

## BIOSENSORS: DESIGN, CLASSIFICATION, AND APPLICATIONS IN THE FOOD INDUSTRY

E. V. Korotkaya

Kemerovo Institute of Food Science and Technology,  
bul'v. Stroitelei 47, Kemerovo, 650056 Russia,  
phone: +7(3842) 39-05-37, e-mail: lena\_short@mail.ru

(Received March 3, 2014; Accepted in revised form April 14, 2014)

**Abstract:** Biosensors are promising analytical tools applicable in clinical diagnostics, food industry, environmental monitoring, and other areas in which rapid and reliable analyses are needed. This review covers the basic types of biosensors and their designs and general operating principles. A classification of biosensors according to the type of transducer they involve and according to the nature of the biological entity used as the recognition element is presented. Methods of immobilization of biological components, namely, adsorption, microencapsulation, inclusion, cross-linking, and covalent binding are briefly characterized. The main areas of application of biosensors in the food industry—food safety and quality assessment, process monitoring, and others—are considered.

**Keywords:** biosensor, transducer, immobilization, foods

UDC [543.92:57.02]:641

DOI 10.12737/5476

### INTRODUCTION

The issue of food safety and quality is becoming increasingly challenging. This is due to the fact that hazardous foods that can contain toxic substances, including those of anthropogenic origin, appear on the market. Environmental pollution caused by sewage from industrial and agricultural enterprises, which contains residual pesticides, fertilizers, growth stimulants, and other harmful substances, leads to accumulation of toxicants in fish, meat, and dairy foods. In addition, in the 1980s some countries began extensive studies in the design of genetically modified organisms (GMOs) intended for the production of foods, animal feeds, and pharmaceutical and veterinary preparations. This highlights the need for extensive supervision aimed at revealing the strains that were created in violation of safety principles and at checking whether a product obtained using a GMO is identical to its natural prototype.

In view of the aforesaid, increasing attention is being focused on development of sufficiently sensitive and selective express analysis methods. Prominent examples of analytical systems combining all of the above merits are biosensors [1–4].

### GENERAL OPERATING PRINCIPLES AND DESIGN OF BIOSENSORS

Biosensors are analytical devices that employ sensitive biological materials to "recognize" certain molecules and provide information on their presence and amount as a signal convenient for recording and processing [4–10].

Any biosensor consists of the following three basic components: recognition element, which is a bio-

selective membrane involving various biological structures; physical transducer; electronic system for signal amplification and recording and for user-friendly data representation [4, 7–17]. Figure 1 presents a general scheme of a biosensor.

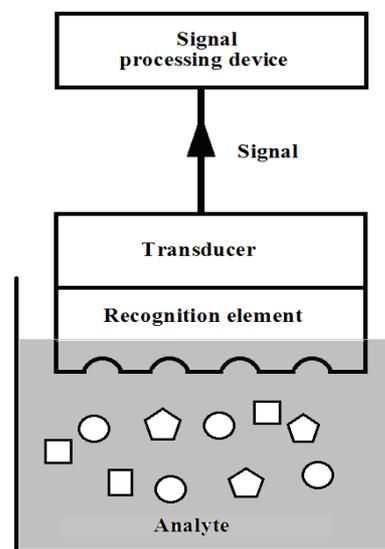


Fig. 1. General scheme of a biosensor.

A recognition element is the basic component of any sensor. It is due to its recognition element that a sensor can selectively respond to one or several analytes among a large number of other substances. All types of biological structures—enzymes, antibodies, receptors, nucleic acids, and even living cells—are used as a recognition element in biosensors.

A transducer converts the changes caused by the reaction between the selective biological layer and the analyte into an electric or optical signal. This signal is then measured using a light-sensitive and/or electronic device.

### CLASSIFICATION OF BIOSENSORS

Biosensors are classified according to transducer type or according to the nature of their biological component. The following four basic transducer types can be distinguished [4, 7–10].

#### *Electrochemical Transducers*

– Potentiometric transducers. The analytical signal in this case is the potential drop between the working electrode and the reference electrode or between two reference electrodes separated by a semipermeable membrane (at a zero current through the electrochemical cell). An ion-selective electrode (ISE) usually serves as the transducer. The most widespread potentiometric biosensors employ pH electrodes.

