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Review Article

Biosensors and Biofuel Cells: Application-Oriented Research (A Review)

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Abstract

The review discusses the results of research into the development of electrochemical microbial-type biosensor analyzers and microbial fuel cells. Research made by international teams is reviewed, but a larger part of presented data contains the results obtained by the Laboratory of Biosensors, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. The major trend in the use of microbial cells had been detection of alcohols, sugars and toxic compounds. As the research evolved, the search expanded into the field of fuel cells, and a series of works united by this direction were carried out. The parameters of the fuel cell anode fabricated from nanomaterials, namely, thermally expanded graphite and carbon nanotubes, were assessed. The development of the fuel cell anode based on *Gluconobacter* membrane fractions was described. Catalytic oxidation of ethanol by *Gluconobacter* membrane fractions was found to be able to occur in mediator-free mode. A charge-accumulation converter system was developed and tested. Evaluation data were obtained on the use of the brown frog *Rana temporaria* as a source for producing electric energy during the oxidation of frog's glucose in the implanted fuel cell. Applications of the developed devices are discussed.

Keywords: Microbial Cells; Biosensors; Biofuel Cells; Anode Nanomaterial; Converter-Based Charge Accumulation; Frog Organism as a Fuel Cell Source

Introduction

More than 50 years have passed since the development of the first biosensor and the beginning of biosensor research as such [1]. At present, this trend of analytical biotechnology is interdisciplinary and not only unites multi-skilled specialists but also includes numerous international teams. A review [2] describes the development of biosensor research in Russia in the 10-year period from 1997 up to 2007. The review summed up Russian research in the field of biological sensors for detection of carbohydrates, alcohols, medicines, enzyme inhibitors, toxicants, heavy metal ions, as well as viruses and microbial cells. As a whole, collected material gave an idea of the main tendencies in biosensor development in Russia of that time.

This review considers the results presented in international publications, but mainly discusses the data obtained at the Laboratory of Biosensors, G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino (IBPhM RAS), within a later, five-year period from 2013 to 2018. As the general trend of research by the Institute consists in studying the properties of microorganisms, Laboratory's R&D was also directed to the use of primarily microbial cells in receptor elements. To register reactions of biomaterial, used was made of electrochemical methods – amperometry based on the application of Clark-type electrodes, graphite paste electrodes, as well as screen printed electrodes; potentiometry based on the use of pH-sensitive field effect transistors; impedance spectroscopy. Biosensor models that made the basis for the possible use in biotechnology, in environment protection services, in medicine and pharmacology were developed. Since 2013, the spectrum of research has expanded into the field related to studies of electricity generation by biofuel cells (BFC).

The aim of this review is to analyze the new trends presented in international publications in biosensors as well as to discuss the latest R&D in biosensors and biofuel cells at the IBPhM RAS.

Biosensors based on whole microbial cells

Analysis of works on microbial biosensors can be found in a number of reviews that discuss in detail the comparative characteristics of microbial and enzyme biosensors, particular features of using signal transducers, immobilization of microorganisms, the most efficient applications of biosensors based on microorganisms in various fields – from monitoring objects of the environment to providing for safety of foods and protection of the human habitat [3,4]. A new wave of optical biosensors based on genetically engineered microorganisms is widely used to assay toxicity and bioavailability [5,6]. The process and the component base of microbial biosensors are constantly improved; nanomaterials leading to increase the sensitivity of biosensors are widely used [7]. Various methodologies based on genetic/protein engineering and synthetic biology are discussed with the view to construct microorganisms with the required signal outputs, sensitivity and selectivity [8].

Speaking in general about biosensors – which are related not only to microbial cells – it should be noted that they help to understand the causes of various diseases, to find ways of their therapy, to monitor biological processes. Such an analysis is possible because biosensors represent devices that enable real-time studies of the behaviour of compounds, including biologically activ substances, which control most physiological processes in the organism. Given the increasing demand for practical and low-cost analytical techniques, biosensors have attracted attention for use in the quality analysis of drugs, medicines, and other analytes of interest in the pharmaceutical area. Biosensors allow quantification not only of the active component in pharmaceutical formulations, but also the analysis of degradation products and metabolites in biological fluids [9]. Although microbial biosensors show promise for application in various detection fields, some limitations still remain such as poor selectivity, low sensitivity and impractical portability. To overcome such limitations, microbial biosensors have been integrated with many recently developed micro/nanotechnologies and applied to a wide range of detection purposes [10].

