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MICROBIOLOGY

Evaluation of the Properties of Potassium Ferrate Used for Water Purification by Luminescence Bioassay

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Abstract—Characteristics of four natural water samples from urban and rural areas and the efficiency of a new purifying agent, potassium ferrate K_2FeO_4, were studied by bacterial luminescence bioassay for 30 minutes. It was revealed that two samples of water from the urban areas are toxic, while the other two samples (one from urban and one from rural environment) are nontoxic. Numerous data obtained on the increase in toxicity index with time allow reasonable conclusions to be made about the chemical nature of substances present in the test water samples. Toxic natural water samples were likely to contain heavy metals and were well purified using potassium ferrate, including via their adsorption. In nontoxic natural water samples, toxic complexes with organic compounds present in water could form at the addition of potassium ferrate. The obtained data call for further studying the properties of potassium ferrate complexes with organic compounds. Bacterial luminescence bioassay is a promising method for the rapid assessment of properties of various water sources (their integral toxicity and presumable chemical composition) and new reagents for their purification (effective concentrations, bactericidal properties, and mechanisms of interacting with heavy metals and organic substances in water).

Keywords: bioassay, bacterial luminescence, water-purifying agent, potassium ferrate, water toxicity.
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INTRODUCTION

Purification of potable and industrial water (wastewater from the production of heavy metals, industrial accidents, natural catastrophes, etc.) is a scientific and practical problem. Biological methods are widely used for the purification of water from biodegradable organic substances; nitrogen, phosphorus, and sulfur compounds; etc. However, they are insufficiently active at the contamination of groundwater with dioxins and their derivatives, phosphonate pesticides, gasoline additives, etc. [1]. At biological treatment plants, water is not always completely purified from microorganisms, hygiene products, household chemicals, pharmaceuticals, etc. These are hazardous substances with pathogenic, carcinogenic, or mutagenic properties. Chlorine, sodium hypochlorite, chlorine dioxide, ozone, hydrogen peroxides, Fenton's reagent, and other agents are used for water purification. Some of them contaminate the environment with chlorine; other can form more toxic products than the original pollutants, and the use of gaseous oxidants is limited by the size of water sources. The use of alkali metal ferrates is a promising method of water purification [2]. Ferrate (VI) salts have oxidative and disinfectant properties; they decompose many toxic substances to low-toxic products and kill microorganisms. The degradation of ferrates in solution results in the formation of low-toxic iron hydroxide, which is precipitated as colloidal aggregates with a very developed surface adsorbing heavy metal ions and particles of organic residues. Their coagulating effect ensures additional purification of water by sorption of many pollutants. The efficiency of ferrates in water purification was shown for such industrial wastes as hydrogen sulfide, ammonia, cyanides and thiocyanates, thioacetamide and thiourea, etc. At present, studies of physicochemical properties of ferrates (VI) are performed, and new preparations, including potassium ferrates, are synthesized [3, 4]. The characterization of the toxicological properties of ferrates and the selection of adequate bioassay objects are of great importance for the development of technology for the industrial application of ferrates in water purification. Therefore, bacterial luminescence bioassay provided to be a successful method for the rapid estimation of the effect of physical factors, chemical substances, and their mixtures in the ecological monitoring of environmental objects [5–7].

In this work, some properties of a reagent for the chemical purification of water by bacterial luminescence bioassay—potassium ferrate K_2FeO_4—(effective

concentrations, assumed mechanism of interaction with environmental pollutants of water, bactericidal properties) were studied on urban and rural water samples.

MATERIALS AND METHODS

Bioassay was performed using the genetically engineered *Escherichia coli* K12 TG1 strain with an artificially created luminous phenotype provided by the built-in lux-operon of marine luminous bacterium *Photobacterium leiognathi* 54D10. The strain was created and kept at the Department of Microbiology of the School of Biology, Moscow State University; it is known as a biosensor of the Ekolyum-06 test system [8]. The bioassay used lyophilized bacterial cells after regeneration for 30 min in 10 mL of cooled sterile distilled water (pH 7.4) and diluted to standard suspension concentrations.

The density of bacterial suspensions was determined by nephelometry (λ = 670 nm) on a KF-77 photocolorimeter (Poland) and expressed as the number of cells in 1 mL (cells/mL) on the calibrated curve.

The pH of water samples was determined by potentiometry.

Potassium ferrate to be used as a water-purifying reagent was prepared in the Department of Radiochemistry, the Department of Microbiology of the School of Chemistry, Moscow State University; the content of K_2FeO_4 was more than 95% [3].