– Voltammetric transducers. Here, the electroactive species oxidation or reduction current is measured. The latter is induced by producing the preset potential drop between the electrodes. In most cases, a constant potential vs. the reference electrode is applied to the working electrode (or a bundle of electrodes). The observed current is proportional either to the volume concentration of electroactive species or to the rate of their disappearance or formation in the biocatalytic layer [4].

– Conductometric transducers. These transducers measure the electrical conductivity of the solution in the course of a biochemical reaction. They are less commonly used in biosensors, particularly when the recognition element is an enzyme. However, they should not be discounted in detection of affine interactions [4].

– Impedimetric transducers. These devices measure the impedance of an electrochemical cell and the variation of this impedance with ac frequency [9].

– Transducers based on field-effect transistors. The systems involving ion-sensitive silicon field-effect transistors are, in essence, conventional potentiometric systems, with the only difference that the input transistor of the electronic circuit of the high-resistance voltmeter is transposed into the solution being analyzed. This considerably enhances the resolving power of the transducer and thereby raises the sensitivity of the biosensor. The biosensitive layer is usually placed directly on the surface of an ion-sensitive membrane as part of the gate of the field-effect transistor [4]. Biologically modified, ion-selective, field-effect transistors provide means to directly determine small peptides and proteins as their characteristic charge [18].

A serious drawback of all potentiometric systems based on the above operating principles is their sensitivity to the buffer capacity of the solution, which markedly restricts their application.

#### *Optical Transducers*

Optical transducers may be based on absorption, fluorescence, luminescence, internal reflection, surface plasmon resonance, or light scattering spectroscopy. For example, an immunosensor based on localized

surface plasmon resonance on gold nanoparticles has been developed for determining casein in milk [19].

#### *Piezoelectric Devices*

Piezoelectric sensors employ crystals that undergo elastic deformation under the action of an electric potential. An alternating potential at a certain frequency generates a standing wave in the crystal. Analyte adsorption on the surface of the crystal, which is covered with a biological recognition element, alters the resonance frequency, and this is an indication of binding taking place. Piezoelectric immunosensors are considered to be among the most sensitive sensors developed to date, for they are capable of detecting antigens in the picogram range (<http://www.tms.org/pubs/journals/JOM/0010/Kumar/Kumar-0010.html>).

#### *Thermometric Transducers*

Measuring the amount of heat with a sensitive thermistor provides means to determine the analyte concentration. Thermal biosensors are quite uncommon.

Any type of biochemical element can be combined with different transducers to obtain a wide variety of biosensors. Up to 80% of the biosensors are electrochemical ones; depending on the nature of their biological component, they are also referred to as enzyme electrodes, immunosensors, and DNA sensors [3, 5, 8, 15].

The recognition elements in biosensors are biological entities that can recognize a single substrate among a multitude of others. This requirement is met by four entities: enzymes, antibodies, nucleic acids, and receptors [8].

#### *Enzymatic Sensors*

These include pure enzyme preparations or biological preparations (tissue or microbial culture homogenates) showing a certain biological activity. The simplest enzymatic biosensor design is used when the substrate or the product of the enzymatic reaction is electrochemically active, capable of being rapidly and reversibly oxidized or reduced on an electrode upon the application of an appropriate potential. According to their functions, enzymatic sensors are subdivided into substrate and inhibitor ones. Substrate biosensors are intended for determination of specific substrates of enzymatic reactions. Examples are glucose determination using a glucose oxidase-based enzymatic sensor and urea determination using a urease sensor [20, 21]. Inhibitor sensors are intended for determination of substances reducing the activity of an enzyme. An example is the determination of organophosphorus pesticides inhibiting acetylcholine hydrolysis catalyzed by acetylcholinesterase [6, 79].

The most common enzymatic biosensors are glucose and urea biosensors [8].

#### *Immunosensors*

Immunoglobulins, which are protective proteins secreted by the immune system of an organism in response to the ingress of alien biological compounds (antigens), are employed in this case as the biochemical receptor. Immunoglobulins, also known as antibodies, form strong complexes with antigens. Immunosensors are used to detect the participants of immunochemical

interaction, namely, the antibodies and antigen. The presence of antibodies in blood is a diagnostic indication of infection or a toxic action of certain substances.

