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A MEDIATOR MICROBIAL BIOSENSOR FOR ASSAYING GENERAL TOXICITY

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Highlights

- A P. *yeei*-based biosensor measured the toxicity of perfumery and cosmetics samples
- The biosensor was more sensitive to investigated toxicants than most known analogues
- The biosensor was resistant to samples of four heavy metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+})
- The results correlated with the data of the standard method

Abstract

A mediator biosensor based on *Paracoccus yeei* bacteria for assaying the toxicity of perfumery and cosmetics samples was developed. An approach to selecting an electron-transport mediator based on the heterogeneous electron transfer constants for investigated mediators (k_s) and the mediator–biomaterial interaction constants ($k_{interact}$) was proposed. Screening of nine compounds as potential mediators showed a ferrocene mediator immobilized in graphite paste to have the highest efficiency of electron transfer to the graphite-paste electrode (the heterogeneous transfer constant, 0.4±0.1 cm/s) and a high constant of interaction with *P. yeei* (0.023±0.001 $dm^3/(g \cdot s)$). A biosensor for toxicity assessment based on the ferrocene mediator and *P. yeei* bacteria was formed. The biosensor was tested on samples of four heavy metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}) and two phenols (phenol and *p*-nitrophenol). Proceeding from the EC_{50} index, it was found that the use of the ferrocene mediator made the biosensor more sensitive to investigated toxicants than most analogues described. Toxicity determination of four perfumery and cosmetics samples by the developed biosensor showed prospects of using this system for real-time toxicity monitoring of samples.

***Keywords*:** biosensor, toxicity, EC_{50}, mediator, *Paracoccus yeei* bacteria VKM B-3302

Introduction

Annual production of a large amount of cosmetic products contributes to the emergence of waste waters with increased contents of biocides, in particular, preservatives used in production of cosmetics [1]. The concentrations of preservatives in commercial waste waters may reach 10^{-6} up to 10^{-3} g/l [2–4]. Recent years have witnessed the appearance of numerous laboratory models of analyzers for general toxicity assessment of water and perfumery / cosmetic products (PCP). The physical and chemical methods of analysis allow quantifying various components with high sensitivity and selectivity; at the same time, they are incapable of assessing the synergistic and antagonistic effects of pollutants, which stimulates the development of biological methods of toxicity assessment [5].

Biological methods of toxicity monitoring use most often living organisms, e.g., fish, daphnia, algae, infusoria, duckweed, animal cells and some bacterial species, as test organisms. The main disadvantage of most biotesting methods is that they are time consuming. The use of microorganisms, whose physiological reactions to pollutants are similar to those in higher organisms, is preferable for toxicity measurements for a number of reasons: relative simplicity, small assay time, low cost [6, 7].

A typical example of a successful use of microorganisms for toxicity assessment is the Microtox commercial analyzer (Modern Water, UK) based on the decreased bioluminescence of bacteria in the presence of toxicants. It should be noted that the test organism used in this kit is sensitive to organic substances, which can easily penetrate bacterial cell walls [6], but is less sensitive to metals [7]. The resistance of *Vibrio fischeri* bacteria to heavy metals relative to other test organisms is due to the fact that the habitat of these microorganisms is sea water [8]. It is rather difficult to vary biomaterial to develop a bioreceptor system sensitive both to metals and organic substances; this can also be attributed to disadvantages of the bioluminescent biosensor. What is more, the biosensor cannot be used for analysis of turbid solutions, which may cause a scattering of the light flux.

Mediator electrochemical microbial biosensors for toxicity assessment attract great attention owing to the simplicity of miniaturization, reliability and low cost [9–13]. The current generated in mediator biosensors is due to the fact that mediators transfer electrons from the bacterial cell to the electrode. Interacting with the microorganism, the mediator gets reduced and subsequently can be oxidized on the electrode surface at a corresponding potential. Thus, in the absence of a toxicant the current generated in the microorganism–mediator–electrode system characterizes the metabolic processes in the cell. When a toxicant is added to the system, the bacterial respiratory activity is inhibited, and, as a consequence, the recorded current decreases. The extent of current change in the presence and absence of a toxicant determines the toxicity index of an investigated sample. In contrast with luminescent biosensors, the mediator type of detection enables using a large number of individual microorganisms and forming bioreceptors based on consortia sensitive both to organic toxicants and heavy metals.