Of special importance in biosensor development are approaches to the creation of systems with extended lifetimes and sensitivities to a broad range of substrates. Such biosensor types, containing *Photobacterium phosphoreum* microbial cells are described in [11]. Cells were immobilized in poly(vinyl alcohol) (PVA) cryogel, and a flow system of supplying solutions were used. The biosensors enabled assessing the presence of Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} ecotoxicants, 2,4-dichlorophenoxyacetic acid, 2,6-dimethylphenol, pentachlorophenol, couma-phos, malathion, chloropyriphos and methyl parathion and possessed high sensitivities coupled with broad ranges of heavy metal (10^{-8}–10^{-4} M) and pesticide (10^{-8}–10^{-5} M) detection.

Examples of some electrochemical microbial cell-based biosensors with account for available information are given in Table 1. When developing them, we primarily considered the topicality of detecting a given substance, the presence or absence of information about the existing models developed by other authors, the simplicity of forming a biosensor, availability of microorganisms conforming the most fully to the detection conditions. Our own previous experience was also integrated. In accordance with this, we developed and studied the characteristics of biosensors based on strains oxidizing carbohydrates and alcohols (Table 1, Part 1); toxic compounds of the type of ethylene diamine tetraacetic acid, thiodiglycol, methylamine, dimethylamine, trimethylamine (Table 1, Part 2); biosensors for detection of biological oxygen demand (BOD) (Table 1, Part 3).

Biosensor detection of ethanol and glucose

Biosensors for detection of ethanol and glucose are required in medicine, food industry and those fields of biotechnology that need fast and accurate assays of these compounds. Developers of biosensor models considered issues of sensitivity, immobilization techniques, lifetimes, effects of electron transport mediators on the signal. Thus, Voronova., *et al*. [12] performed a comparative analysis of the characteristics of biosensors based on cells, adapted by primary growth on alcohol substrate, methylotrophic yeasts and alcohol dehydrogenase (AO), used to determine the ethanol content. The biosensor based on strain *Pichia angusta* VKM Y-2518 proved to be the most efficient. Adaptation enabled formation of biosensors based on AO and *P. angusta* VKM Y-2518, which in practice possessed no sensitivity to carbohydrates and organic acids. The interfering compound in ethanol detection was methanol.

	Analyzed compound	Microorganism	Transducer; immobilization	Lower limit of detection, mM	Detection range, mM	Interfering compounds	Optimal conditions	Stability; usage	Refs.
	Table 1, Part 1 (detection of glucose and ethanol)								
1	Ethanol	*Pichia angusta (P. angusta* VKM Y-1397, *P. angusta* VKM Y-2518, *P. angusta* VKM Y-2559)	Clark oxygen electrode; adsorption on Whatman GF/A chromatographic paper	0.012	0.5–5.0	Insensitivity to carbohydrates, organic acids; interfering compound, methanol	pH 7.2–7.6, 30 mM phosphate buffer	5 days; ethanol detection in alcoholic beverage	[12]
2	Ethanol (methanol)	*Pichia angusta* VKM Y-2559, *Pichia angusta* VKM Y-2518, *Pichia angusta* VKM Y-1397, *Hansenula polymorpha* NCYC 495 ln	Clark oxygen electrode; adsorption on Whatman GF/A paper, nitrocellulose membrane chemically modified by DEAE dextran and benzoquinone	0.05 (glass fibre), 0.2 (membrane)	0.05–1.18 (glass fibre), 0.2–1.53 (membrane)	Glycerol, glucose, fructose	pH 7.6; concentration of salts, 60 mM	21 days, 61% of the initial amplitude (adsorption on Whatman GF/A); ethanol in samples of ethanol production	[13,14]
3	Glucose	*Gluconobacter oxydans* sbsp. *industrius* VKM B-1280	Carbon paste electrode; mediators under study: ferrocene, 1,1'-dimethyl-ferrocene, 2,5-dibromo-1,4-benzoquinone, 2-methyl-1,4-benzoquinone	–	–	–	Use of water-insoluble mediators	2,5-Dibromo-1,4-benzoquinone is the most efficient of mediators under study	[16]
4	Glucose, ethanol	*Gluconobacter oxydans* sbsp. *industrius* VKM B-1280 and membrane fractions	Carbon paste electrode; ferrocene mediators	–	–	–	Mediators with donor and acceptor substituents	Ferrocene was tested for measurement of BOD in rye distillers' grains	[17]
5	Detection of ethanol in acetic medium	*Gluconobacter oxydans* sbsp. *industrius* VKM B-1280	Clark oxygen electrode; adsorption on Whatman GF/A paper	–	0.0125–2.00	–	pH 6–7; 30-mM Na-K phosphate buffer	Assessment of ethanol content in samples of commercially produced acetic acid	[18]
	Table 1, Part 2 (detection of toxic compounds)								
6	ε-Caprolactam	*Pseudomonas putida* BS394 modified by degradative plasmids pBS26, pBS265, pBS276	Clark oxygen electrode; adsorption on Whatman GF/A paper	0.02	4.5	Aminocapronate, 3-adipinate, 4-cyclohexanol, 5-cyclohexanone	***pH*** of the medium, 7.6; concentration of buffer solution salts, 33 mM	15 days (drop of activity by 25%); detection of ε-caprolactam in model media	[20]
7	Ethylene diamine tetraacetic acid (EDTA). Degradation of EDTA and its complex with Ba^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}	Bacterial strain *Chelativorans oligotrophicus* LPM-4. Degradation of Cd-EDTA and Ni-EDTA complexes by strain *Chelativorans oligotrophicus* LPM-4 with consumption of oxygen was first shown	Clark oxygen electrode; adsorption on Whatman GF/A paper	0.5	Sensitivity, 4.33 nA·min/mM	–	The first enzyme of EDTA degradation is monoamine oxidase oxidizing EDTA to ethylene diamine triacetate and glyoxylate	Laboratory studies	[21]
8	Thiodiglycol	*Alcaligenes xylosoxydans* subsp. *denitrificans* TD2	Clark oxygen electrode; adsorption on Whatman GF/A paper	05	1.0–50.0	Thioglycolic and glutamic acids, ethanol	pH 7.8	3 days	[22]
9	Methylamine (MA), dimethyl-amine (DMA), trimethyl-amine (TMA)	*Methylopila musalis* VKM B-2646	Clark oxygen electrode; adsorption on Whatman GF/A paper	11.0 for MA; 16.0 for DMA; 6.0 for TMA	–	Formaldehyde, methanol, ethanol. glucose	–	5 days	[23]

