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Application of Rapid Bioassay Method for Assessing Its Water Purification by Ferrate (VI) Potassium

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ABSTRACT
Appreciated integral toxicity of four water samples taken from various sources, urban and rural environment, and explored some of the properties of the reagent chemical purification of water - potassium ferrate K_2FeO_4. These data allow suggesting for practical use test system based on bacterial luminescence for express evaluation of the toxicity of chemical reagents used for water purification, selection of their effective concentrations and optimal processing time of water samples.
***Keywords*:** biological testing, bacterial luminescence test, ferrate potassium.

I. INTRODUCTION

Currently, the problem of water purification, both drinking and industrial-technical, highly relevant. Industrial and domestic water pollution are increasing (waste of heavy metals without proper recycling, industrial accidents, natural disasters, terrorist threats to water supplies and so on.) That requires compliance with water quality standards, search for new methods of its purification technologies (Henze et al., 2004). For water purification chlorine, sodium hypochlorite, chlorine dioxide, ozone, hydrogen peroxide, Fenton's reagent, and others are used. Some of these reagents cause chlorine pollution, others may result the formation of even more toxic by-products than the starting pollutants and gaseous oxidants could be used onlyfor water sources of limited volume. A new and very promising method of water purification is based on the use of ferrate (VI) alkali metal having an oxidizing and disinfecting effect (Jiang et al. 2002; Perfiliev 2002;Macova Z. et al. 2009). Decomposition products of the ferrates solution is ferric hydroxide, which is released in the form of colloidal aggregates with a very large surface area, effectively adsorbing heavy metal ions and particles of organic residues insuspensions. Their coagulative action provides additional water purification by adsorption of pollutants.

For the technology development in order to use ferrates water purification on an industrial scale are fundamental characteristics of their toxicological properties with a selection of convenient methods and objects bioassay. In all developed countries, legislated the use of methods of biological testing, sets dangerous xenobiotics on living organisms. In this regard, it is interesting systems based luminescent bacteria which are already well established as biological indicators environmental monitoring of water samples expressing the toxicity of various chemicals, compounds and the action of physical factors (ionizing radiation, electromagnetic radiation) (Steinbergat al. 1995, Medvedeva at al. 2009; Zarubina 2013; Zarubina and Sorokina 2015).

On the basis of genetically engineered a strain of *Escherichia coli* K12 TG1 created luminous biotest developed new biosensor test systems "Ecolum." They allow without osmoprotectant (solution of NaCl) and at higher temperatures, in contrast, for example, a well-known test systems «Microtox», the sensor which is a natural marine luminescent bacteria(Danilov at al. 2002).

In the present study we evaluated the toxicity of four water samples taken from various sources, urban and rural environment, and investigated the effectiveness of chemical treatment using a reagent - Potassium Ferrate K_2FeO_4.

II. MATERIAL AND METHODS.

The bioassay was performed using as a test organism engineered genetically engineered a strain of *Escherichia coli* K12 TG1 created a luminous phenotype provided by built-in *lux*-operon marine luminous bacteria *Photobacterium leiognathi* 54D10. The strain was obtained and stored at the Department of Microbiology, Faculty

of Biology, Moscow State University and known as a biosensor test system "Ecolum-06"(Danilov at al. 2002). The bioassay using the standard slurry after rehydration lyophilized biosensorbacteria for 30 minutes in 10 ml of sterile distilled water (pH = 7.0 - 7.4) and dilution of the slurry to $6.5 \cdot 10^7$ cell biosensor/ml.

The density of bacterial suspensions (cells/ml) was determined by nephelometric (λ = 670 nm) photoelectrocolorimeter KF77 (Poland) and expressed as the number of cells in 1 ml of the calibration curve.

Determination of the pH of aqueous samples was performed potentiometric ally.

The potassium ferrate, K_2FeO_4, was synthesized electrolytically by anodic dissolution of metallic iron in concentrated KOH solution content K_2FeO_4 > 95% (Macova Z. at al. 2009).

Water samples were collected from rural and urban sources in the early spring period: 1 - natural water of the Desna river basin in Moscow - the river; 7 - the same water sample after making potassium ferrate. 2 - a stream of loam to the city in Khoroshevskoye district of Moscow; 8 - the same water sample after making potassium ferrate; 3 - a small seasonal brook with a light-brown water in the Black Earth region of Istria garden; 9 - the same water sample after making potassium ferrate. 4 - mixture of snow and slush of water taken in the area of Moscow State University, 10 - the same water sample after making potassium ferrate.