The aim of this study was to develop a laboratory model of biosensor for determining the toxicity of PCP extracts using *Paracoccus yeei* bacteria VKM B-3302. These bacteria have been successfully used to assess the quality of water by the biochemical oxygen demand index BOD_5 [14]. This research is highly topical and can make the basis of a method for determining the toxicity of communal waste waters containing high PCP levels. A biosensor based on *Psychrobacter* sp. bacteria isolated from activated sludge makes it possible, as it has been shown in [9], to successfully calculate the toxicity index of waste waters coming to treatment facilities, so as not to reduce the biodegrading capacity of activated sludge [9].

Materials and methods

Reagents and materials

Tryptone (Panreac, Spain), yeast extract (Helicon, Russia), sodium chloride (Diaem, Russia), agar-agar (Panreac, Spain) were used to grow *P. yeei* microorganisms. Graphite powder (Fluka, Germany), paraffin oil (Fluka, Germany), dialysis membrane (MWCO, 14 kDa) (Roth, Germany) were applied to form a working carbon-paste electrode.

All salts used to grow duckweed (the Swedish standard (SIS) growth medium [15]) – $NaNO_3$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$,Na_2CO_3, H_3BO_3, $MnCl_2 \cdot 4H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, Na_2-EDTA$\cdot 2H_2O$ – were of chemically pure grade (Diaem, Russia).

As electron-transfer mediators, we used ferrocene (Aldrich, Germany), 1,1′-dimethylferrocene (Aldrich, Germany), ferrocenecarboxaldehyde (Aldrich, Germany), ferroceneacetonitrile (Aldrich, Germany), neutral red (Diaem, Russia), 2,6-

dichlorophenolindophenol (Diaem, Russia), thionine (Diaem, Russia), methylene blue (Diaem, Russia), potassium hexacyanoferrate(III) (Diaem, Russia).

Microorganisms

P. yeei bacteria VKM B-3302 have been isolated from activated sludge from wastewater treatment facilities of TulaGorVodokanal Water Services and identified in our previous work [14]. Samples of duckweed for inoculation were collected in the Belousov Central Recreation and Entertainment Park, Tula, in accordance with regulatory documents [15].

Cultivation of microbial cells

The bacteria were grown on a Luria–Bertani rich mineral medium. The medium was sterilized by autoclaving at a pressure of 1 atm for 45 min. Cells were grown aerobically for 18–20 h in 750-cm^3 shaken flasks at a temperature of 29°C (optical density, A_{600} = 0.6). Biomass produced was then centrifuged at room temperature for 10 min (8000 rpm). Further on, the centrifugate was washed with a 20-mM phosphate buffer, pH 6.8. Sedimented cells were transferred to fresh portions of the buffer, distributed by portions and sedimented on an Eppendorf centrifuge for 5 min at 8000 rpm. Washed biomass was weighed and kept in microtubes at a temperature of –25°C.

Formation of the working electrode

The working electrode was formed according to the technique described in [14] by filling a plastic tube (internal area, 6.3 mm^2) with the prepared graphite powder–mineral oil paste. Electrodes based on low-soluble mediators (ferrocene, 1,1′-dimethylferrocene, ferrocenecarboxaldehyde, ferroceneacetonitrile) were formed by adding a 1% solution of mediator in acetone to 100 mg of graphite powder; after acetone evaporated, 40 μl of paraffin oil was added and the paste was mixed up. This modified paste was used to fill the plastic tube of the working electrode. To form electrodes based on soluble mediators (thionine, neutral red, methylene blue, 2,6-dichlorophenolindophenol, potassium hexacyanoferrate (III)), a non-modified paste was prepared (100 mg of graphite powder was mixed with 40 μl of paraffin oil) and also used to fill the plastic tube of the working electrode.

Microbial cells were immobilized on the electrode surface as follows. The suspension in the amount of 3 μl with the cell content of 200 mg wet weight per ml was applied onto the working surface of the electrode and dried at room temperature for 15 min. Cells were retained on the electrode surface by means of a dialysis membrane, which was fixed using a plastic ring.