10	Dichloromethane	Four methylobacterial strains checked: *Methylobacterium dichloromethanicum* DM4, *M. extorquens* DM17, *Methylopila helvetica* DM6 and *Ancylobacter dichloromethanicus* DM16. The best with respect to dichloromethane degradation proved to be strain *M. dichloromethanicum* DM4	pH-Sensitive field effect transistor (registers disengagement of hydrochloric acid)	–	0.6–8.8	1.0–9.0	pH 6.0–8.5	Waste water assays	[24]
	Table 1, Part 3 (detection of BOD)								
11	BOD_5 of food production effluents enriched in starch	*Gluconobacter oxydans* sbsp. *industrius* VKM B-1280	Clark oxygen electrode; adsorption on Whatman GF/A paper	–	3.0–30.0 mg O_2/l for GGM (glucose-glutamic mixture)	–	10–12 min, time of single measurement	The developed laboratory models may be effectively used for a fast assessment of BOD in food industry wastewater	[27]
	BOD_5, comparative assessment of strains	*Candida maltosa* VKM Y-2359, *C. blankii* VKM Y-2675, *Debaryomyces hansenii* VKM Y-2482	Clark oxygen electrode; adsorption on Whatman GF/A paper	–	For *Candida maltose*, 9.3–422; for *C. blankii*, 3.0–56; for *Debary-omyces hanse-nii*, 2.2–177	–	The maximum biosen-sor response is ob-served at pH 6.5 for *C. maltosa*; at pH 6.8 for *C. blankii*, and at pH 7.0 for *D. hansenii*	Water treatment facilities, glucose-molasses plants	[28]
12	BOD_5, immobilization	Yeast cells *Debaryomyces hansenii* VKM Y-2482	Clark oxygen electrode; immobilization in poly(vinyl alcohol) modified by N- vinylpyr-rolidone	0.7 mg O_2/dm^3	0.7–207 mg O_2/ dm^3	–	Modification provided for a high sensitivity and stability of the bioreceptor	30 days; 34 substrates checked: alcohols, carbo-hydrates, carboxylic acids, amino acids, nitrophenols and surfactants that may occur in wastewaters	[29]
13	BOD_5, microbial co-culture-based biosensors	Yeast co-culture-based biosensor: *Pichia angusta*, *Arxula adeninivorans* and *Debaryomyces hansenii*	Clark oxygen electrode; immobilization in poly(vinyl alcohol) modified by N- vinylpyr-rolidone, adsorption on Whatman GF/A paper	2.2–2.4 mg O_2/ dm^3	2.4–80.0 mg O_2/ dm^3	–	–	17–19 days; 23 substrates checked: alcohols, carbohydrates, carboxylic acids, amino acids, nitrophenols and surfactants that may occur in wastewaters. Analysis of fermentation products and water samples	[30]
14	BOD_5, BFC-based biosensor	*Gluconobacter oxydans* sbsp. *industrius* VKM B-1280	-	-	-	-	-	Water treatment facili-ties; electricity generation	[35]
15	BOD_5 activated sludge-based biosen-sor	Activated sludge microorganisms						Water treatment facilities	[31]

Table 1: Examples of some amperometric-type microbial biosensors for detection of organic compounds.