Antigens can be determined not only in biological liquids, but also in other media, including the natural environment. Provided that there are specific antibodies, immunosensors can detect practically any compound, showing a high specificity and selectivity.

#### *DNA Sensors*

The biochemical components of DNA sensors are nucleic acids (DNA). Most frequently, they are not natural components isolated from a living organism, but their fragments called DNA probes or DNA primers. They are selected so that they reflect the specificity of the DNA structure as a whole. DNA probes are synthesized by DNA amplification via a polymerase chain reaction. They can be additionally modified so as to enhance their stability or facilitate their introduction into a biosensor. Oligonucleotide sequences having no natural analogue, selected according to their capability to interact with certain biomolecules, are also used in DNA sensors. These synthetic nucleic receptors received the name of aptamers [23, 24]. Since present-day science is unable to predict the aptamer structure required for each particular ligand, one has to synthesize all possible oligonucleotides for obtaining an aptamer (imposing a reasonable limit of, e.g., 40–100 nucleotides) and then select those which bind most strongly to the target.

Another purpose of DNA sensors is to reveal proteins and nonmacromolecular compounds specifically interacting with certain DNA fragments. These objects include regulatory proteins, tumor markers damaging DNA, and many anti-cancer drugs. Aptamers are nearly as specific as antibodies and exceed them in stability. The aptamer-based DNA sensors are called aptasensors. DNA sensors are used to determine the nucleotide sequence in a target DNA molecule that is complementary to the probe. This provides means to reliably diagnose pathogenic microorganisms and viruses and to solve problems of fine genetic diagnostics [3, 15, 25]. Examples of the latter application are affiliation, detection of genetic disorders, and detection of products made from genetically modified organisms.

#### *Microbial Biosensors*

In most common microbial biosensors, the biological component is separated from the recording device. This is due to the fact that the response of microorganisms to variations in the chemical composition of the medium is rather sluggish compared to the response of enzymes or antibodies, because the former is mediated by matter transfer across a biomembrane. For this reason, it is necessary to create a higher concentration of living cells than is allowed by the geometry of the transducer. A microbial biosensor may be a columnar or membrane reactor or a suspension of microorganisms in a solution with an immersed sensor [8, 14]. The microorganisms employed in these sensors can execute various

functions. They can convert the analyte using enzymes they secrete into the culture medium during their metabolism or enzymes remaining in their living cells. These sensors are similar to enzymatic sensors, with the only difference that a group of enzymes, not necessarily a single one, may be involved in the conversion of the substrate [14, 16]. The action of microorganisms is based on the fact that they change their respiratory activity as they assimilate organic substances. These microbial biosensors are called respiratory biosensors. They are used in the determination of the total amount of oxidizable organics in, e.g., wastewater [14, 26–28]. Respiratory microbial biosensors are also usable in the determination of antimicrobial agents suppressing microbial respiration.

Microbial oxidation reactions are low-selective, because, as distinct from individual enzymes, unicellular organisms can decompose various organic substances at similar rates. Genetic engineering makes it possible to design microorganisms producing certain enzymes whose activity can be measured in the same way as in the case of enzymatic sensors. This is how the stability of enzymes can be enhanced and their concentration can be increased in the case of low-stability proteins. The best known examples of these sensors include toxin determination systems based on the inhibition of luciferase, a microbial enzyme that generates luminescence during the oxidation of some substrates [14, 29–31].

Another area of application of microbial sensors is investigation of the effect of substances on a cell as a model of a multicellular organism. These biosensors are also employed in toxicological studies to estimate the median lethal concentration of toxicants and in the optimization of individual doses of antibiotics and the amounts of antimicrobial and antifungal additives for paints and finishing materials. Finally, microbial biosensors are used to estimate the condition of natural microorganism communities, for example, in monitoring the performance of biological wastewater treatment systems.