Recording of current–voltage curves

Cyclic voltammograms were recorded using an Ekotest-VA voltammetric analyzer (Ekoniks-Ekspert, Russia) in a three-electrode configuration. A carbon-paste electrode with immobilized cells served as a working electrode; a platinum electrode, as an auxiliary electrode. A saturated silver chloride (Ag/AgCl) electrode, relative to which all voltammograms are presented, was used as a reference electrode. Measurements were conducted at a scan rate of 10 up to 200 mV/s in a potassium–sodium–phosphate buffer (pH 6.8) at a temperature of 22°C. The compartment volume was 15 ml.

Biosensor measurements of the toxicity index

Measurements were carried out using an IPC-micro galvanopotentiostat (SPE Volta, Russia). Biosensor responses were registered using a two-electrode configuration. A carbon-paste electrode modified by an insoluble mediator with immobilized cells served as a working electrode; a saturated silver chloride electrode, as a reference electrode.

Soluble mediators (2,6-dichlorophenolindophenol, thionine, neutral red, potassium hexacyanoferrate(III), methylene blue) were added into the potassium–sodium–phosphate buffer

in which the measurements were performed. The measurements made use of potentials found earlier [14]. The measurement temperature was 20°C; the compartment volume, 5 cm^3.

After a stable level of current was set, 500 μl of an assayed solution containing the oxidized substrate (glucose; concentration in the measuring cuvette, 11 mM) and the required amount of toxicant were pipetted into the compartment. The compartment was washed with a potassium–sodium–phosphate buffer solution after each measurement. The inhibition index (%Inh) served as an analytical signal. The index was calculated by the formula

$$\%Inh = (1 - I_2/I_1)\cdot 100\%, \quad (1)$$

where I_1 is the response of the sensor at the introduction of a solution containing glucose (concentration in the measuring cuvette, 11 mM); I_2, the response of the sensor at the introduction of a solution containing glucose (concentration in the measuring cuvette, 11 mM) and the required amount of toxicant.

Preparation of PCP samples

Four samples were chosen as objects of study: two samples of shampoo, a liquid toilet soap and a cosmetic cream. The samples were prepared as follows: a certain amount of a sample was diluted with distilled water and thermostatted at 37°C. The data are given in Table 1.

PUT **Table 1** HERE

After the aqueous solutions of PCP were prepared, they were further diluted with distilled water to a concentration of 5 mg/cm^3 (if the primary dilution of the sample was 1:20) and to a concentration of 10 mg/cm^3 (if the primary dilution of the sample was 1:10). For these concentrations of the samples, the toxicity indices were determined using a biosensor and by the standard biotesting method.

Biotesting by using duckweed as a test organism for determining the toxicity index

Biotesting using duckweed was carried out in accordance with regulatory documents [15]. Duckweed cultivated for three weeks in mother culture was inoculated under aceptic conditions. In a similar way, inoculation into a control container (without a toxicant) was done. Experimental and control containers were continuously illuminated with a fluorescent lamp for 7 days of incubation. Experimental containers were arranged in the incubator in a random way. The maintained temperature was 24±2°C.

The inhibition of the specific growth rate ($\%Inh_p$) was calculated in accordance with the equation:

$$\%Inh_p = [(\mu_c - \mu_t)/\mu_c]100\%, \quad (2)$$

where μ_c is the specific growth rate in the control group, min^{-1}; μ_t, the specific growth rate in the experimental group, min^{-1}.

A sample was considered to be toxic if the extent of inhibition exceeded 20%.

Results and discussion

Selection of an electron-transfer mediator for forming a bioreceptor system

When choosing an electron-transfer mediator, it is necessary to take into account that the analytical signal is of an integral character and is based on biochemical and electrochemical processes occurring in the analyte–microorganism–mediator–electrode system. Proceeding from this, we carried out a quantitative assessment of the efficiency of nine electron-transfer mediators; in particular, we determined the constants of heterogeneous electron transfer to the electrode and the constants of mediator–biomaterial interaction.

To determine the microorganism–mediator interaction constants, use was made of the cyclic voltammetry method, applying the Kulys modelling for these systems [16]. The interaction constant was determined by the equation:

$$\frac{Ip_1}{Ip_2} = \sqrt{\frac{k_{interact}[\mathrm{E}]RT}{nFv}} \qquad (3)$$

where $I\mathrm{p}_1$ is the anodic peak current in the presence of substrate ; $I\mathrm{p}_2$, the anodic peak current in the absence of substrate; k_{interact}, the mediator–biomaterial interaction rate constant; *n*, number of transferring electrons; ν, scan rate; *F*, Faraday's constant; *R*, universal gas constant; [*E*], bacterial titer.