Natural water samples collected from rural and urban sources in early spring were used: (1) an original water sample from the Desna River in the Moskva River basin, (1*) an analogous water sample after the addition of potassium ferrate to a concentration of 21.6 μg/mL, (2) an original water sample from a brook on loamy soil in the Khoroshevskii district of Moscow, (2*) an analogous sample of water after the addition of potassium ferrate to a concentration of 44.8 μg/mL, (3) an original water sample from a brook in the chernozemic agricultural region near the city of Istra, (3*) an analogous sample of water after the addition of potassium ferrate at a concentration of 32 μg/mL, (4) an original sample of snow–water mixture taken near Moscow State University, (4*) an analogous sample of water after the addition of potassium ferrate at a concentration of 51.2 μg/mL. The samples were mixed thoroughly and kept at room temperature (18–20°C) for 2 weeks before biotesting. The water samples, the time of their storage, and the concentrations of potassium ferrate used for the treatments were selected arbitrarily, with consideration for the possibility of estimating the efficiency of the concentrations and the stability of bactericidal properties of the water-purifying reagent.

The luminescence intensity of bacteria (pulses/s) was recorded with a Biotoks-6MS luminometer (Russia).

The integral toxicities of original water samples and analogous samples with potassium ferrate after their similar storage were measured at room temperature (20°C) for 5, 15, and 30 min. Bacterial cell suspension (0.1 mL) was mixed with 0.9 mL of the test water solution in tubes of 1.5 mL, and biosensor (6.5×10^7 cells/mL) was added to test and control samples.

All of the studied water samples were estimated in two experimental treatments with different controls. A general control containing 0.1 mL of bacterial cell suspension and 0.9 mL of distilled water was used in first treatment. To each experimental sample, 0.1 mL of bacterial cell suspension and 0.9 mL of test solution with or without potassium ferrate were added. In the second treatment, the contribution of the reagent concentrations to the purification of water was directly estimated. Bacterial cell suspension (0.1 mL) and 0.9 mL of the test water were mixed in the control tube. The test samples were analogous to the control samples, but they contained potassium ferrate. Analysis was performed at the fixed exposure time for each control and test water sample, and their luminescence intensities were recorded simultaneously in triplicate.

The toxicity index (T) during the interaction of biosensor with the test water sample was determined automatically on the Biotoks luminometer using the formula $T = 100 \cdot (Ic - I)/Ic$, where Ic and I denote the luminescence intensities of the control and test samples, respectively. The toxicity estimates were classified into three groups: $T < 20$, the sample is nontoxic; $20 < T < 50$, the sample is toxic; $T > 50$, the sample is very toxic. Stimulation of test organism luminescence (i.e., a negative T value) was observed sometimes.

The bactericidal properties of potassium ferrate in water samples 1, 2, 3, and 4 were estimated from the survival of luminous bacterial cells (number of CFU) grown on the Luria–Bertani medium with 100 μg/mL ampicillin at 32°C for 24 h. Water samples treated with potassium ferrate were studied after their storage for 2 weeks and biotesting (30 min).

RESULTS AND DISCUSSION

Potassium ferrate, a known strong oxidant, lost its disinfecting properties after storage for 14 days. In water samples 1–4 pretreated with this reagent, the preservation of the cell viability of luminous Gran-negative bacteria *Escherichia coli* K12 TG1 (from the number of CFU) was observed after the interaction for 30 min.

All water samples (original and test ones) had pH 7.0–7.4, which corresponded to the recommendations on the use of this bioassay method [8].

The estimated toxicities of natural water samples 1–4 and analogous water samples treated with potassium ferrate (1*–4*) are shown in Fig. 1. The T values of original water samples 1 and 2 after 30 min