#### *Biosensors Based on Supramolecular Structures of a Cell*

These biosensors occupy an intermediate place between enzyme and DNA sensors, since they involve intracellular entities that have a fairly complex hierarchical structure. These entities include lipid membranes with built-in receptors, cell organelles (mitochondria and chloroplasts), polyenzyme complexes, etc. These biosensors have not found wide application yet, because their biological components, when isolated from their natural medium, are insufficiently stable to maintain the operating parameters of a sensor for a long time. They are used in the investigation of biochemical processes, for example, for verifying the mechanism of the toxic action of pollutants and for determining the pathway via which an action potential or another biochemical signal is transmitted from a cell. These biosensors include phytotoxicity sensors involving components of the photosynthetic apparatus of plant cells.

## IMMOBILIZATION OF BIOLOGICAL COMPONENTS

For a biosensor to operate reliably, its biomaterial should be bound to the transducer surface. This operation is referred to as immobilization of a biological component.

Immobilization means bringing a biomaterial (enzymes, antibodies, nucleic acids, oligonucleotides) into insoluble form by incorporating it in an inert support or by chemically or physically binding it to the transducer surface.

There are five basic methods of immobilizing biomaterials [8–10].

1. *Adsorption*. This is the simplest method that does not need any substantial pretreatment of sensor components or use of special-purpose chemicals. Alumina, activated carbon, clay, cellulose, collodion, silica gel, glass, hydroxylapatite, and many other substances are known to adsorb enzymes without affecting their native conformation.

Both physical adsorption and chemical adsorption (chemisorption) are usable in this case. In physical adsorption, the biomaterial is held on the surface by Coulomb, van der Waals, or ionic interactions or hydrogen bonding. In the case of immobilization of cellular structures, the adsorptive binding of cells to the surface of a pretreated polymer can be so strong that the removal of the cells from the polymer surface causes their lysis.

The result of adsorptive immobilization is largely determined by the properties of the transducer surface, including its charge, the presence of polar groups, its redox potential, and its energetic uniformity. Adsorption does not afford a high concentration of a biological component. In order to increase the amount of biological component adsorbed, the transducer is pretreated so as to generate charged or polar groups enhancing biomaterial adsorption. This is done by using various methods of oxidation and surface modification with polymers or functionalizing reagents. For example, the oxidation of gold and carbon electrodes increases their protein, nucleic acid, and microorganism adsorption capacity.

A considerably stronger binding between the biomaterial and the support is provided by chemisorption yielding covalent bonds (see below). The recognition elements prepared by adsorption are very sensitive to pH, temperature, ionic strength, and substrate concentration variations.

Adsorption is mainly used at the research stage, when even a weak binding between the biological material and the transducer is sufficient and the sensor is not intended for long-term operation.

2. *Microencapsulation*. This is one of the widespread methods of making electrochemical sensors. A biomaterial is placed near the transducer (electrode) so that it is separated from the rest of the solution by a semipermeable membrane allowing analyte molecules and the products of the catalytic reaction to pass through. For this purpose a direct or inverse emulsion is initially prepared from a polymer solution in an organic solvent and an aqueous solution of the biological component. The emulsion is dried to

obtain a membrane whose polymeric matrix incorporates microcapsules of water containing biomolecules and nonmacromolecular electrolyte ions. This immobilization method leaves intact the hydrophilic environment of the biopolymer at all immobilization stages, making it possible to achieve a sufficiently high residual activity of the enzyme. The immobilized enzyme is actually free but is localized in a certain part of the measurement cell.

Several types of membranes are used in microencapsulation. Along with cellulose acetate (dialysis) membranes, which are impermeable to protein molecules and slow down transport of many nonmacromolecular compounds, membranes made from polycarbonate (Nucleopore), the natural protein collagen, and from polytetrafluoroethylene (Teflon) are employed, the latter being permeable only to some gases [8]. Negatively charged membranes used in glucose sensors are made from the Nafion polymer (<http://www.biosensoracademy.com/rus/readarticle.php>).

This immobilization method is readily applicable to various sensor models. It ensures a reproducible performance of the enzyme, protecting it against contamination and degradation. On the whole, microencapsulated enzymes are resistant to variations of the pH, temperature, ionic strength, and chemical composition of the medium. Nevertheless, some molecules and species, such as small gas and electrolyte molecules, pass through the membrane.