Note: all strains used were from the All-Russian Collection of Microorganisms (VKM, IBPhM RAS).

The characteristics of the receptor elements and biosensors based on the yeast *Hansenula polymorpha* NCYC 495 ln for ethanol assays were investigated. Cells were immobilized by adsorption on glass fibre and benzoquinone- and diethylaminoethyl dextran (DEAE dextran)-modified nitrocellulose membrane [13,14]. The used immobilization techniques met the conditions of cell fixation and were simpler in forming first-generation biosensors than incorporation into conducting polymers, e.g., into polypyrrole. The integrated data on this type of immobilization used to develop second- and third-generation biosensors can be found in a review [15].

Amperometric biosensors based on carbon paste electrodes containing electron transport mediators were considered. The possibility was shown of using strain *Gluconobacter oxydans* sbsp. *industrius* VKM B-1280 in a carbon paste electrode in combination with water-insoluble mediators – ferrocene, 1,1-dimethylferrocene, 2,5-dibromo-1,4-benzoquinone, 2-methyl-1,4-benzoquinone – for electrocatalytic detection of glucose [16]. The mediator 2,5-dibromo-1,4-benzoquinone provided the highest catalytic activity. Ferrocene-type mediators, having donor and acceptor substituents, were dealt with in [17]. That work showed that 1,1′-ferrocene dimethanol and ferrocene monocarbonic acid – mediators with acceptor substituents – were the most efficient as compared with donor substituents and were preferable for detection of sugars and alcohols.

Gluconobacter bacteria are widely used in various biotechnological processes, in particular, in production of acetic acid from alcohol-containing products. At the initial stage of the fermentation process the reactor contains the maximum amount of ethanol (about 10%), which goes down during the rise in the content of acetic acid. A method was proposed for the biosensor assessment of the acetic acid production process by measuring the content of alcohol in the reactor. The method is based on the fact that *Gluconobacter* bacteria do not oxidize acetic acid but, herewith, the ethanol oxidation rate does not depend on the concentration of acetic acid. A biosensor based on the bacterium *G. oxydans* sbsp. *industrius* VKM B-1280 made it possible to determine ethanol within the range of 0.0125–2.00 mM. For real measurements of samples taken from the reactor they would have to be diluted 80-fold. The content of ethanol in samples of commercial acetic acid produced by various manufacturers was assessed [18]. This approach forms the basis for the development of an express assay of the fermentation process and can be used in acetic acid production.

Although microbial biosensors make use of hundreds of strains from various genera, one would like to note the features of *Gluconobacter oxydans* bacteria. These cells are of significant interest for developing both biosensors and biofuel cells. *G. oxydans* bacteria exhibit a unique metabolism for quick and incomplete oxidation of a wide range of different compounds (aldoses, ketoses, mono- and polyalcohols, etc.). Such biotransformation efficiency with simple biomass production led to industrial applications of these bacteria in the production of several important commodities. Their respiratory activity can also be successfully studied and used in the field of bioelectrochemistry. In a review [19], the authors set it their goal to present various strategies to improve the selectivity of assays using intact/treated cells of *G. oxydans* to introduce the application of *G. oxydans*-based biosensors in selective monitoring of analytes during biotransformation processes and to provide information about utilizable sugars in fermentation media or in biological oxygen demand determination. The concluding part of that review describes a potential application of *G. oxydans* cells in the generation of electricity from complex fuels within microbial fuel cells by an advanced direct electron transfer route between bacterial cells and electrodes. Many of these important data were taken into consideration in R&D at the Laboratory of Biosensors [16–18,27,43].

Biosensor detection of xenobiotics

Detection of xenobiotics is required in monitoring the environment for preservation and protection of human health. In [20], ε-caprolactam – a cyclic amide of ε-aminocaproic acid – was chosen as a target object. Caprolactam serves for formation of polyamide resin used for production of capron fibre. It was shown that a microbial biosensor based on cells containing ε-caprolactam-degradative plasmid can be an important tool for its detection on objects of the environment. Strain *Pseudomonas putida* BS394 modified by plasmids pBS26, pBS265, pBS276 was used to form the biosensor.

