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Biosensors – basics and use

Abstract. The work is devoted to the existing variety of biosensors used to control environmental pollution. The physical principles of enzymatic, microbial and DNA sensors are reviewed. It is concluded that it is possible to create biosensors based on *Drosophila melanogaster* genetic constructs, with the similar properties to microbiological ones.
Key words: biosensor, enzyme, DNA, immobilization, environment, toxicant, GFP, luciferase.

Introduction

Recently, increasing interest in biosensors, that are new analytical devices to process ecological information.

Biosensor is a device in which the sensitive layer contains a biological material, such as enzymes, tissue, bacteria, yeast antigens\antibodies, liposomes, organelles, receptors, DNA [1]. They immediately react to the presence of the analyte.

In 2003 the total world market sales amounted to 7.2 billion biosensors. US dollars, with an annual increase of 10-14% over the next eight years [2, 3].

A biosensor is a composite device consisting of two major components: biochemical converter performs the function of biological recognition, and physical converter (transducer) which converts an electrical signal into concentration using a special apparatus (Figure 1). There is a wide variety of physical transducers: electrochemical, spectroscopic, thermal, piezoelectric, transducers for surface acoustic waves, etc. If the transmitter uses a physical change in absorbance biolayer, is called a biosensor, e.g., a fiber, since the measured signal is transmitted to the measuring device via an optical fiber [4].

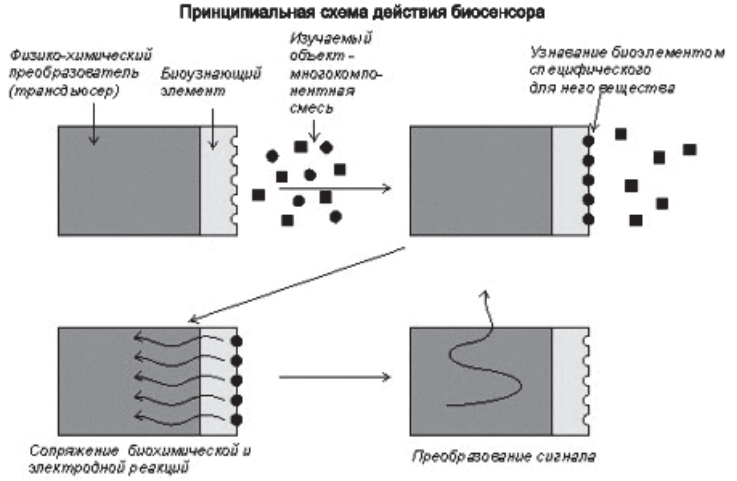

Figure 1 – Schematic diagram of the steps of the biosensor [5]

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Main part

Biosensors can be grouped according to their biological elements or transducer elements (Table 1). The biological components include enzymes, antibodies, microorganisms, biological tissues and organelles.

Antibody-based biosensors also exist, called immunosensors.

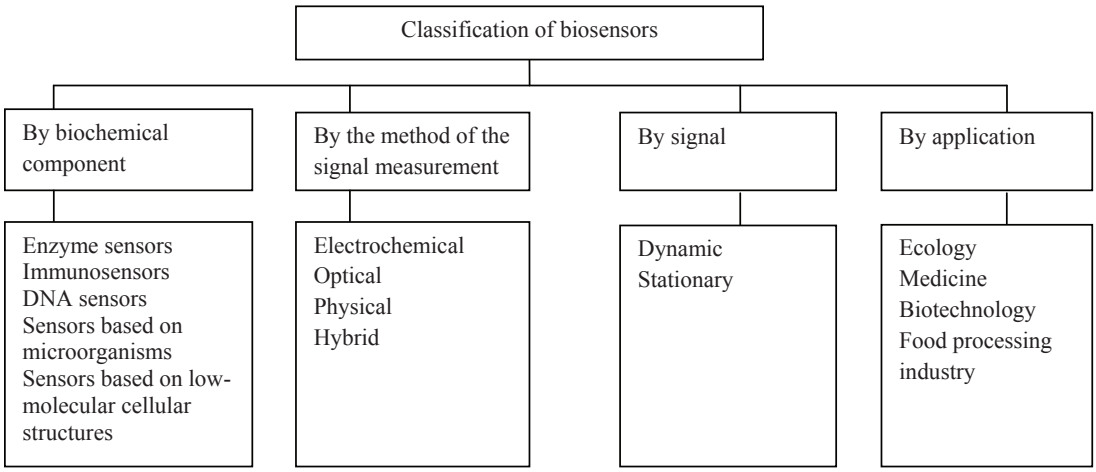
Biosensors can be classified according to the nature of the biochemical converter – enzyme electrodes, immunosensors and DNA sensors [6].

The enzyme sensors include biological material exhibiting biological activity. Enzyme sensors in turn, are divided into substrate and inhibitor. Substrate biosensors are substrates for determining enzymatic reactions, and to determine the inhibitory substances which reduce the activity of the enzyme. Immunosensors, used as a receptor immunoglobulins – protective proteins. They are used to determine interaction – antibody and antigen. In the presence of specific antibodies such sensors can detect virtually any compound.