Water purification. In 125 ml each, the same initial natural water samples were added potassium ferrate in the form of powder at the following concentrations: 2.7 mg of a water sample 7; 5.6 mg water sample 8; 4.0 mg water sample 9 and 6.4 mg water sample 10. Samples were mixed thoroughly and stored for two weeks at room temperature (18 – 20^0C). When this comes from the ability to evaluate the efficiency and stability of insertion concentration reagent water purification properties over time.

The intensity of the luminescence of bacteria - biosensors were recorded with a luminometer "Biotoks-6ms" (Russia), registering it impulses/sec.

Measuring integrated toxicity test was performed using the - system by bacterial luminescence at room temperature (20^0C) for 5, 15 and 30 minutes for all samples of natural waters and similar parallel with potassium ferrate samples after storage for 14 days. As one general control (K), distilled water (pH = 7.2). In such an Eppendorf (volume 1.5 ml) was poured into 0.1 ml of a suspension of bacterial cells and 0.9 ml of the test solution. In the control, tube was added 0.1 ml of bacterial suspension was added and 0.9 ml of distilled water. Analysis was performed for a fixed exposure time for each control and experimental samples studied water. To obtain reliable data simultaneously recorded bioluminescence control and experimental samples in 3 replications.

Toxicity Index (T) during the time of interaction of the biosensor with the sample water is determined automatically by the program luminometer "Biotoks" according to the formula:
$T = 100 * (I_k - I) / Ik$, where I_k and I - intensity illumination control and experience, respectively. Assessment of toxicity were classified into three groups:
1. The value of T <20 - the sample is non-toxic;
2. The value of T> 20 but <50 - the sample is toxic;
3. The value of T> 50 - the sample is very toxic.

Sometimes there is stimulation luminescence test - organism, T value with a negative sign. The recommendations when stimulated luminescence biosensor when bioassay based on bacterial luminescence offer to conclude the absence of toxicity of the test sample(Danilov at al. 2002).

Cell viability was assessed by the number of colony forming units (CFU) using the serial dilution method and plating both experimental and control samples on Petri dishes with LB agar supplemented with 100 μg/mL of ampicillin and culturing biosensor cells for 24 h at 32°C. Were plated bacteria biosensor from samples of each sample of water treated with potassium ferrate, after storage for two weeks followed by 30 min bioassay analysis.

III. RESULTS AND DISCUSSION

Evaluating the efficacy of potassium ferrate concentrations introduced for water and the stability of its properties over time was studied bacterial survival - biosensors in water samples treated with the reagent. Studies have shown that water samples with potassium ferrate after treatment reagent and 14 days of their age has bactericidal. In these water samples during bioassay after 30 minutes of interaction with cells of a biosensor, Gram-negative bacteria *Escherichia coli* K12 TG1 remained viable (in number of CFU). Obviously, potassium ferrate, known as a strong oxidant, having a disinfectant action and, after 14 days in aqueous samples lose these properties.

All test water samples had pH 6.9 - 7.4, which corresponds to the recommendations of the bioassay using this method (Danilov at al. 2002). The results of evaluation of toxicity of natural water samples (1 - 4) and similar water samples treated with potassium ferrate (7 - 10) at 14 days of storage are given in the Table 1, Figure 1. Natural water samples 1 and 2 were toxic, 30 min analysis indices of toxicity (T) ≈ 40 - 50, respectively.