It is known that accumulation of ethylenediaminetetraacetate (EDTA) in ground waters leads to deteriorate the quality of potable water, as well as to transfer heavy and toxic metal ions into a dissolved state. Using an obligate EDTA degrader *Chelativorans oligotrophicus* LPM-4 immobilized on a Clark oxygen electrode, the

oxidative activity of bacteria was investigated. The strain was first shown to degrade Cd–EDTA and Ni–EDTA complexes (for other complexes with metals the effect has been known). The biosensor model can be useful in laboratory studies [21].

Thiodiglycol (beta′-dihydroxydiethyl sulfide, TDG) is a product of hydrolysis of mustard gas, a blistering agent. TDG is degraded by representatives of a small number of taxonomic groups of microorganisms. They mainly include representatives of *Alcaligenes* and *Pseudomonas.* The work [22] set its aim to study the behaviour of cells in a biosensor model. The biosensor made use of immobilized bacteria *Alcaligenes xylosoxydans* subsp. *denitrificans* TD2 as the basis of a receptor and a Clark oxygen electrode as a transducer. It was shown that the biosensor had an almost linear calibration curve within the range of 1.0–50.0 mM TDG. It is assumed to be used for laboratory studies.

Methylated aliphatic amines, as common precursors in organic synthesis, are used in production of insecticides, vulcanization accelerators, medicinal products, solvents. Methylamine (MA) is used to produce fungicides, tanning substances, dyes, rocket fuels. Dimethylamine (DMA) is applied in production of herbicides and detergents. Trimethylamine (TMA), in production of bactericides, fodder additives, reagents for flotation processes. Released into the environment, methylated aliphatic amines inflict significant damage to living organisms. Considering these data, it can be said that there was a must to develop an analytical device for MA/DMA/TMA assays by means of a biosensor simple in design and easy to operate. When developing such a biosensor, aerobic methylobacteria *Methylopila musalis* VKM B-2646 were used as biological material. The duration of a single assay did not exceed 10 min [23].

The broad application of dichloromethane (DCM) requires a constant control of its content in municipal and industrial effluents. DCM belongs to the class of halogenated methane derivatives and is a widespread pollutant. It is known to possess a high toxicity for mammals. To develop a breadboard of a biosensor, dichloromethane-degrading bacterial cells were immobilized on the measuring surface of a pH-sensitive field effect transistor. The presence of dichloromethane in the medium caused a change in the output signal of the transistor due to the emergence of H^+ ions in the medium as the result of DCM utilization by methylobacteria [24].

Biosensor determination of biological oxygen demand (BOD)

The BOD index is used in monitoring pollutions of the environment [25,26] and is determined as the amount of oxygen required for oxidation of organic components contained in water. BOD measurements using biosensor devices are thousands of times more rapid than the classical techniques; they take from 2 up to 15 min. BOD_5 measurements (five-day tests) by the classical method require about 5 days. In studies of this issue, it was found that the use of *G. oxydans* bacterial cells in a BOD biosensor led to a possibility of stable BOD monitoring and it could be used for detection of food production wastewaters, enriched in starch, under laboratory conditions [27].

Another work [28] presents the characteristics of BOD biosensors based on the yeast cultures *Candida maltosa* VKM Y-2359, *Candida blankii* VKM Y-2675 and *Debaryomyces hansenii* VKM Y-2482. These studies led to a conclusion that of three strains, the use of *D. hansenii* as the basis for a biosensor receptor element for BOD detection in municipal and biotechnological wastewaters is the best.

To assess the behaviour of microbial cells under various immobilization conditions, an immobilization scheme based on the use of polyvinyl alcohol modified by N-vinylpyrrolidone was proposed. Combination of polymers made it possible to produce receptor elements, which preserved the cell viability for a long time and had a high sensitivity and stability [29].

The use of a yeast co-culture-based biosensor for determination of waste water contamination levels (microorganisms of *P. angusta*, *A. adeninivorans* and *D. hansenii* were used in the experiment) showed that the co-cultures possessed broad substrate specificities and enabled assays of water and fermentation products within a broad BOD range (2.4–80 mg/dm^3) with a high correlation to the standard method (R = 0.9988). The use of the mentioned co-cultures immobilized in *N*-vinylpyrrolidone-modified poly(vinyl alcohol) enabled developing a BOD biosensor possessing the characteristics not inferior to those in the known biosensors [30]. It should be noted that in a BOD analyzer the use of several cultures simultaneously, isolated, e.g., from activated sludge, leads to the development of a more stable and highly sensitive BOD device [31].