3. *Inclusion*. Biomaterial inclusion into a forming polymer matrix is actually a universal method applicable to various types of recognition elements. The polymer can be deposited from an organic solvent by diluting the solution with water or from a microemulsion by drying it on the sensor surface. The polymer can also be obtained by gelation from a gelatin, agar, polyacrylamide, or alginate solution or by polycondensation of some organic esters or chloroanhydrides [32]. The latter technique is called sol–gel immobilization (Fig. 2).

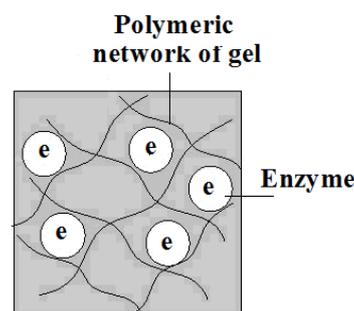


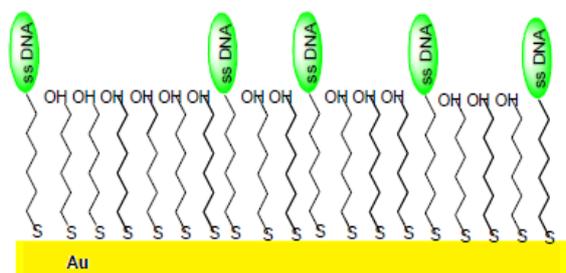
Fig. 2. Enzyme inclusion in gel pores.

A matrix consisting of a synthetic polymer is prepared in the presence of a biomaterial. A cross-linking agent is usually added in order to unite separate polymer strands into a three-dimensional network. The biologically active molecules find themselves entrapped in the polymer bulk. An obvious advantage of this method is its universality. Its drawback is that the network impedes diffusion and hampers analyte permeation. In addition, if the molecules included into

the network are not chemically bonded to it, they can be washed away.

Proteins and nucleic acids are immobilized in polymers of *o*- and *m*-phenylenediamine, *p*-aminophenol, thionine, other phenothiazine and phenoxazine dyes, and derivatives of pyrrole, thiophene, and aniline.

The inclusion of biomolecules in polyionic complexes results from complexation in the layer-by-layer deposition of polyelectrolytes from solution. The native environment of the enzyme is retained in synthetic lipid membranes, specifically, Langmuir–Blodgett (LB) films that are similar in composition and properties to natural biomembranes. These membranes are used as a model in the investigation of membrane processes, as well as in protein and nucleic acid immobilization [33]. LB films as such have a low mechanical strength; for this reason, they are deposited onto the surface of an inert hydrophobic polymer (polyvinyl chloride, Teflon).



**Fig. 3.** Self-assembled monolayer of  $C_nH_{2n+1}$  alkanethiol on the surface of the gold electrode of a DNA sensor.

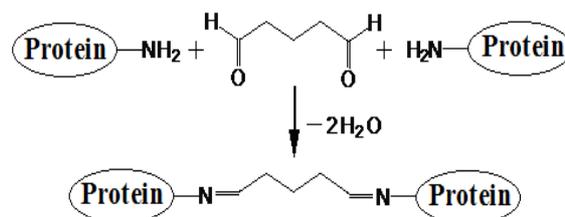
*Self-assembled monolayers (SAMs)* differ from LB films in that they are more strongly bound to the support [34, 35]. Monolayer formation begins with the interaction between the polar moiety (head) of separate molecules and the support surface (Fig. 3). The subsequent ordering of the molecules in the monolayer plane, or layer self-assembling, is due to the van der Waals interaction between the hydrophobic moieties (tails) of the molecules. Various materials, including silicon, metals, and oxides, can serve as the support [36]. SAMs are usable as a matrix for inclusion of biopolymers and hydrophobic nonmacromolecular compounds.

*Photopolymerized layers* are prepared by depositing a homogeneous mixture of monomers and a biological component onto a support and by exposing the mixture to UV radiation. This procedure exerts a weaker denaturing effect on the biomaterial than the chemical initiation of the same reaction.