To determine the rate constants, the dependences of the ratio of anodic peak currents in the presence and absence of substrate ($I\mathrm{p}_1/I\mathrm{p}_2$) on the value of $1/v^{1/2}$ were obtained; by the tangent of the slope of the linear regression, k_{interact} was found. A typical voltammogram and a calculation plot are shown in Fig. 1.

PUT FIG. 1 A, B HERE

The obtained values of the mediator–*P. yeei* interaction constants are given in Table 2. On the whole, it can be noted that soluble mediators interact with cells much faster than low-soluble mediators of the ferrocene series, which is probably due to the ability of phenazine- and phenothiazine-series mediators to penetrate into the cell easier, thus providing for high rates of investigated mediators' interaction with bacterial cells [17].

To determine the heterogeneous constant of electron transfer to the electrode, we investigated the mediator–electrode system, in which electrons are transferred in several stages: diffusion of mediator to the electrode surface, adsorption of mediator on the electrode, transfer of electron to the electrode, desorption of the reaction product and its diffusion [18]. The rate-determining stage is the slowest. Identification of the limiting stage by analyzing the dependence of the peak current on the scan rate made it possible to use the Nicholson model [19] and the Laviron model [20] to find the heterogeneous electron transfer constant (Table 2).

The first step of conversion of the obtained voltammograms was to investigate the dependence of the peak current (I_{p}) on the scan rate (v). If the linear dependence was achieved in the coordinates of I_{p} vs v, then the further calculation of the heterogeneous constants was conducted using the Laviron model; if in the coordinates of I_{p} vs $v^{1/2}$, the Nicholson model.

An intermediate step of the calculation was to find the transfer coefficients α for the cathodic process and (1 – α) for the anodic process. In the case of the Nicholson model, the Tafel equation transforms to (4), and in the case of the Laviron model, to (5):

$$E_{\mathrm{c}} = \mathrm{const} + b/2\log v, \qquad (4)$$

$$E_{\mathrm{c}} = \mathrm{const} + b\log v, \qquad (5)$$

where E_{c} is the potential of the cathodic peak, V; v, the scan rate, V/s; const, the sum of constant magnitudes independent of the scan rate; $b = 2.3RT/(\alpha nF)$.

The concluding step in the calculation of the heterogeneous electron transfer constants was the use of the Nicholson formula (6) and the Laviron formula (7) for the calculation of the constants:

$$k_S = \psi\sqrt{\pi\frac{nFv}{RT}D}\,, \qquad (6)$$

$$\log(k_S) = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log(\frac{RT}{nFv}) - \frac{\alpha(1-\alpha)nF\Delta E}{2.3RT}\,, \qquad (7)$$

where *n* is the number of electrons; *F*, Faraday's constant, C/mol; *D*, the diffusion coefficient of an electrochemical substance, cm^2/s; *R*, the universal gas constant, J/(mol·K); *v*, the scan rate,

mV/s; *T*, temperature, K; α, the value of the transfer coefficient; ψ, the parameter affecting the peak potential difference (Δ*E*, mV, calculated by the Nicholson equation [19]).

The obtained values of the heterogeneous constants of electron transfer to the carbon-paste electrode are given in Table 2.

PUT **Table 2** HERE

The fastest transfer of electrons is achieved in the ferrocene–carbon paste electrode system. On the whole, it can be noted that for ferrocene and its derivatives the heterogeneous transfer constants are higher than for the corresponding soluble mediators. This is probably due to the immobilization of mediator near the electrode surface and, correspondingly, to a change in the character of the electron transfer rate-determining stage. Owing to adsorption, electroactive molecules of ferrocene derivatives are close to the surface of the electrode, while for soluble mediators the transfer of electrons is significantly affected by the diffusion mobility of mediators; the higher the mobility, the greater the heterogeneous electron transfer constant is [21, 22].