Microbial biofuel cells

Biofuel cells (BFC) are attributed to systems, the basis of whose functioning is the bioelectrocatalysis reaction, which is based on the interphase transfer of charge and enables generation of electric energy at the oxidation of organic compounds – sugars, alcohols; substances that occur in effluents, dyes etc. A distinctive feature of BFC is that for oxidation they take up organic compounds of biological material – enzymes, whole (including microbial) cells. BFC are attributed to the class of electric storage batteries, which differ from traditional chemical batteries by a number of parameters, one of which is that the BFC is a rechargeable source. After the main portion of fuel has been oxidized, a new portion is added, and the system continues to function. This fact is what attracts attention of practice-oriented people.

The theoretical principles and examples of applied usage of microbial cells in BFC are described in detail in a monograph [32]. The modern development of the materials science technology leads to publications that describe applications of nanomaterials for forming biosensors and BFC; thus, a detailed analysis and recommendations on the use of graphene for forming BFC electrodes is given in a review [33]. Microbes can be fed with waste products rich in organic matter (domestic wastewater, lignocellulosic biomass, brewery wastewater, starch processing wastewater, landfill leachates etc.) to generate electricity. Microbial BFC can also be used for wastewater treatment, as biosensors and for production of secondary fuel like hydrogen [34,35]. Gathered data made the basis of R&D by the Laboratory of Biosensors [36].

Thermally expanded graphite as a microbial BFC anode nanomaterial. Formation of the BFC anode based on *G. oxydans* membrane fractions

An important thing in the development of BFC is to choose material of the electrode. Electrode material should possess a number of properties: have a high electrical conductivity, chemical resistance, large specific surface and specific adsorption capacity; to possess a biocompatibility. A graphene-like material, thermally expanded graphite (TEG), is promising in this respect. Its production is reduced to the incorporation of sulfuric or nitric acid in the presence of hydrogen peroxide, potassium permanganite or some other compounds between layers of graphite's lattice. Then, oxidized graphite is subjected to a thermal treatment, high-speed heating at a rate of 400–600°C/s. The high heating rate leads to a sharp evolution of gaseous intercalate-decomposition products, and graphite particles are cleaved under the action of temperature virtually to graphene layers [37]. Owing to the produced laminated fibrous structure, TEG is well pressed and molded. Fabrication of BFC electrodes from it is reduced to a simple compression of TEG powder at a pressure of 100–150 Bar. TEG has a high specific surface reaching values of 2000 m^2/g. Using TEG material as an electrode, it could be assumed that the high specific surface would provide its high surface concentration in immobilization of biocatalyst. The use of TEG in combination with biocatalyst utilized for electrooxidation of organic compounds has not been described in the literature.

As the experiment showed, immobilization of cells on an electrode fabricated from TEG led to an increase of current, which can be seen on the current–voltage curves. The BFC power also increased. At the immobilization on TEG, the maximal power was 6 $\mu W/cm^2$ (at an BFC internal impedance of 166 Ohm), which exceeded the value of 2.5 $\mu W/cm^2$ (internal impedance, 416 Ohm) obtained for a control electrode fabricated from spectral graphite. Presumably, its role in the rise of power was also played by the high specific surface of the TEG electrode and the possibility to increase, because of this, the amount of immobilized catalyst [38].

In continuation of this research, a work was done in which membrane fractions (MF) of *Gluconobacter* cells were used as anode biocatalyst. Membrane fractions are produced by disrupting whole bacterial cells by sonication. In essence, MF are parts of the cell membrane and contain various cell membrane structures, including membrane PQQ-dependent dehydrogenases. Their metabolic activity is similar to that of whole cells.

A number of recent works observed the effect of ethanol direct mediator-free electrooxidation with participation of PQQ-dependent alcohol dehydrogenase immobilized on multiwalled carbon nanotubes [39]. Earlier, direct mediator-free electrooxidation of ethanol by PQQ-dependent alcohol dehydrogenase immobilized on spectral graphite was presented in [40]. A mediator-free transfer of electrons in a BFC based on glucose dehydrogenase and laccase adsorbed on carbon nanotubes was also shown [41,42].