**4. Cross-linking.** In this method, the biomaterial is chemically bound to a solid support or to a gel using so-called bifunctional reagents, for example, glutaraldehyde [13, 37, 38]. An example of such cross-linking is the action of glutaraldehyde, which forms Schiff bases with amino, hydroxyl, and thiol groups of proteins and nucleic acids (Fig. 4).

Various types of polymers (gelatin, agar, cyclodextrins, polyvinyl chloride, polyacrylamide, and

many other polymers and gels) were investigated in detail as matrices for a biomaterial, but none of them was found to be perfect [39]. As in the case of encapsulation, substrate diffusion through the resulting material may be rather slow. Biologically active compounds in these materials can gradually degrade. Another disadvantage of the method is that the resulting materials have poor mechanical characteristics. At the same time, this method can be of use in enhancing the stability of adsorbed biomaterials.



**Fig. 4.** Interaction between glutaraldehyde and an amino group of a protein.

**5. Covalent bonding.** Covalent bonding is likely the most widespread immobilization method. As follows from its name, it means the formation of a covalent bond between a biomaterial and a support. The choice of chemicals to be used in this immobilization method depends on the molecules to be bonded and on the support material. Covalent bonding is usually carried out in three steps: the first step is purification of the support and functionalization of its surface with the necessary groups, the second step is biomaterial deposition, and the third one is removal of weakly bound molecules with a pure solvent. Obviously, the sequence of chemical reactions should be chosen so that the bonds formed at the early stages persist at the later stages. The following support materials are used to produce sensors: metals, (usually gold, silver, or platinum), glass, carbon, polysaccharides (cellulose and its derivatives), nylon, poly(methyl methacrylate), and materials having free  $-NH_2$ ,  $-SH$ , or  $-COOH$  groups or imidazol groups.

Usually proteins are covalently bonded through nucleophilic functional groups in their side amino acid chains that have no effect on their enzymatic activity. Covalent bonds form mainly at moderate temperatures, low ionic strengths, and physiologic pH values. In order to protect the active site of the enzyme during the reaction, the latter is conducted in the presence of a substrate.

The covalent immobilization of DNA and oligonucleotides is carried out by cross-linking them with chitosan to obtain a multitude of amide bonds [37]. Methods of covalent bonding of DNA to aminodextrins and silanized supports were also developed [40].

A widespread method is modification of terminal nucleotide residues. The introduction of thiol groups into these nucleotide residues provides means to obtain, via chemisorption on gold, regular layers of oligonucleotides that are mostly oriented orthogonally to the surface.

The main advantage of covalent bonding is that, on the one hand, it ensures strong biomaterial–support

binding and prevents biomaterial loss and, on the other hand, it provides means to produce sensors with a long service life [8].

In some cases, a biomaterial can be bound to the transducer by several methods. It is always necessary to investigate the efficiency of binding by each method; in particular, it is necessary to compare the activity of an enzyme in solution and the activity of the same enzyme in the immobilized state. A binding method and a matrix (support) should be chosen before completing the fabrication of the sensor.

### APPLICATIONS IN THE FOOD INDUSTRY

The food industry needs express analysis methods for checking the quality and safety of foods, for process monitoring, for increasing the product yield, for energy input optimization, and for raising the process automation level. Determination of chemical and biological contaminants in foods is of paramount importance for ensuring healthy nutrition for people.

The biosensors employed in the food industry are primarily intended for determination of contaminants, also covering a few important food components, such as sugars, alcohols, amino acids, phenolic compounds, lactic acid, malic acid, ascorbic acid, and acetic acid [8, 41, 42]. It is, therefore, necessary to invest in development of food quality biosensors, since they proved to be a viable alternative to conventional analytical methods, such as chromatography [41, 43]. However, very few biosensors are playing a significant role in quality control in the food industry. Considerable effort should be put into development of inexpensive and sufficiently reliable biosensors capable of operating under real conditions [44].

As was mentioned above, the sensors used in the food industry are mostly intended for food safety analysis (detection of contaminants, allergens, toxins, pathogenic microorganisms, detergents, etc.), for determination of the composition of foods and raw materials, and for fermentation process control (Table 1) [45].