DNA sensors comprise nucleic acids as component. It is not natural components derived from a living organism and their fragments, called DNA probes or DNA primers. The main task of DNA sensors is to detect low molecular weight compounds and proteins that interact with specific regions of DNA.

Microbial biosensors are used to evaluate the state of natural microbial communities, to monitor treatment plants [1, 7].

Table 1 – Classification of biosensors



In enzyme biosensors the substance to be detected diffuse through the semipermeable membrane thin layer of a biocatalyst, wherein the enzymatic reaction occurs. The product of the enzymatic reaction is determined by the electrode on the surface of which is fixed an enzyme, such a device is called enzyme electrode (Figure 2) [8].

Enzyme sensors are used in eco-analytical control to determine toxic compounds – environmental pollutants of anthropogenic origin, and to assess the level of contamination. (Figure 3) to select a part of the enzyme biosensor is determined on the basis of what enzyme systems are attacked at its receipt toxicants into the body [9, 10].

Estimation of substrates – environmental contaminants has the advantage as compared with inhibitor biosensor since after the measurement is not necessary in the regeneration of the enzyme inhibitor-related (Table 2) [11].

Nucleic acids are capable of forming different connections to define compounds [12]. DNA reacts with biomolecules highly specific, due to the cooperative interaction of hydrogen, electrostatic and donor-acceptor bonds and hydrophobic interactions [13].

Creating a DNA sensor solves a number of urgent bioanalytical and medical problems:

1. Identification of the biomaterial on the sequence of nucleotides (genome sequencing, establishment of paternity, the diagnosis of microorganisms).

2. Diagnosis and treatment of cancer (screening anticancer drugs).

3. Identification of anticancer effect of pharmacological agents and DNA-damaging factors [14].

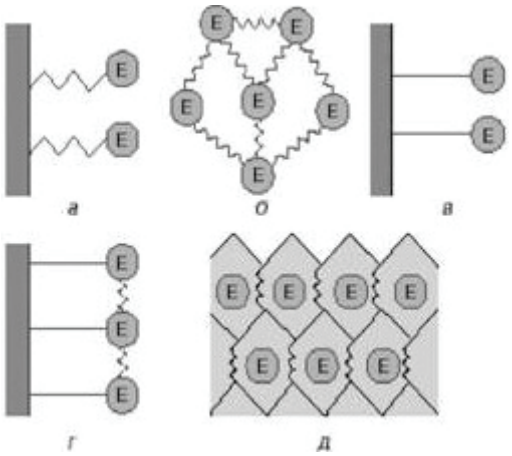


Figure 2 – Schematic representation of the methods of immobilization of enzymes in biosensors: a-covalent binding to the surface of the electrode, b – stitching, c- adsorption on the support (electrode), d – covalent binding and sewing in the substrate (electrode), e -railway carrier capture (in a polymer film). Budnikov GK Biosensors as a new type of analytical devices // Soros Educational Journal. -1996. – No. 12 (26) – pp. 26-32

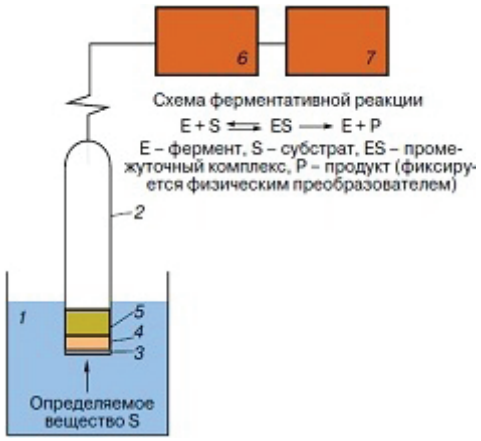


Figure 3 – 1 test solution; 2 biosensor body, 3 – semipermeable membrane, 4- biomaterial layer, 5 – physical transducer, 6 – signal amplifier 7 – recorder. Budnikov G.K. Biosensors as a new type of analytical devices // Soros Educational Journal. – 1996. – №12 (26) – C. 26-32

Table 2 – Enzyme substrates for determining – pollutants. Evtyugin G.A., Budnikov G.K., Stoykova E.E. Fundamentals of biosensors: Textbook – Kazan. – 2007. – P.80.

Enzyme	Substrate	Enzyme	Substrate
Sulfite oxidase	Sulfites	Cytochrome	Sulfites
Laccase	Phenols, amines	Cytochrome P_{450}	Amines, alcohols
Peroxidase	Phenols, amines	Phosphate tree esterase	Pesticides
Tyrosinase	Phenols, amines	Amine oxidase	Amines
Urease	Urea	Rhodanese	Hydrogen sulfide

Interaction of DNA analyte, there are three approaches to registration:

Direct determination of the fact of complex formation based on the change in the mass of DNA adsorbed on the surface of the sensor – the piezoelectric element. This method is suitable for registration of hybridization process – the interaction of certain sequences of nucleotides which are complementary to each other. Detection of the changes in DNA (conformational changes partial hydrolysis or partial oxidation of methylation specific nucleotides) in optical, electrochemical or other characteristics of the nucleotide sequence [15].