Adsorption of membrane fractions on TEG, carbon nanomaterial of a high specific surface, made it possible to hope that statistically a required contact of biological material and conducting surface, which will lead to mediator-free electrooxidation of substrate, will be achieved. As substrate, ethanol was chosen, whose efficient oxidation with participation of MF and redox mediators was shown in [17]. In [43], electrooxidation of ethanol on electroconducting biocomposite material based on TEG and *Gluconobacter oxydans* VKM B-1280 membrane fractions was studied. Using cyclic voltammetry and chronopotentiometry, the characteristics of bioelectrodes were studied. It was first shown that electrooxidation of ethanol on a bioanode that contained membrane fractions immobilized on TEG could proceed both in the mode of direct and mediator-based electrocatalysis.

Converter-based accumulation of BFC energy

A comprehensive consideration of the properties of enzyme and biofuel cells is given in a review [44]. For BFC, a classification in accordance with the nature of electrode reactions and the character of biochemical reactions was proposed. Special attention is given to the fact that biofuel cells belong to low-power devices. Many BFC are known to be characterized by the developed power on the order of microwatts obtained from a square centimetre of the electrode [32]. In [45], the authors described a method of accumulating electric energy, which was used by investigators from Clarkson University and which was a partial case of energy accumulation at the connection of a high-value capacitor to a BFC battery. The method was characterized by a simplicity and required no processing of the BFC signal by means of special apparatus. It should be noted that, herewith, the described method did not lead to a rise of BFC voltage.

In [46], a novel, not described earlier, method of accumulation was described, in accordance with which a BQ25504 (Texas Instruments) converter, producing the transformation of direct current, was connected to the BFC. The converter accumulated (increased voltage) and stored electric energy generated by unstable sources. An earlier work showed the efficiency of the BQ25504 converter in the processing of output voltage from a photocell, a solar battery, whose instability is related to that of the light flux [48]. From the description of the BQ25504 parameters it follows that the device should also work with energy sources having an extremely low, microwatt level of output energy. It is important to note that the electronic circuit of the converter is fed from the same source of energy, to which it is connected, i.e., the converter requires no connection to an external power supply. In [46], the first steps were made in the converter-based accumulation of microbial BFC electric energy. The accumulation mode was studied using capacitors of 100, 1000 and 6800 μF, as well as an ultracapacitor of 1 F. The system was found to enable increasing the BFC voltage from an open-circuit potential (400–500 mV) up to a specified value, which was set to be 3.2 V. The time required to accumulate the set voltage at the converter output depends on the value of the accumulation capacitor. It is ~13 min when using a 100-μF capacitor and ~2 h for a 6800-μF capacitor. The 6800-μF capacitor charged in this way contained a charge of 21×10^{-3} C that provided for accumulated energy of 32.7 mJ, which enabled, in a short-time mode (~30 s), to maintain the flash of an L-1154SURDK light-emitting diode (Kingbright; 2.0 V, 20 mA) or a short-time rotation of an M25E-4L electric motor (MITSUMI; 3.0 V, 100 mA). It should be noted that the number of microbial cells at the BFC anode was equal to 200 mg of wet weight, and the initial concentration of ethanol was 20 mM. In accordance with the available data obtained from the literature sources, such studies have not been conducted until now. The obtained results form the basis for implementing an application of the converter-based accumulation of energy, e.g., in an implanted BFC. The developed electricity accumuator also enabed accumulating energy for test data transfer [47].

Development of miniature electrodes. Start-up studies of a microbial BFC implanted into the brown frog *Rana temporaria*

In R&D of the recent years, there is a tendency to develop implanted BFC. It is assumed that an integrated solution of this task will be based on the use of sugars, primarily glucose, that are present in a living organism, as an oxidized substrate [49,50]. The meaning of this approach is that in the functioning of a BFC the amount of consumed fuel is insignificant and would not lead to a significant loss of glucose or another metabolite in a living system. Herewith, the expected benefit is evident, because the need for the surgical replacement of power supply sources in such devices as pacemakers, micropumps, etc., may in fact disappear or the replacement period be significantly extended. A promising application is considered to be the use of BFC for autonomous systems of artificial intellect – robots [51].

The first enzyme BFC implanted into the rat peritoneal cavity was described in 2010 [52,53]. Later works presented the results by two groups of researchers, whose experiments used various species of invertebrates: one group, under the leadership of Daniel Scherson from CaseWestern Reserve University (USA), cockroaches [51]; the other, of Evgeny Katz from the University of Clarkson (USA), molluscs [45], snails [54], lobsters [55]; this group also developed an enzyme BFC that generated energy at the oxidation of glucose and fructose in an orange; the data of the BFC were transmitted to the experimenter via a radiochannel [56].