Potassium ferrate used as a reagent for the purification of water, at a concentration of 2.7 mg/125 ml enough purified water sample 7 (T ≈ 20). It should be noted that the index of toxicity (T) of 20, according to "threshold" value of toxicity, which is more than the sample recognize the toxic(Zarubina at al. 2013; Danilov at al. 2002). Perhaps for complete cleaning water sample 7 should use the potassium ferrate in a higher concentration than 2.7 mg/125 ml. The reagent potassium ferrate, in a concentration of 5.6 mg / 125 ml water sample is completely cleared 8, which was non-toxic. By the nature of the growth of the index of toxicity bioassay in time (5, 15, 30 min), it can be assumed that the natural water samples 1 and 2 contain heavy metals (Dedushenko et al. 2002; Zarubina at al. 2013; Zarubina and Sorokina 2015). Based on the known mechanism of action ferrates(Jiang et al. 2002; Perfiliev 2002) and our data bioassay can be assumed that the water has been emptied potassium ferrate because of the sorption of heavy metals, which, obviously, is the contamination of the source water samples. Natural water samples 3 - light brown brook countryside and 4 - water mud (after the melting of snow in it) were non-toxic. Water sample 4 slightly stimulated luminescence biosensor (the value of T ≈ - 10). Stimulation of the luminescence intensity of luminescent bacteria from the action of many substances in low concentrations observed in the past by many authors. The mechanism of stimulation is not clear and complicates the interpretation of results (Danilov at al. 2002). In the analysis of the proposed recommendations to draw the conclusion about the absence of toxicity in the samples(Zarubina at al. 2013; Danilov at al. 2002). Non-toxic natural water samples (3 and 4) after the introduction of water in similar samples (9 and 10) ferrate in potassium concentration 4.0 mg/125 ml and 6.4 mg/125 ml, respectively, become toxic (T ≈ 40). The toxicity of these water samples in time bioassay (5, 15 and 30 min), virtually unchanged (Table1). This may indicate the presence of non-toxic natural water samples of some substances of organic nature (Zarubina and Sorokina 2015; Danilov at al. 2002) and the subsequent connection of potassium ferrate with these substances. A consequence of this connection, obviously, is the formation of toxic compounds (Table 1, Figure 1). The natural, non-toxic water sample 4 (a mixture of water and snow) after making potassium ferrate in the same water sample 10 with potassium ferrate concentration of 6.4 mg / 125 ml are toxic. Perhaps this natural nontoxic water sample also contained in the composition of any substances of organic nature (Zarubina and Sorokina 2015; Danilov at al. 2002), which interact with the potassium ferrate to form toxic compounds. Additionally, this toxicity of the water sample may be associated, and with an excess amount of the reagent used in a concentration of 6.4 mg/125 ml (Table 1, Figure 1).

IV. CONCLUSIONS.

1. The data indicate that the water study natural sources in 14 days after making them ferrate potassium concentrations 2.7, 4.0, 5.6 and 6.4 mg / 125 ml not have a bactericidal effect (identified by the number of CFU for growing bacteria - biosensor test - system "Ecolum" bioassay analysis after 30 minutes).
2. The bioassay for 30 min. using test - systems based on the bacterial luminescence natural water samples showed toxicity two city water samples and one non-toxicity of the urban environment and one of the rural environment of water samples. The concentrations of potassium ferrate as water treatment agent revealed that the concentration of 2.4 mg/125 ml of water is insufficient, and 6.4 mg / 125 ml of water - excess.
2. The bioassay for 30 minutes, allowed assuming the chemical nature of the substances contained in the samples of water, which agrees well with the literature data. Thus, the toxic water samples likely to contain heavy metals and are well-cleaned potassium ferrate the known mechanism of sorption. The test samples of non-toxic water when making potassium ferrate in them were probably formed complexes with toxic organic compounds.
4. These data suggest a rapid method bioassay using the test-system "Ecolum" based luminescent bacteria for the selection and evaluation of effective chemical treatment of water, the selection of their effective concentration and treatment time reagent water sources.

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Table 1. Comparison of the toxicity indices of natural water samples T (1 - 4) and similar samples (7 - 10) water treated ferrate * Time bioassay analysis using test - systems based on the bacterial luminescence.

Analysis time, min	The test samples of water							
	1	7*	2	8*	3	9*	4	10*
5	**29** ± 7	21 ± 2	0 - 17	12 ± 4	9 ± 1	**47 ± 1**	- 13 ± - 2	**43** ± 6
15	**39** ± 2	19 ± 4	20 ± 3	8 ± 1	8 ± 2	**49 ± 2**	- 3 ± - 2	**42** ± 4
30	**51** ± 8	**24** ± 2	**38** ± 2	9 ± 2	13 ± 2	**44 ± 1**	- 10 ± -2	**40** ± 5

Figure 1. Compare of the toxicity indices four natural water sources (1 - 4) and a similar sample of water (7 - 10), after the action of the potassium ferrate - using bioassay test 30 minutes - systems based on the bacterial luminescence: Dark columns - cell biosensor natural water sample (1 - 4). Light columns - water sample with potassium ferrate (7 - 10).