**Table 1.** Main applications of biosensors in the food industry

Food safety		
Xenobiotics • Additives • Drugs • Pesticides and fertilizers • Other contaminants: dioxins, PCB's, PAH's, heavy metals, and biotoxins	Bacterial toxins • Mycotoxins • Marine toxins	Pathogens • Viruses • Bacteria • Protozoa
Food quality		
Food composition: • Sugars • Amino acids • Alcohols • Organic acids • Cholesterol	Shelf life: • Polyphenols and fatty acids (rancidity) • Sugars and organic acids (maturation) • Biogenic amines (freshness index) • Aliina (garlic and onions)	
Technological processes		
• Sugars (fermentation and pasteurization) • Amino acids (fermentation) • Lactic acid (cheese making) • Alcohols (fermentation)		
Other applications		
• GMO • Reproductive cycle of animals		

Food security is among the most important elements of the national security of any country. Food security can be conventionally divided into the following three components: ability to domestically manufacture a sufficient amount of food, protectability of the food industry against external and internal impacts, and ability of the government to control the quality of the foods sold to the population. In view of this, food and raw material quality control is of primary importance. For this purpose, it is necessary to have express, accurate, informative, and reliable analytical methods meeting present-day requirements. The amounts and types of food additives used in food production are regulated by the legislation of each particular country. Detection and quantification of food additives are essential for preventing manufacturers from abusive use of these components and for revealing substances that can cause allergy in certain groups of people. Table 2 presents examples of biosensors employed in the determination of various

toxicants in foods and raw materials [14, 38, 41].

The safety regulations imposed on horticulture and animal husbandry products, including grain, milk, and meat, are becoming more stringent every year, and, accordingly, microtoxin control in foods is becoming more exacting. For example, a method for determining microtoxin traces has been developed for milk quality control. This method employs a bioluminescent biosensor based on a strain of the genetically modified yeast *Saccharomyces cerevisiae* [46].

Piezoelectric quartz crystal immunosensors proved to be convenient tools for biochemical and clinical examinations of patients, for food and drug certification, and for environmental monitoring. They are also used in the kinetic study of biochemical interactions and in the characterization of immunoreagent cross-coupling [21, 38, 47–50]. The practice of employing piezoelectric immunosensors demonstrated that these analytical devices have a high potential for determining residual amounts of pollutants (Table 2) [38].

**Table 2.** Biosensors for toxicant detection in foods

Analyte	Biological component/matrix	Transducer	Detection limit, linear-response concentration range	Reference
Estrogenic mycotoxin	<i>Saccharomyces cerevisiae</i>	Luminescent	1–258 nM	[46]
Acetochlor	Hapten–protein conjugate/4-aminothiophenol or succinimidyl propionate	Piezoelectric quartz crystal	20 ng/mL	[51]
	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.02 ng/mL	[52]
Alachlor	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.02 ng/mL	[53]
Butachlor	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.002 ng/mL	[54]
Bisphenol A	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.5 ng/mL	[55]
Sulfamethoxazole	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	1–50 ng/mL	[57, 58]

A large group of piezoelectric quartz crystal biosensors is intended for detection of pesticides: acetochlor in surface and potable water [51] and in milk and apple juice [52], alachlor in sausages [53], and butachlor in rice [54]. The detection limit is 0.02 ng/mL for alachlor and acetochlor and 0.002 ng/mL for butachlor, so these sensors can be used in the determination of herbicides at their TLV levels and below in surface, potable, and ground water.

At present, manufacturers of foods and plastic packaging materials widely use detergents, emulsifiers, and pigments, thus polluting the foods and environment with endocrine disruptors (bisphenol A, nonylphenol, linear alkylbenzenesulfonates, esters of phthalic acid, etc.). Dergunova et al. [55, 56] have developed piezoelectric quartz crystal immunosensors for the detection of trace nonylphenol and bisphenol A concentrations in aqueous solutions in the flow injection mode. These sensors provide means to detect nonylphenol and bisphenol A at a concentration of 0.8 and 0.5 ng/mL, respectively, in foods stored in plastic packages.

Use of antimicrobial drugs (sulfanilamides) in the medical treatment of cattle and poultry causes accumulation of these compounds in agricultural products. A sensor was suggested for the detection of residual amounts of sulfa drugs (0.15 mg/mL) in foods (milk, chicken meat, eggs) [57, 58].