It should be noted that the heterogeneous electron transfer constants for ferrocene and its derivatives dissolved in one solvent and prepared for ultramicroelectrodes do not in fact depend on the functional groups of ferrocenes. This can be explained by the fact that the rate-determining stage is of a diffusion character; in view of the close size of mediator molecules, the mobilities of these compounds at the electrode surface have close values [21]. In the investigated ferrocene–carbon paste electrode system, we should take into account the solubilities of ferrocene derivatives in the considered solution, as well as those of the corresponding cations. With this consideration in mind, the methyl groups in 1,1′-dimethylferrocene should increase the oxidation reaction rate; at the same time, these groups increase the hydrophobicity of this compound, which shall lead to decrease the rate of the transfer of the formed cation to solution [23, 24].

Thus, out of nine mediators, ferrocene is the most promising for developing a bioreceptor system based on *P. yeei* bacteria, because it transfers electrons to the electrode and interacts with microorganisms at a high rate. It should be noted that the *P. yeei*–ferrocene bioreceptor system was successfully applied at the development of a biosensor for detection of BOD_5 (this biosensor possessed the lowest lower boundary of assayed BOD concentrations (1.3 mg O_2/dm^3)) [14]. Thus, it can be assumed that the requirement of a high reaction rate with participation of an electron transport mediator is one of the important mediator-choosing criteria that determine the high sensitivity of a bioreceptor system.

Assessment of the sensitivity of the developed mediator biosensors for determining the toxicity of particular toxicants

A bioreceptor system based on ferrocene and *P. yeei* was used to form a biosensor for assessing the toxicity of some heavy metals and organic substances. An extent of the decrease in the glucose uptake rate by microorganisms in the presence and absence of an inhibitor was taken to be the inhibition index. As toxicants, heavy metal ions were taken, which are used as model toxicants for various test systems: four types of heavy metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}) and two types of phenols (phenol and *p*-nitrophenol) [5]. Inhibitory curves for each toxicant were plotted (Fig. 2).

PUT Fig. 2 HERE

Using the obtained results, we found the toxicant concentrations (EC_{50}) causing a decrease of the activity of biosensor's receptor element by 50% (Table 3). The experimental results showed that the order of toxicity for individual toxicant was ranked as *p*-nitrophenol > phenol ≈ Pb^{2+} > Cd^{2+} > Cu^{2+} > Zn^{2+}. The obtained results of the toxic effect of the Cd^{2+} and Cu^{2+} ions correlate with the data of [10] and [25], respectively. Besides, the developed system exhibits a larger sensitivity to

Pb^{2+} ions and organic toxicants phenol and *p*-nitrophenol than other mediator biosensors. Error bars represent the standard deviation of six measurements (two technical replicates of three biological replicates).

PUT **Table 3** HERE

Comparing the sensitivity of the developed biosensor with other test organisms (duckweed and luminescent bacteria), we should note that the developed biosensor is more sensitive to heavy metals than luminescent bacteria (except for the action of Zn^{2+} ions). On the whole, biosensor methods of toxicity assessment are characterized by a lower sensitivity to heavy metals than using duckweed as a test organism [7]. Nevertheless, the development of biosensor analyzers is topical, which is due to the reagent-free manner of the method and the possibility of uninterrupted monitoring of the quality of water, as well as the high rapidity of analysis, which makes it possible to prevent the propagation of various contaminations.

Analysis of PCP samples using the developed mediator biosensor

Four samples of PCP were analyzed using the developed biosensor and by the biotesting method using duckweed as a test organism. The registered parameter was the toxicity index determined as a degree of a decrease in the activity of biosensor's bioreceptor element determined by formula (1), and a degree of inhibition of duckweed's specific growth rate (the reference method) calculated using formula (2). The obtained results are presented in Table 4. A sample was considered to be non-toxic if the toxicity index did not exceed 20%.

PUT **Table 4** HERE

The sample of shampoo No 2 had a strong toxic effect on the specific growth rate of duckweed and on the activity of the receptor element. Statistical analysis of experimental data (Fisher's test and a modified Student's *t*-test) showed an insignificant difference in the results of analysis by both methods, therefore the developed biosensor can be used as an alternative to the standard method of analysis. Thus, the biosensor based on *P. yeei* bacteria and ferrocene mediator can be used for developing a prototype analyzer to enable the determination of the toxicity index.

