BIOSENSORS IN FIEDBACK CONTROL OF WATER PURIFICATION

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Abstarct
It was analyzed number of biosensors intended for the determination of total toxicity of some objects of the environment. To control of total toxicity of the environment it was proposed biosensors based on the measurement of bioluminescence of bacteria (*Vibrio fisheri* and *Ph. Phosphoreum*) as well as on the estimation of the level of chemiluminescence of *Daphnia magna* living medium. These biosensors may be as stationary and portable devices with the use of fiber optics. They were examined at the control of water purification contaminated by some detergents.

Introduction
The fact of global dissemination of chemical substances is confirmed by their revealing in Arctic where they never manufactured and where their concentration achieves a threatening level for wild nature and peoples (1). The similar situation may be observed at the simultaneous combustion of huge mass of organic substances or at different types of explosions. In these cases a lot of toxic elements of organic and non-organic nature may be accumulated in environment. At that it may be as close to place of catastrophe and on a long distance from it. Taking into account facts above mentioned it is important to be in opposite of forthcoming catastrophe and the main approach in this case is accomplishment of constant control of the environment. It may be only achieved by use of instrumental analytical devices. Among them biosensors draw attention since they may provide high selective, sensitive, very fast, simple and cheapest analysis. Biosensors may fulfill all demands of modern practice. Being proposed about 40 years ago biosensors are intensively developed and today new their generations appeared. Today it is very actually to apply biosensors for feedback control of different processes and, in particular, control of water purification from toxic agents. In this article the experimental data about application of some biosensors for control of total toxicity of water before and after its purification from some surface active substances (SAS).

Methods
To control of total toxicity of the environment and especially water it is proposed instrumental biotests: 1) based on the measurement of luminol dependent chemiluminescence of sample after staying in its *Daphnia* and 2) the determination of bioluminescence of some microorganisms (*Vibrio sp., Vibrio ficsheri* F1 and *Ph. Phosphoreum*). In the experiments it was used *Daphnia magna* Straus (*Cladocera, Daphnia*), which were kept in medium according to the International standard rules (ISO 6341:1996(E)) (2) *Daphnia's* (5 animals) were placed in 10 ml of medium contained some quantity of analysed sample on 24 h at the certain temperature.
In this investigations *Photobacterium phosphoreum* K3 (IMB B-7071), *Vibrio fischeri* F1 (IMB B-7070) and *Vibrio fischeri* Sh1 purified from Black sea (3) were used. The cultivation of bacteria and their preparation for the analysis are accomplished according to method described early (4). The bioluminescent (BL) analysis was fulfilled by the two different ways: a) with the use of the stationary chemiluminometer (ChML-3, Ukraine) and with the portable device based on the fiber optics. In first case the device included signal amplification block on the basis of photoelectric counter (PhEC-176, Ukraine), block for the injection of chemical reagents and control block with digital microprocessor of signals treatment, which allowed us to have maximally automated process. Signals from chemiluminometer were registered by personal computer switched trough special interface.
At the registration of intensity of bioluminescence by stationary device the samples contented 0.8 ml of the tested SAS in 2,5% solution of NaCl, 0,1 ml of 0.5 M phosphate (pH 7.0) or phosphate citrate (pH 5.5) buffers and 0.2 ml of bacterial suspension including $5x10^5$ cells/ml. In second case (with

work with portable device) bacteria (10^5 cells) were immobilized in sepharose gel (about 0.1 ml) deposited at the end of fiber optics. In both case the intensity of bioluminescence (I) was registered through 30-120 min. The level of toxicity was presented as the concentration, which caused 50% decrease of the intensity of bioluminescence (EC_{50}) (5). The bioluminescent signal was recorded and processed with the help of chemiluminometer.

In this work the cationic (miramistin, ethonii, dekametaksidin, chlorheksidin), anionic (alkylbenzolsulphonate sodium – ABS, dodecylsulfonate sodium – SDS) and non-ionic (t-octylphenoxypolyethoxyethanol – Triton X-100, polyoxyethylene-20-sorbital monooleate – Tween-80 and polyoxyethylated nonylphenol – OP-10) SAS were used. The treatment of SAS solution (50±3 mg/dm^3) by combination of O_3 and UV-radiation was curried out with the help of laboratory device (6) in the reactors of bloated type (volume 0,4, 4,0 и 11,0 дм^3) supplied by dipping source of UV-radiation (lamps DB-15 and DRB-15). Reactor allowed the circulation of treated solution and wash out of formed foam. The velocity of solution circulation was 0,6 L/min. Ozonator had productivity of 1g/h of O_3 and provided concentration of this gas about 18-32 g/L. The rate of O_3 introduction in some experiments was 1,8 mg/(dm^3·min), intensity of UV-radiation was 5 Vat/дм^3 (intensive treatment). In other cases the rate of O_3 introduction was 0,3 – 0,4 mg/(дм^3·min) and intensity of UV-radiation was 5 Vat/dm^3.

