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Application of rapid bioassay method for assessing its water purification by ferrate (VI) potassium

Zarubina A.P. [*], Sorokina E.V.[*], Perfiliev Y.D[**],
[*] *(Department of Biology, M. V. Lomonosov Moscow State University,*
119899 Russia Moscow, Leninskie Gory, 1, build. 12,
Email: sorokina_ev77@mail.ru)
[**] *(Department of Chemistry M. V. Lomonosov Moscow State University, 119899 Russia Moscow, Leninskie Gory, 1, build. 3*
Email: perf@radio.chem.msu.ru)

ABSTRACT: *Integral toxicity of four water samples taken from various sources, urban and rural environment was evaluated, and some of the properties of potassium ferrate K_2FeO_4 as the reagent for chemical purification of water were explored. These data allow to suggest bacterial luminescence based test system for practical use. Thetest system is suitable for rapid evaluation of toxicity of the used for water purification chemical agents, as well as for selection of their effective concentrations and for optimization of treatment time.*
Keywords: *biological testing, bacterial luminescence test, ferrate potassium.*

I. INTRODUCTION

Currently, the problem of water purification, both drinking and industrial-technical is highly relevant, taking in account increasing water pollution. To identify environmental problems of water quality it is necessary to develop new methods ofwater testing and newtechnologies of its purification [1]. It is known that for water purification different chemicals - 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 in the formation of even more toxic by-products, and besides of this gaseous oxidants could be used only for water sources of limited volume. A new and very promising method of water purification is based on the use of alkali metal ferrates (VI) having an oxidizing and disinfecting effect [2]. 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 suspended organic particles. Their coagulative action provides additional water purification by adsorption of pollutants[3].

For the development of water purification technologies utilizing ferrates on an industrial scale, some fundamental characteristics of their toxicological properties should be studied and convenient methods and objects bioassay should be chosen.

In all developed countries, the use of methods for biological testing of dangerous xenobiotics action on living organisms is legislated. In this regard, interestingtest systems based on luminous bacteria and already widely used as bio indicators for environmental monitoring of water bodies. Their use allows evaluate the toxicity of various chemicals, their mixtures and the effect of some physical factors (ionizing radiation, electromagnetic radiation) [4].

On the basis of genetical engineeringa strain of *Escherichia coli* K12 TG1 havingartificially created luminous phenotype new biosensor test systems «Ecolum» was developed. It allows to perform analysis without osmoprotectant (solution of NaCl) and at higher temperatures, in contrast, for example, a well-known test system «Microtox» using natural marine luminescent bacteria as a biosensors[5].

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. MATERIALS AND METHODS

The bioassay was performed using a test organism genetically engineered strain of Escherichia coli K12 TG1 with artificially created 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» [6]. The bioassay uses the standard suspension prepared after rehydration of the lyophilized biosensor for 30 minutes in 10 ml of sterile distilled water (pH 7.0 - 7.4) and dilution of the suspension to 6.5 $*10^7$ cells/mlbiosensor/ml was carried out.

The density of bacterial suspensions (cells/ml) was determined by nephelometric (λ = 670 nm) photoelectrocolorimeter KF77 using calibration curve.

pH value of water samples was measured by potentiometry.

The potassium ferrate, K_2FeO_4, was synthesized electrolytically by anodic dissolution of metallic iron in concentrated KOH solution.The K_2FeO_4 concentration exeeded 95% in final solution [2, 6].

Water samples were collected from rural and urban sources in the early spring period: 1 - natural water of the Desna river belonging to the Moskva river basin; 5 - the same water sample after potassium ferrate addition. 2 - a streamlet flowing over the loamy ground in urban area of Moscow; 6 - the same water sample after potassium ferrate addition; 3 - a small seasonal brook with a light-brown water located in black soil region of Istra town; 7 - the same water sample after potassium ferrate addition. 4 - snow slush taken in the area of Moscow State University, 8 - the same water sample after potassium ferrate addition.