Conclusion

To assess the efficiency of electron transfer from *P. yeei* bacteria to the electrode, we found the constants of the heterogeneous transfer of electrons in the carbon-paste electrode–mediator system and the interaction constants in the *P. yeei*–electrode system for nine compounds potentially capable of mediator transfer of electrons. The use of ferrocene immobilized into graphite paste was established to be characterized by both the high rates of heterogeneous transfer (0.4±0.1 cm/s) and the high rate of interaction with the investigated microorganisms (0.023±0.001 $dm^3/(g \cdot s)$). The proposed approach makes it possible to quantitatively assess the efficiency of mediator bioelectrocatalysis and can be used both for immobilized and non-immobilized mediators.

Successful use of the *P. yeei*–ferrocene system in bioelectroanalysis has been supported by earlier research in the development of a BOD biosensor, and this study was the first to show the possibility of using *P. yeei* bacteria, isolated from activated sludge, together with the ferrocene mediator as a bioreceptor element of a mediator biosensor for toxicity index assessment.

The values of EC_{50} were found for four types of heavy metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}) and two types of phenols (phenol and *p*-nitrophenol). On the whole, the developed bioreceptor system showed a high sensitivity to the tested toxicants but did not outperform duckweed as a test organism. The biosensor based on *P. yeei* bacteria and the ferrocene mediator for assaying integral

toxicity is reagentless; it also enables an uninterrupted real-time monitoring of the toxicity index, which enables prevention of the emergence and propagation of contaminations.

Analysis of four PCP samples showed that the biosensor based on ferrocene mediator and *P. yeei* bacteria generates data that differ statistically insignificantly from biotesting data. The proposed combination of bacterial cells and a mediator can be used subsequently for developing a prototype analyzer making possible the determination of the toxicity index.

Author Agreement

for the manuscript "A mediator microbial biosensor for assaying general toxicity"
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The authors fully agree with the rules of submission of the manuscript to the Journal of Enzyme and Microbial Technology.

Conflicts of Interest:
The authors declare no conflict of interest.

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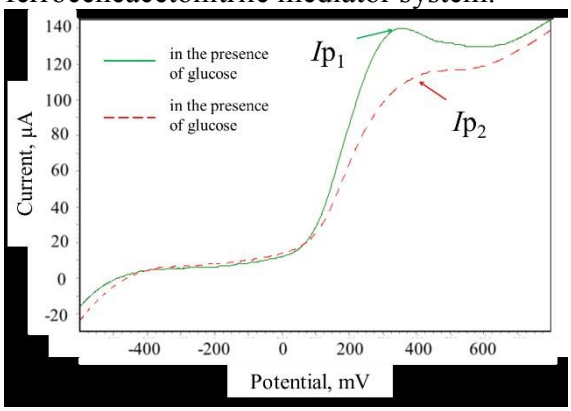
LEGENDS TO FIGURES

Fig. 1. Determination of the mediator–*P. yeei* interaction constant by cyclic voltammetry. A, typical voltammogram; B, dependence of the ratio of the peak currents in the presence and absence of substrate on the inverse value of the root of the scan rate $1/v^{1/2}$ in the *P. yeei*–ferroceneacetonitrile mediator system.



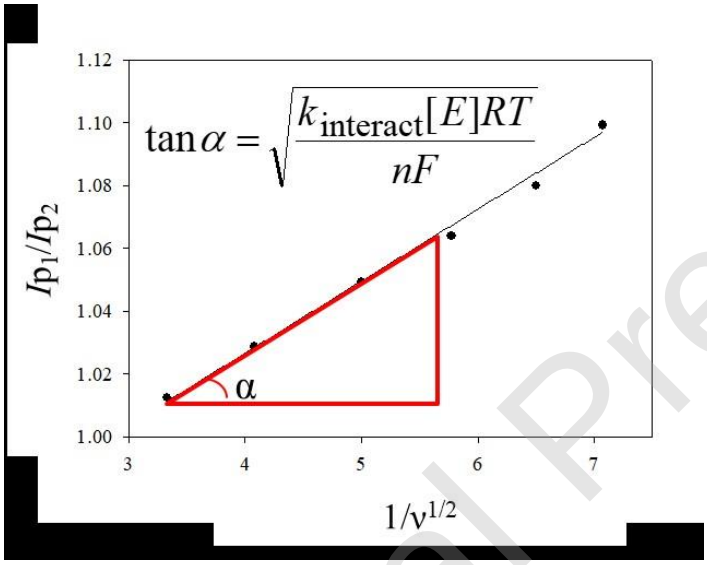


Fig. 2. An inhibitory curve exemplified by Cd^{2+}.