Adsorption-biosorption afterpurification of SAS solution is accomplished by filtration of its through two columns connected in series and filled by the activated carbon. The first column was filed by pure active carbon and second one - by carbon with immobilized bacteria of *Pseudomonas* strain. The velocity of filtration was 0,5 m/h and duration of the incubation of solution with carbon was 30-100 min. The concentration of anionic SAS was determined by photometric method according to the reaction of complexing with the methyl blue (7). The content of oxyethylated non-ionic SAS was found by the reaction with phosphormolibdenic acid (8). The concentration of SAS with aromatic ring (ABS, Triton X-100, OP-10) was controlled according to the adsorption in ultraviolet field (at the wavelength of A_{224}, A_{225}) with the help of SPECORD UV-VIS.

Results

Daphnia biotest was examined at the estimation of general toxicity of the solution of polyoxyethylated nonylphenol (OP-10) before and after it destroying by ozone and combination of ozone with the ultraviolet. It can see (Table 1) that the treatment above indicated was accompanied by decreasing of content of surfactant and simultaneous changing of pH value in the solution as well as it caused by the generation of hydrogen peroxide forming.

Table 1. Characterization of the solution of OP-10 before and after its photo-chemical degradation.

Type of treatment	Residual OP-10	pH	H_2O_2, mg/l	Toxicity according to:	
				Daphnia immobilization, %	chemiluminescent test, Rel.u.
None	50,0	5,7	0	73	630
None	50,0	9,3	0	100	690
None	50,0	9	0	89	640
O_3, 10 min	35,0	7,1	0,5	70	295
O_3, 20 min	32,5	7,1	1,3	53	58
O_3, 30 min	25,0	6,9	1,7	51	56
O_3, 33 min	14,25	5,1	3,1	20	22
O_3, 48 min	11,0	6,8	2	27	29
O_3/UV	40,0	9	?	85	590
O_3/UV; 9,5 min	40,0	5,6	0,6	-	240
O_3/UV; 10 min	26,0	7,2	0,9	40	46
O_3/UV; 20 min	24,0	7,0	1,9	20	20
O_3/UV; 49 min	11,25	6,7	3,2	13	18

To eliminate the role of pH value on *Daphnia* activity we equalized its value in each sample up to level of 7,75-7,80 by sample titration with appropriate substances. To keep needed ionic strength, buffer capacity and pH of the analyzed samples the concentrated salts (according to International standard) were added to them in 1/4 of initial volume. The analysis of the obtained results gives to us the possibility to conclude that the toxicity of OP-10 solution decreases with the decreasing of

residual quantity of surfactant. It is pointed out that results obtained by developed biotest have a very good correlation with ones obtained by traditional standard method. This fact allows to us recommend to use the developed biotest based on the analysis of chemiluminescence of *Daphnia* living medium for the determination of water toxicity. The proposed method is very simple and may be used for express screening when the time of *Daphnia* staying could be shortened.

In case of the determination of some surfactants by the bioluminescence method the cells of *V. sp., V. ficsheri* F1 *and Ph. phosphoreum* were taken as sensitive structures. It was stated that tween-20 does not effect on the level of bioluminescence of any cells. Moreover this process was activated up to 1%, in particular, in case of cells of *Ph. phosphoreum.* Triton X-100 and, especially, brij-35 have inhibited bioluminescence of all investigated cells. It was find the level of 50% inhibition of bioluminescence by Triton X-100 and brij-35. It may see that *V. fischeri* has a maximum sensitivity to Triton X-100 and brij-35 and this cells allow to determine much less than 0.01% of surfactants above mentioned.

