at the landfill areas. Pollution of the drinking and service water sources with wastewater prevents normal usage of these sources and brings serious sanitary and epidemic threats as water is considered as one of the main ways of the bacterial and virus infections proliferation [1].

New type of contamination became important in Ukraine after the Chornobyl nuclear catastrophe, which left strong radionuclide anthropogenic pollution in many areas. This caused qualitative and quantitative changes in some natural microbiocenoses and resulted higher aggressivity of some enteropathogenic strains [2].

All these factors contribute into higher risk of contagion with various enteric infections. Moreover, a chance contamination with genetically modified organisms becomes more probable and this can cause new, uninvestigated diseases [2]. Besides, some bacteria can gain chlorine tolerance as a result of the genetic changes and survive after the chlorine water disinfection, which is still widely used in Ukraine as a stage of the drinking water production.

Water chlorination has some serious shortcomings [3]: chlorine does not provide needful effectiveness against some types of microbes and ensure only selective contamination of some vital centres of bacteria. This contamination is quite slow because of the low diffusion rate of chlorine into cytoplasm. Chlorine is also almost completely passive against viruses.

Many countries have stopped a practice of chlorination and use water ozonization instead. Ozone is very effective disinfection agent and it reveals strong bactericide and virucide activity together with quite high effectiveness in decomposition of many organic pollutants to rather nontoxic products [3-5].

Therefore, this work was aimed onto investigation of the destruction kinetics of some microbial taxonomic groups under effect of ozone and influence of ozone on some microbial proteins.

2. Materials and methods

Ozone was generated by the barrier and non-barrier discharge tube generators designed in Institute of Cryobiology and Cryomedicine Problems of NAS of Ukraine and Institute of the Plasma Electronics and New Methods of Acceleration of Kharkiv Physico-Technical Institute.

Ozone concentration was measured by the spectrophotometry method through the Hartley light absorption band (255 nm) value. This method can be applied to the ozone concentration measurement in the gaseous and liquid phases [6].

Water samples were contaminated with the following microbial cultures *Escherichia coli, Staphylococcus aureus, Candida albicans, Bacillus subtilis* and then gaseous mixtures with the ozone concentration 6,8-7,0 mg/l were barbotaged through the samples. The barbotage time was different for various microbes [7].

It was found that the complete deactivation of the bacteria *Escherichia coli, Staphylococcus aureus* and yeast-like fungi *Candida albicans* required 60 min of barbotage while the spore forms of *Bacillus subtilis* required 120 min [7].

The area of ozonization is widening but details of its antimicrobic activity are still disputable. It is supposed that biochemical bactericide effect of ozone can be caused by the catalytic oxidation of the bacterial proteins. Ozone exerts an impact on the entire redox system of bacteria and their protoplasm while chlorine influences ferments of the microbial cell only. This concept can be proved by the high antiviral activity of ozone.

Bactericide effect of low doses of ozone is rather insignificant for the bacteria cultures in the organic-free water but it jumps to much higher level as soon as concentration of ozone reaches some critical value. Then further deactivation of bacteria goes on with significantly lower intensity. For example, two separate phases can be identified in a process of *Escherichia coli* deactivation in the bidistilled water: initial fast deactivation followed by continuous and much slower decontamination. This pattern depends on several parameters: amount of ozone consumed by bacteria, concentration of the dissolved ozone in water and content of ozone in the ozone-air mixture. It was found [6] that kinetics of the initial fast deactivation of *Escherichia coli* is governed by the amount of ozone consumed by the bacteria. On the other hand, this parameter was found non-influential for the second phase of deactivation. Long and less active "tailoring deactivation" can be related to protection of the survived bacteria with the bacterial cells lysis products. Initial consumption of ozone is very active and the majority of bacteria die out during this stage. Besides, kinetics of deactivation also depends on the microbes' nature and presence of some organic agents. They can react with ozone and form secondary products with higher bactericide activity. For instance, ethylenes can react with ozone and form hydroperoxides which reveal higher bactericide activity than ozone. In contrary, humic acids react with ozone and do not form any secondary bactericides so, amount of ozone required for effective decontamination should be increased in case any humic compounds are present in the system. The higher is concentration of the humic acids, the longer latent stage without any tangible bactericide activity will be registered after ozonization [6].