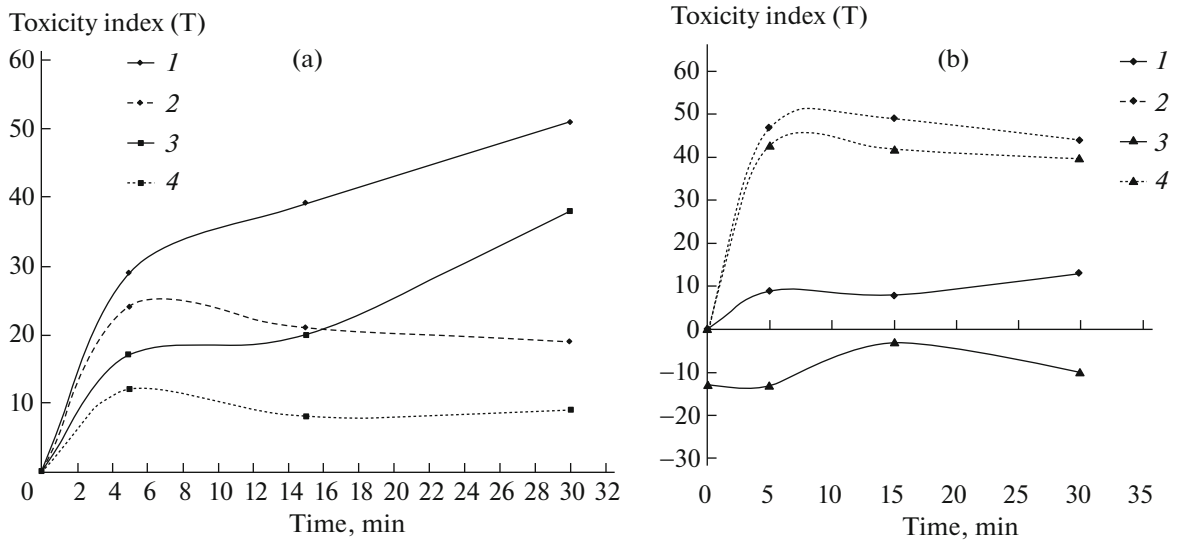

Fig. 1. Estimation of toxicity indices for (1–4) natural water samples and analogous water samples (1*–4*) treated with potassium ferrate using a bacterial luminescence test system. Distilled water was used as a control sample.

of analysis were 50 and 40, respectively; i.e., their toxicity was revealed. Potassium ferrate at 21.6 and 44.8 μg/mL purified water samples 1 and 2, respectively, which became nontoxic (Fig. 1). From the increase in the T value with bioassay time (5, 15, and 30 min), it may be supposed that original water samples 1 and 2 contained heavy metals [8]. One of the acting mechanisms of potassium ferrate as a water-purifying regent is the adsorption of heavy metals by iron hydroxide resulting from the decomposition of ferrates [2]. From available literature data and our results, it may be supposed that natural water samples 1 and 2 were contaminated with heavy metals and purified via the adsorption of heavy metals by potassium ferrate. We revealed that natural water samples 3 and 4 are nontoxic. Water sample 4 showed an insignificant stimulation of biosensor luminescence (T ~ –10) (Fig. 1). However, nontoxic water samples 3* and 4* treated with potassium ferrate at concentrations of 32.0 and 51.2 μg/mL, respectively, became toxic (T ~ 40). Toxicity parameters of these water samples remained almost unchanged during 30 min (Fig. 1), which can indirectly indicate the presence of organic substances [8]. The toxicity of water samples 3* and 4* treated with potassium ferrate was probably due to the interaction of the toxic reagent compound with organic substances present in the original nontoxic samples (Fig. 1).

The direct effect of potassium ferrate on the studied water samples (contribution to toxicity) is revealed in the second experimental treatment using natural water samples as controls for the corresponding test soils treated with potassium ferrate (Fig. 2). Potassium ferrate in water samples 1* and 2* behaves as a reagent purifying natural water from pollutants. Luminescence stimulation of test object is observed in this case (Fig. 2). The manifestation of water toxicity after the treatment with potassium ferrate is noted for samples 3* and 4* (Fig. 1). It is found that potassium ferrate at a concentration of 32 μg/mL has no direct effect on the toxicity of water sample 3* (Fig. 2). The toxicity of water is obviously due to the formation of regent complex with any organic substance present in this natural water sample (Fig. 1). The toxicity of water sample 4* treated with potassium ferrate at a concentration of 51.2 μg/mL can be related both to the effect of the reagent forming a toxic compound with an organic substance from this natural water and to the excessive content of the reagent (Fig. 2). An insignificant direct effect of potassium ferrate is observed in sample 4* (T ~ 45, Fig. 1; T~ 30, Fig. 2). It can be seen (Fig. 2) that potassium ferrate stimulates the luminescence of bacterial biosensor (except for sample 4*).

Many authors noted stimulation of luminescence intensity in luminous bacteria under the effect of many substances present at low concentrations [9]. The mechanism of stimulation is not clear, which complicates the interpretation of results. Recommendations on luminescence bioassays propose concluding on the absence of toxicity in the studied samples [8]. However, under the effect of substances significantly stimulating the luminescence intensity of bacterial biotest, the stimulation of their luminous system functioning can be related to the competition of their respiratory system for reduced flavin. This results in the inhibition of electron transport in the respiratory chain; the electron flow decreases in this chain and, hence, increases in the chain of the luminescent system. Thus, the intensity of bioluminescence increases, and the T acquires a negative value [9]. It should be noted that the concept of hormesis, when different substances in low concentrations affect some functions of the organ-