The sensitivity of *V. fischeri* F1 to some chemical compounds, in particular to above indicated detergents, may be increased by adding to the analyzed samples the substances, which are able to modulate their sensitivity, as well as by optimization of phase of bacteria growth and by fulfillment of testing in subacid conditions. It was stated that the preliminary incubation of bioluminescent bacteria with small quantities of cationic SAS which have any effect on the level of their bioluminescence increases the sensitivity of these bacteria to influence of toxic agents. So, adding miramistine (0,5 mg/l) to *V. fischeri* F1 results in decreasing value of EC_{50} for Op-10 in 57-200 times (Table. 2). Maybe it is connected with increasing penetrability of bacterial membrane at the influence of miramistine. Unfortunately such effect was absent in case of sodium salt of alkylbensolsulphate (ABS). It is stipulated for some interaction of cationic and anionic substances without changes of cell membranes.

Table 2. Changes of EC_{50} for OP-10 and ABS at the adding miramistine

Time of incubation, min	EC_{50} for OP-10, mg/l		EC_{50} for ABS, mg/l	
	control	with miramistine	control	with miramistine
10	80	1,4	40	45
20	70	1,2	36	42
30	60	0,3	30	40

It is well known during different stages of growth bacteria their sensitivity to toxic agents may be changed. It was studied bacteria at the logarithmic, stationary and later stationary stages. It was found that logarithmic stage is more suitable for analysis. Bacteria of *V. fischeri* F1 at this stage have more high sensitivity to the above indicated detergents (in 3-4 times for ABS and in 5-6 times for OP-10) in comparison with that which are on the stationary phase of growth (Table 3).

The extreme value of pH at which bacteria bioluminescence remains stable is 5,0-5,5.

The values of EC_{50} for OP-10 and ABS at the pH of 5,5 were less in 25-50 times in comparison with that obtained at the physiological pH.

Thus, developed approach gives possibility to extend the sensitivity of bioluminescent bacteria to some chemical substances and to determine content of these substances in the environment at the low level concentration.

Table 3. The sensitivity of *V. fischeri* F1 to the effect of surface active substances on the logarithmic (1), stationary (2) and later stationary (3) phase of growth.

Time of influence, min	EC_{50} for ABS, mg/l			EC_{50} for OP-10, mg/l		
	phase of growth			phase of growth		
	1	2	3	1	2	3
30	10	40	50	16	80	n/d
60	9	36	45	10	70	n/d
90	9	30	45	10	60	n/d

Notice: n/d – EC_{50} was not determined due to low sensitivity of bacteria.

The determination of EC_{50} value according to the obtained graphical dependences allowed obtaining the next results. At 30 min of incubation the middle values of EC_{50} for OP-10 and ABS (three measurements) were equal 1,40±0,26 and 2,47±0,99 mg/L, respectively. At the increase of incubation time up to 60 min it was obtained the next values of EC_{50}: 1,33±0,23 mg/L for OP-10 and 1,43±0,29 mg/L for ABS. At the pH of 5,5 these values were in 25-50 times low comparing with that obtained at the neutral pH. In this case they were comparable with that, which were typical for cationic SAS.

The preliminary worked out optimal conditions of the analysis were used at the determination of the total toxicity of ABS and OP-10 solutions during their purification by such physical-chemical factors as combination of O_3 with UV-radiation. The toxicity of SAS and the products of their destruction were estimated at the measurement of the bioluminescence intensity of *V. fischeri* F1 relative to this parameter at the incubation of these bacteria in 2,5% of solution of NaCl (I =100 %).

It was shown (Table 4) that at the neutral level of pH the ABS solutions after their treatment by O_3 together with UV-radiation in comparison with original ones did not practically reduce intensity of bioluminescence if the level of this substance destruction was no more than 50%. Nevertheless, at the residual concentration of ABS less than 20 mg/dm^3 the inhibition of bioluminescence was evident (about 14%). At the extreme destruction of this substance (on 80-90%) it was registered the decrease of the intensity of bioluminescence up to two times. The observed increase of the toxicity of samples at the O_3/UV-radiation influence during 30 min or longer is probably resulted in the formation of very toxic products of ABS destruction. Partially it may be stipulated by the formation of organic peroxides or/and H_2O_2.

So, the sensitivity of bioluminescent test to the influence of ABS at the neutral level of pH testifies its efficiency for control of water purification process. Moreover, this test may be probably applied for the analysis of the toxicity of wastewater with similar content of anionic SAS. The fulfilment of analysis at the pH of 5,5 is resulted in to complete extinguishing of the bioluminescence in the presence all samples and demanded additional selection of degree of sample dilution.