In microbial biosensors are used as the source of microorganisms enzymatic reaction. In comparison with the enzymatic biosensors have a number of advantages:

1. Excluded are costly operations for isolation and purification of enzyme preparations
2. Improved stability of enzymes in living cells
3. The presence of cofactors in the cell required for enzyme function
4. The simplicity and versatility of methods for measuring enzyme activity in terms of cell activity (concentration of the major metabolites such as hydrogen ions, carbon dioxide and ammonia, respiratory activity)

Amperometric, potentiometric and conductometric electrochemical devices are microbial biosensors [1].

In electrochemical microbial sensors the oxygen electrode is used in amperometric microbial sensors as the transducer. Such sensors are often used to measure the total content of easily oxidizable organic compounds, the BOD indicator analogue [16]. Sensors based on micro-organisms to measure the BOD include bacteria-destructors *Torulopsis candida, Trichosporon cutaneum, Pseudomonas putida, Klebsiella oxytoca, Bacillus subtilis, Arxula adeninivorans, Serratia mercescens, Hansennula anomala*, and thermophilic bacteria and yeasts. It is best to use several different strains, as well as natural microbial communities, such as community of activated sludge biological treatment facilities. Signal is a measurement of the concentration of dissolved oxygen, the field of administration of the substrate to be tested in the wastewater treatment process. BOD-testers have a high stability and fast response signal signal [17].

Biosensors based on *Trichosporon brassicae, Acetobacter aceti, Candida vini, Gluconobacter suboxydans, Aspergillus niger, Saccharomyces ellipsoideus, Pichia methanolica*, serve to define and ethanol have high sensitivity but low selectivity [18]. To improve the selectivity of the sensors used genetically modified microorganisms with inducer-dependent production of certain enzymes. It was proposed a selective method for the determination of copper ions using a sensor based on a recombinant strain of *S. cerevisiae*, which is composed of the plasmid with Cu and induced promoter *lacZ* gene [19]. This recombinant strain in presence of copper ions acquires the ability to oxidize lactose, thereby increasing the oxygen consumption [20].

Another application of amperometric biosensors microbial is a definition of compounds that inhibit the growth of microorganisms. As a result, the sensors have been proposed for the determination of hydrocyanic acid and cyanides inhibitory activity *Nitrosomonas europaea*, *Thiobacillus ferrooxidans, Saccharomyces cerevisiae and Pseudomonas fluorescens* [21, 22].

The most sensitive bioluminescent systems which utilize luminescent bacteria are composed of the enzyme luciferase. To activate the gene *lux* – a luciferase reporter one can use the inductor and the constitutive type's approaches. The gene expression in the inductor type is determined by the presence of the substance tested. The level of bioluminescence and the level of production of luciferase depended upon activator concentration [23]. This type of regulation applies to amperometric microbial sensors. In the second case, the promoter is present in living cells with active metabolism; its activity depends on the presence of the compound, and depending on the level of bioluminescence is modulated. This method is used in a generalized assessment of environmental pollution. Bioavailability is determined by the toxicity of heavy metal ions thus microbial sensors provide realistic results than methods of chemical analysis. Thus, the proposed bioluminescent microbial sensor for evaluating nickel and cobalt ions based on the strain of *Ralstonia eutropha* AE2515, which was introduced regulatory gene cnrYXN, having a relationship with a reporter system luxCDBAE. To estimate the concentration of mercury (II) it was used the bacteria containing an operon and a system merR luxCDBAE. The emergence of bioluminescence based on the induction of promoter mer, which is activated when Hg ion binds to the MerR. Lux gene expression is also used to assess the overall state of stress-causing microbes in unfavorable external environment, as well as the detection of DNA-damaging factors. This principle is based on microbial sensors carrying plasmid with a gene *lux Vibrio fischeri*. Their luminescence is in-

creased by the action of toxic compounds [24-26].

Similarly, gene expression of *lux* fluorescent sensors used microbial organisms genetically modified with the gene *GFP*, which encodes green fluorescent protein synthesis (green fluorescent protein, GFP) [27, 28]. Is very stable and is used as an indicator of the effects of various microorganisms. But one of the drawbacks of GFP is the presence of time between protein synthesis and its fluorescence. These GFP-based biosensors are often used for evaluation of bioavailable iron for plants, determining arsenite, toluene and its derivatives. Fluorescence detection is used as the fluorescent marine organisms, the glow of which depends on the oxygen concentration [29, 30].

Conclusion

In our opinion the range of possible optosensors can be significantly extended by using model genetic objects. Indeed, the genetic collection P-element insertion (in particular the enhancer-trap and GFP-trap) in *Drosophila* currently gives a possibility to record the activity of many different genes. On the other hand, the genome-wide analysis of gene expression changes under the influence of some typical environmental pollutants in *Drosophila*, carried out by A. Moskalev et al. in 2014, showed that there are significant differences in the spectrum of expressed genes in response to various pollutants. This makes it possible to create a collection of lines corresponding to a specific contaminant through the fluorescent signal of some tissues and thereby realize optosenor at the level of higher eukaryotes. To record the fluorescent signal the A fluorescence microscope low resolution can be used.

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