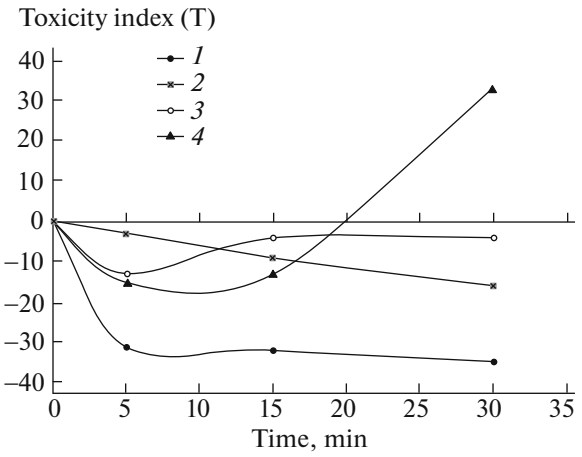

Fig. 2. (1*–4*) Direct contribution of potassium ferrate to the toxicity index of each water sample. Analogous natural water samples without potassium ferrate were used as control samples.

ism and stimulate different functions, is well known [10]. Some authors consider nontoxic the stimulating effect of xenobiotics up to a level of 30% [11].

Thus, the bioassay using a bacterial luminescence test system for 30 min allowed determining the quality of the studied natural water (toxicity, chemical composition of assumed substances) and studying some properties of potassium ferrate (active concentrations, storage time, potential mechanism of its action as a water-purifying reagent depending on the chemical composition of water). The obtained data can orient researchers to study the complexation of potassium ferrate with organic substances. Luminescence bioassay can be considered as a promising rapid method for the comparison of different water-purifying reagents, the selection of efficient concentrations, and the assessment of the stability and treatment duration of test samples.

In conclusion, we would like to note the following advantages of the used bacterial luminescence bioassay over other biotests:

(1) Rapidity (analysis time 5–30 min).

(2) Good correlation of analytical results with other biotest data. The coefficient of correlation between the acute toxicity parameter EC_{50} (effective concentration for the inhibition of 50% of bacterial luminescence intensity) and the LD_{50} (lethal dose, 50%) for eukaryotic bioassays is 0.8 to 0.95 [7].

(3) Low volume of the test sample (1–0.1 mL and lower); good accuracy and reproducibility of results (error no more than 10%).

(4) Assessment of the metabolic status of bacterial cell as a characteristic of the whole organism and not only a parameter of luminescent system functioning.

(5) Automatic determination of T for the studied xenobiotics using up-to-date luminometers.

(6) Convenience and simplicity of analysis (low number of preparation operations, automatic determination of T on a luminometer).

(7) Stability and standard nature of lyophilized biotest cells.

(8) Harmlessness of the technology.

(9) Revelation of the toxic effect of xenobiotics (temporary or permanent effect).

(10) Revelation of accumulating, additive, or synergetic effect of two and more xenobiotics.

(11) Determination of the chemical nature of the sample. From T changes in the course of bioassay (5, 15, 30 min), the chemical nature of the test sample can be suggested (the presence of heavy metals results in an abrupt increase in toxicity; the toxicity of organic compounds increases gradually and is followed by stabilization or some decrease).

(12) Availability and economic efficiency of the assessment of physical factor effects (e.g., biological effects of ionizing radiation or nonthermal electromagnetic radiation) and toxicity of chemical substances and their mixtures.

(13) Wide opportunity of analysis and sanitary control for the regular real-time or on-line monitoring of individual samples and environmental objects (water, soil, air) under laboratory and field conditions.

(14) Use of the studied xenobiotics as model organisms with a wider opportunity for studying the mechanisms of their action on biological systems [9, 12].

The significant quenching of bacterial luminescence under the effect of toxic xenobiotics indicates risks of their use. However, correlation between the death of bacteria under the effect of xenobiotic and the significant toxicity of the xenobiotic (complete luminescence quenching) is observed only at the loss of bacterial liability (dead bacteria do not luminesce) and verified by additional microbiological methods [13–15].

In some countries, bacteria luminescence bioassays are certified under different trademarks. In Russia, a test with marine luminous bacterium *Photobacterium leiognathi* is certified [16]. Biosensors of the Ecolum test system were prepared on the basis of natural luminous bacteria and genetically engineered *Escherichia coli* K12 TG1 strains with cloned lux-operons from different natural luminous bacteria [8]. Genetically engineered biotests of these luminescent bacteria with artificially created luminous phenotype allow the analysis to be performed without osmoprotector (NaCl solution) at higher temperatures (up to 37°C) than the used biotests of marine luminous bacteria (15°C). Procedures for measuring the integral toxicity of water, soils, air, chemical materials, and goods using the Ecolum test system have a calibration certificate (4/7-93) and are registered in the Department of Sanitary and Epidemiological Supervision of Russian Federation (nos. 11-1/131-09, 11-1/132-09,

11-1/133-09, and 11-1/134-09) and in the State Committee for Environmental Protection, certificate of the Russian Federal Agency on Technical Regulating and Metrology no. 01.19.231/2001. They have been introduced in nature-protecting organizations and sanitary and epidemic control agencies and are oriented to the use of domestic reagents and equipment.

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