The OP-10 solution at the concentration of 50±3 mg/dm^3 and at the neutral pH (7,0) possessed a low toxicity. The intensity of the bioluminescence of *V. fischeri* F1 decreased on ~ 28 %. Partial oxidizing of OP-10 by the O_3/UV-radiation in the subacid medium (pH ~ 6,0) to the residual concentration of 20-30 mg/dm^3 (according to the determination at the wavelength of A_{224}) has low effect on the level of bioluminescence. The toxicity of these solutions was lower than original one. At the destruction of OP-10 up to 80-90% only the level of bioluminescence decreased almost in 10 times. At the intensive regime of treatment by O_3/UV-radiation only the toxicity of the samples did not increase even if the level of OP-10 destruction was about 80%.

Table 4. Characteristics of OP-10 solutions at the different ways of oxidized tretment.

Time of treatment (min)	Initial characteristics			Bioluminescence, %		
	Concentration of OP-10, mg/l	pH	H_2O_2, mg/l	pH 7,0, (n/d*)	pH 5,5	
					dilution 1:2	(n/d*)
Control	50,00	5,9	0,0	72,3	5,2	0,1
O_3/UV-radiation, min						
4	16,00	5,0	3,8	83,3	11,9	0,1
9,5	40,00	5,6	0,0	83,3	5,9	0,1
10	6,50	4,3	6,0	92,2	17,0	0,1
28	13,50	4,7	1,3	83,3	8,9	0,1
47	10,80	4,5	2,5	7,2	5,2	7,5
BAC, min						
30			-	105,8	102,0	105,0
50			-	103,2	95,5	108,0

So, the bioluminescent testing at the neutral pH gives possibility to estimate the formation of high toxic products, which may appear in course of long time of influence of the O_3/UV-radiation.

In case of the use of phosphate-citrate buffers with pH of 5,5 the bioluminescence of *V. fischeri* F1bacteria was completely suppressed by samples of OP-10 obtained at all types of oxidative treatment. After dilution of these samples in 2 times (1 part of SAS solution and 1 part of buffer) the

intensity of bioluminescence for partially oxidized solutions of OP-10 was higher than for initial ones. Nevertheless, the level of bioluminescence did not exceed 20% in comparison with that in control (when bacteria of *V. fischeri* F1 were incubated in 2,5% of solution of NaCl). In similar investigations with the use of *Daphnia magna* it was shown that at the oxidative destruction of OP-10 by the O_3/UV-radiation the toxicity of its solutions essentially decreased. Taken into account that bioluminescence is resulted in process of enzymatic oxidisation which is closely connected with electron-transport ways in cell as well as considering the possibility of competition SAS (and probably products of their oxidisation) with the mirimistine aldehyde for binding with active centre of luciferase it may suppose the formation of specific inhibitors of bacterial bioluminescence in course of oxidative destruction of OP-10.

Absolutely other level of toxicity was observed after filtration of partially oxidised solutions of SAS through biologically active carbon (ABC). After contact of such solutions with ABC during 15-60 min the level of their toxicity sharply decreased.

It is well known that preliminary rather shallow oxidation of SAS and other organic substances allows increasing resources of work of adsorption of biological filters due to decrease of biological resistance of above indicated substances, increase velocity of mass transfer and biological degradable processes. That is why the developed approach for the determination of total toxicity may be very effectively used at the final stage of technological process of water purification, in particular on the stage after biological sorption. It can give possibility to avoid non-controlled influence of different intermediate substances formed on the stage of oxidisation on the level of bacterial bioluminescence. At the some time the registration of bioluminescence can allow to optimise conditions of the preliminary oxidisation of SAS after their removing from solution by biological sorption.

Discussion

So, the use of bacterial bioluminescence at pH of 7,0 for testing of anionic and non-ionic SAS shown that the partial destruction (up to 50% from initial concentration) of these substances by O_3 in combination with the UV-radiation doe's not accompany by increase of total toxicity of the treated samples. Such destruction of SAS is acceptable for their following biological sorption.

The developed both instrumental approach (based on the determination of intensities of chemiluminescence of *Daphnia* staying medium and bioluminescence of *V. fisheri*) gives possibility to effective control of general toxicity of SAS. The portable device based on fibre optics can be used for control of water toxicity in field conditions.

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