Water purification was performed by addition of potassium ferrate in the powder form powder to 125 ml of corresponding water samples: 2.7 mg to water sample 5; 5.6 mg to water sample 6; 4.0 mg to water sample 7, and 6.4 mg to water sample 8.

Samples were mixed thoroughly and stored for two weeks at room temperature ($18 – 20^0$C).

The intensity of the biosensors luminescence of biosensors in impulses / sec was recorded with a luminometer «Biotox - 06» (Russia).

Integrated toxicity test was performed for 5, 15 and 30 minutes for all samples. 0.1 ml of the bacterial suspension and 0.9 ml of the tested solution were mixed inEppendorf tubes (1.5 ml). Distilled water (pH 7.2) was used as a general control (K).Bioluminescencemeasurement was performed for a fixed exposure time for each sample. To obtain reliable data, simultaneously control and experimental samples bioluminescence was recorded in triplicate.

Toxicity Index (T) during the time of interaction of the biosensor with the sample water wascalculated automatically by the software of luminometer «Biotox» according to the formula:

$$T = 100 (Ik - I) /Ik,$$

where Ik and I - intensity luminescence control and experience, respectively.

Range of Toxicity Index values was divided into three intervals: the value of T <20 - non-toxic; T > 20 but < 50 - toxic; > 50 - very toxic.

Sometimes,stimulation of biosensor luminescence was observed, when T value is negative. In case of the stimulation it is recommendedto conclude the absence of toxicity of the tested sample [4].

Biosensor survivalwas evaluated for each sample of potassium ferrate treated water after 30 min bioassay using determination the number of grown luminous colony forming units (CFU). For CFU culture, solid agar medium LB with 100 μg/ml ampicillin was used. CFU counting was performed after culture incubation at 32^0Cfor 24 h.

III. RESULTS AND DISCUSSION

Evaluating the efficiency 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). 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 [4]. The results of evaluation of toxicity of natural water samples (1 - 4) and similar water samples treated with potassium ferrate (5-8) at 14 days of storage are given in the Table. 1,- & Fig. 1.

Table 1. Comparison of the toxicity indices of natural water samples T (1 - 4) and similar samples (5-8) water treated ferrate * Time bioassay analysis using test - systems based on the bacterial luminescence.

Analysis time, min	The test samples of water							
	1	5*	2	6*	3	7*	4	8*
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

Natural water samples 1 and 2 were toxic, 30 min analysis indices of toxicity (T) is ≈ 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 [1, 7]. Perhaps for complete cleaning water sample 5 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 completely cleared 6, which was non-toxic. By the nature of the growth of the index of toxicity bioassay in time (5, 15, 30 min), it could be assumed that the natural water samples 1 and 2 contained heavy metals [8]. Based on the known mechanism of action ferrates [3, 6] and our data bioassay it could be assumed that the water has been emptied potassium ferrate as result 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 was observed in the past by many authors. The mechanism of stimulation is not clear and complicates the interpretation of results [9]. The conclusion about the absence of toxicity in samples [1, 8]. Non-toxic natural water samples (3 and 4) after the introduction of water in similar samples (7 and 8) 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) was virtually unchanged (Table 1). This might indicate the presence of non-toxic natural water samples of some substances of organic nature [8] and the subsequent connection of potassium ferrate with these substances. A consequence of this connection, obviously, was the formation of toxic compounds (Table 1, Fig. 1).

The natural, non-toxic water sample 4 (a mixture of water and snow) after making potassium ferrate in the same water sample 8 with potassium ferrate concentration of 6.4 mg/125 ml were toxic. Perhaps this natural nontoxic water sample also contained in the composition of any substances of organic nature, which interact with the potassium ferrate to form toxic compounds [10]. 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. Fig. 1).