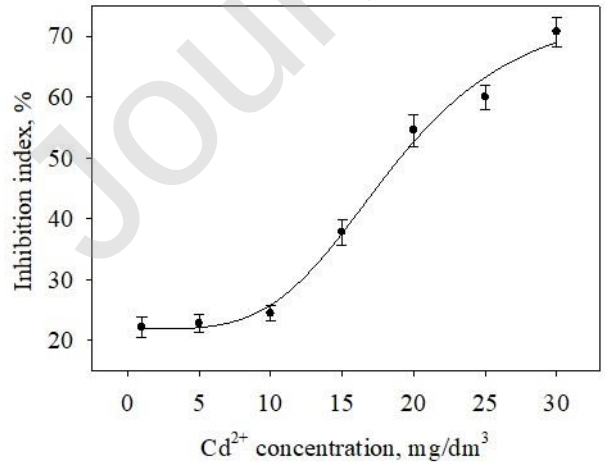


Table 1. Preparation of PCP samples.

Name	Sample weight (g) or volume (cm^3)	Distilled water volume, cm^3	Primary dilution	Thermostatting time, min
Shampoo	1 g	20	1:20	10
Liquid toilet soap	1 g	20	1:20	10
Cosmetic cream	1 cm^3	10	1:10	60

Table 2. The mediator–bacterium interaction constants ($k_{interact}$) and the constants of heterogeneous transfer of electrons (k_s) to the carbon-paste electrode (scan rate, 100 mV/s).

Mediator	$k_{interact}$, $dm^3/(g \cdot s)$	k_s, $cm \cdot s^{-1}$
Methylene blue	0.021±0.001	0.025±0.009
Potassium ferricyanide	0.019±0.003	0.0067±0.0009
Thionine	0.013±0.004	0.022±0.005
2,6-Dichlorophenolindophenol	0.013±0.002	0.069±0.004
Neutral red	0.013±0.003	0.017±0.005
Ferrocene	0.023±0.001	0.4±0.1
1,1′-Dimethylferrocene	0.0038±0.0009	0.07±0.01
Ferrocenecarboxaldehyde	0.007±0.001	0.03±0.01
Ferroceneacetonitrile	0.0014±0.0001	0.14±0.05

Table 3. The developed receptor system as compared with known analogues (based on the EC_{50} parameter).

Mediator/biomaterial	EC_{50} of the toxicant, mg/dm^3						Ref.
	Pb^{2+}	Cd^{2+}	Cu^{2+}	Zn^{2+}	Phenol	*p*-Nitrophenol	
Ferrocene/*P. yeei*	9.9	18.2	21.1	47.5	9.9	2.1	This work
p-Benzoquinone/*E. coli*, *B. subtilis*, *S. cerevisiae*	nd*	20.5	16.5	–	–	–	[10]
Menadione and potassium hexacyanoferrate(III)/ *S. cerevisiae*, *E. coli*	34.6	13.9	10.1	–	44.5	–	[12]
Thionine/*E. coli*	34.4	36.2	20.2	53.2	–	–	[25]
p-Benzoquinone/*Psychro-bacter* sp. isolated from activated sludge	110	47.3	2.6	10.9	–	–	[9]
Potassium hexacyano-ferrate(III)/Activated sludge	–	13.4	19.8	1.19	–	–	[11]
p-Benzoquinone/*E. coli*	–	79	44	–	–	–	[26]
Duckweed (*Lemna*)	5.5	0.33	0.33	0.9	–	–	[7]
Vibrio fischeri	36.0	52.5	34.4	4.64	–	–	[7]

*nd, not determined

Table 4. General toxicity assays of investigated samples.

Sample	Toxicity index (reference method), %	Toxicity index (biosensor method), %
Shampoo No 1	13±3 (nontoxic)	15±2 (nontoxic)
Shampoo No 2	40±4 (toxic)	37±2 (toxic)
Toilet soap	21±2 (toxic)	20±1 (toxic)
Cosmetic cream	18±2 (nontoxic)	19±2 (nontoxic)