Work [57] was carried out in search of ways for the use of BFC in immobilized state. The work investigated the generation of a bioelectrocatalytic potential at the oxidation of substrates when using the peritoneal fluid of the brown frog *Rana temporaria* as a base electrolyte. Glucose and ethanol were tested as oxidized substrates. The experiment was conducted in two stages: at the first stage, the measurements were carried out on peritoneal fluid extracted from the frog; at the second, electrodes immobilized in the animal organism were used. To form a bioelectrode (bioanode), we used immobilized bacterial cells *Gluconobacter oxydans* VKM B-1280. To assess the electrical activity, cyclic voltammograms and chronopotentiograms were registered. The three-electrode scheme of measurements was used. Electrodes from spectral graphite with immobilized bacteria served as an anode. A standard silver chlorine electrode was a reference electrode; a platinum plate, an auxiliary electrode.

The level of glucose in the brown frog depends on many factors, including on the time of the year. It is known that the concentration of glucose in frog's peritoneal fluid can be from 4 (in the norm) up to 200 mM (in a cooled animal) [58], which theoretically is sufficient for the generation of anode potential and difference of potentials by an implanted BFC. The measurements were carried out in February–March 2015. An average level of glucose in frog's peritoneal fluid at that time of year is within the range of 20–60 mM. The work [57] describes an experiment with frog's peritoneal fluid, which was performed for the first time. Answers to questions were obtained, which can be formulated as follows. First, peritoneal fluid of the brown frog *Rana temporaria* as an electrolyte satisfies the conditions required for charge transfer and makes it possible to perform the reaction of the bioelectrocatalytic generation of potential. Second, peritoneal fluid of the frog contains no components inhibiting the activity of biocatalyst, in this case, *G. oxydans* bacterial cells. Third, based on the generation of potential in the presence of the mediator, it can be concluded that peritoneal fluid contains a glucose concentration sufficient for the generation, and introduction of an additional portion of glucose only insignificantly accelerates the generation process. Fourth, test *in vitro* experiments with peritoneal fluid of the brown frog showed that this type of amphibia enables implantation of BFC and generation of electric energy owing to the oxidation of inner substrates, i.e., glucose. The conducted test experiments enabled considering further tests with implantation of BFC anode and cathode into the brown frog *Rana temporaria*.

Measurements of potential difference generation by a BFC implanted into a spinalized frog were the second part of this experiment. Electrodes were fabricated from TEG. They featured a small area (the anode and cathode areas were the same, of the order of 0.5 cm^2, i.e., the size of the electrodes was 5×5 mm^2). The current–voltage and power curves plotted for these electrodes showed that the specific power for them was of the same value as for large-size electrodes, i.e., ~5 $\mu W/cm^2$. Fabrication of small-size electrodes was stimulated by the aim to minimize animal's trauma during their incorporation into the peritoneal cavity. To enable the generation of signal during the formation of the anode, a water-soluble mediator dimethyl ferrocene was introduced into the TEG. For this purpose, the mediator was dissolved in acetone at a concentration of 10 mg/ml. TEG electrodes were immersed into this solution for 30 min. The acetone solution evaporated during the drying. A membrane-free registration was used. A microbial BFC implanted into the brown frog *Rana temporaria* was shown to enable generation of a potential of the order of 50 mV. Time required for generation was 600–800 s [59].

The above listed features – generation of electric energy by a BFC based on frog's peritoneal fluid, as well as the functioning of an implanted BFC in a brown frog enable considering the frog as a promising object for developments of novel types of implanted devices. According to the available data, no works on the assessment of the suitability of frog's peritoneal fluid for generation of bioelectrocatalytic potential, as well as implantation of a BFC into the frog, has been done until now.

Conclusion

The presented review considered issues associated with the development of microbial biosensors and microbial biofuel cells. Based on which assayed compounds can be detected by the developed biosensor models, it can be stated that the approach based on the use of microorganisms for these tasks is expedient. Development of microbial biosensor models once again emphasized the use of microorganisms' positive quality – to use them for oxidation of substances, without a special search for and isolation of enzymes.

When developing microbial biofuel cells, results were obtained that can be of applied interest. Thus, membrane fractions can be used to form the anode; it was shown that thermally expanded graphite – a nanomaterial well known but not used in BFC earlier, which increases its efficiency – can be used in formation of the anode; to increase the efficiency and to accumulate the power of the BFC, an efficient way is the use of a converter for sources of microwatt power; implantation of a BFC into the brown frog continues the chain of examples of BFC incorporation into the animal organism.

Given the increasing demand for practical and low-cost analytical techniques, biosensors have attracted attention for use in the quality analysis of drugs, medicines, and other analytes of interest in the pharmaceutical area. The obtained results expand our knowledge and, to this or that extent, can be used in practice.

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