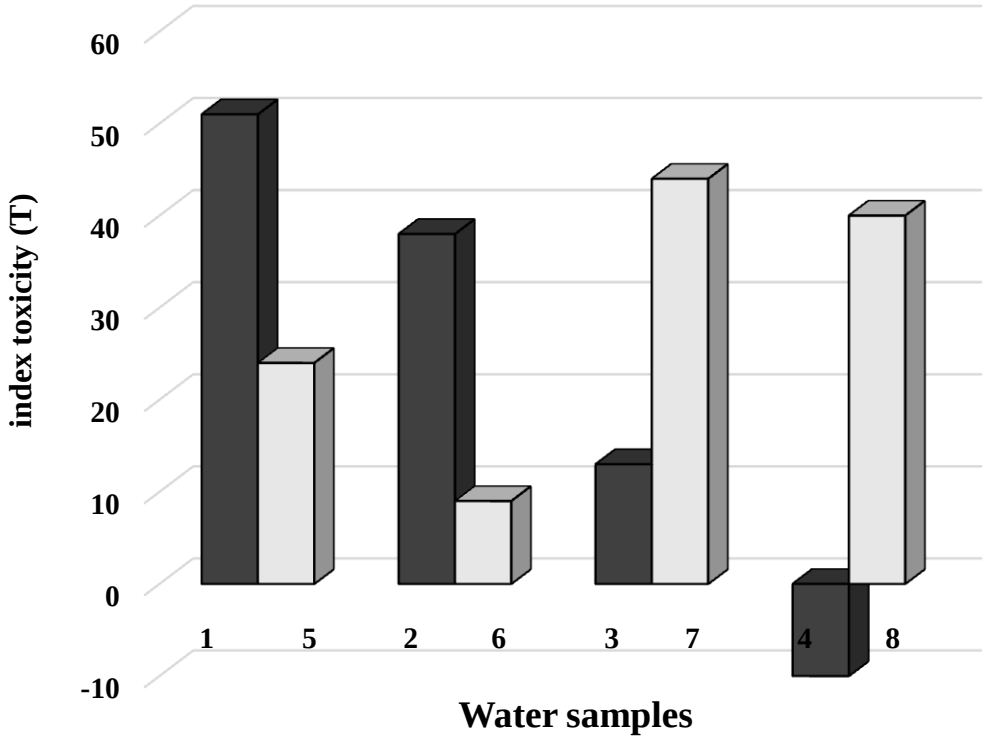

Fig. 1 Comparison of the toxicity indices of four natural water sources (1 - 4) and a similar sample of water (5-8), after the action of the potassium ferrate - 30 minutes using bioassay test - systems based on the bacterial luminescence: Dark columns - cell biosensor every natural water sample (1 - 4). Light columns - prototypes treated potassium ferrate (5-8).

IV. CONCLUSIONS

1. These data indicate that the study of natural water sources, within 14 days after the addition of potassium ferrate concentrations of 2.7, 4.0, 5.6 and 6.4 mg / 125 ml did not have a bactericidal effect (which is determined by the number of CFU for growing bacteria - biosensor test - system "Ekolyum" analysis of biological analysis in 30 minutes).

2. The bioassay for 30 minutes using test - systems based on the bacterial luminescence natural water samples showed toxicity of two water samples and one sample of the urban environment and one of the rural environment of water samples. The concentrations of potassium ferrate for water treating revealed that the concentration of 2.4 mg/125 ml of water is insufficient, and 6.4 mg/125 ml of water - excess.

3. The bioassay for 30 minutes, allowed assuming the chemical nature of the substances contained in the samples of water, which well agrees with the literature data. Thus, the toxic water samples are likely to contain heavy metals and well-cleaned potassium ferrate the known mechanism of sorption. The test samples of non-toxic water when addition potassium ferrate in them probably formed complexes with toxic organic compounds.

4. These data suggested a rapid method bioassay using the test-system "Ecolyum" based luminescent bacteria for the selection and evaluation the effective chemical treatment of water, the selection of the effective concentration and treatment time reagent water sources.

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