3. Results and discussion

The model investigation of influence of ozone on the conformational changes in the microbial proteins (serum albumin) has been carried out to clarify details of the ozone effect on bacteria [8].

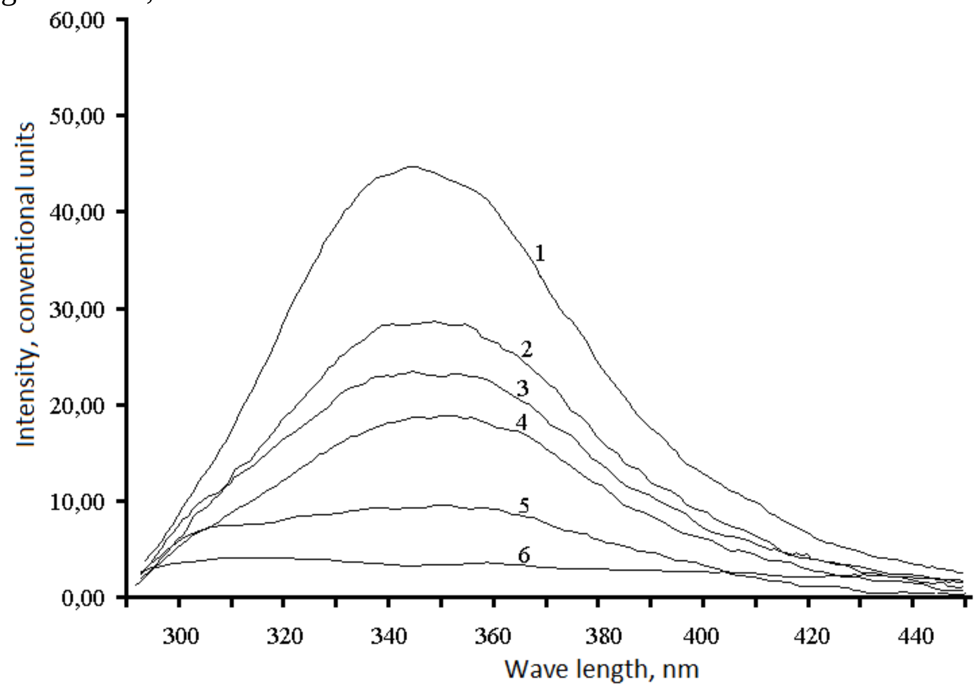

Figure 1. The intrinsic fluorescence spectra of BSA after bubbling of the ozone-oxygen mixture (ozone content 7,0 mg/l) during various periods of time. 1 – control experiment (no ozone mixture bubbled); 2 – 0,5; 3 – 1; 4 – 2,5; 5 – 4; 6 – 6 min. Exciting radiation wavelength – 280 nm.

The bull serum albumin (BSA) has been exposed to the high doses of ozone that should result in deactivation of microbes. BSA samples were dissolved in the buffer-free physiological solution and then the ozone-oxygen mixture with 7,0 mg/l of ozone was bubbled during various time periods. Activity of BSA was measured after the bubbling using the fluorescence spectra [8].

These spectra are shown in Fig. 1 for various times of the ozone exposition. The wavelength of the exciting radiation was 280 nm and the wavelength of the fluorescence maximum was 338 nm. As seen in Fig. 1, the fluorescence intensity decreases after the ozone exposition and no fluorescence can be registered after 6 min long exposition [8].

4. Conclusion

Analysis of the fluorescence spectra shows that destruction of the protein globule results in its complete decomposition down to amino-acids. It can be supposed that ozone acts on the other bacterial proteins similarly.

The results of this investigation can be helpful for better understanding of the biochemical effect of ozone on microbes, which promotes developing of more effective water decontamination ozone technologies. It is well known that environmental safety is one of the main competitive advantages of ozone.

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