A considerable number of publications have been devoted to heavy-metal biosensors. Voltammetric biosensors have been developed for detecting lead [59–61] and copper [62, 63]; fluorescent biosensors, for detecting zinc and copper [64, 65]; bioluminescent [66] and amperometric biosensors, for detecting mercury [67].

Biosensors for food quality control and food production monitoring are characterized in Table 3.

Biosensors are fairly widely employed in solving scientific and practical problems in alcohol manufacturing [14, 68]. The quality of ethanol

production processes at all stages is characterized by dynamics of the time variation of the concentrations of dissolved starch, sugars, ethanol, and methanol, which are parameters determinable with biosensors. Ethanol determination in fermentation process control can be carried out using various types of biosensors. Enzymatic sensors for estimating the ethanol concentration may be based on alcohol dehydrogenase or alcohol oxidase immobilized on an appropriate transducer. An amperometric biosensor for ethanol determination in the vapor phase, based on alcohol dehydrogenase and nicotinamide adenine dinucleotide (NAD<sup>+</sup>) as a cofactor was presented by Park et al. [69]. Ethanol detection in the vapor phase was possible in the 20–800 ppm range. An ethanol biosensor based on alcohol oxidase and a Clark oxygen electrode was described by Morozova et al. [70]. The measurement range of this electrode is from 0.05 to 10 mM. An amperometric biosensor involving *Candida tropicalis* cells immobilized in gelatin using glutaraldehyde allows ethanol to be determined in the 0.5–7.5 mM range [71]. Here, ethanol determination is based on measuring the difference between the respiratory activities of the cells in the presence and absence of ethanol. Valach et al. [72] designed a new microbial amperometric biosensor for flow injection determination of ethanol. Hammerle et al. [73] developed an amperometric biosensor based on alcohol oxidase, involving the methylotrophic yeast *Pichia pastoris* as the catalyst for ethanol conversion into hydrogen peroxide. This biosensor can qualitatively determine the total volatile alcohol content of apple juice by analyzing the gas phase over the sample, requiring no preliminary absorption or concentration. A biosensor based on *Methylobacterium organophilum* immobilized on a thin membrane and an oxygen electrode was also developed for ethanol quantification. The linear response range of this biosensor is 0.050–7.5 mmol/L [74].

**Table 3.** Food quality control biosensors

Analyte	Biological component/matrix	Transducer	Detection limit, linear-response concentration range	Reference
Ethanol	Alcohol dehydrogenase and (NAD <sup>+</sup> )	Amperometric	20–800 ppm	[69]
	Alcohol oxidase		0.05–10 mM	[70]
	<i>Candida tropicalis</i> /gelatin, glutaraldehyde	Amperometric	0.5–7.5 mM	[71]
	<i>Gluconobacter oxydans</i>	Amperometric	10 $\mu$ M – 1.5 mM	[72]
	<i>Methylobacterium organophilium</i>	Oxygen electrode	0.050–7.5 mM	[74]
	Alcohol oxidases	Amperometric	0.7–12.3 mM	[77]
Volatile alcohols	<i>Pichia pastoris</i>	Amperometric	0.10–30 mM	[73]
Sugars	Glucose oxidases	Amperometric	0.5–2.5 mM	[77]
D-Glucose and D-xylose	XDH-bacteria	Voltammetric	0.25–6 mM (–0.5 V) 0.24–4 mM (+0.55 V)	[76]
Clotting activity of rennet	Casein micelles/gold electrode	Impedimetric	–	[82]
Lactate	Lactate oxidase	Amperometric	$5 \cdot 10^{-7}$ – $5 \cdot 10^{-4}$ M	[83]
Casein	Anti-casein antibody/gold-capped nanoparticle substrate	LSPR	0.1–10 mg/mL	[19]
Oxalate	Oxalate oxidase/chitosan	Potentiometric	–	[41]
	Oxalate oxidase/gold nanoparticles	Amperometric	1–800 $\mu$ M	[41]
Amygdalin	$\beta$ -Glucosidases	Potentiometric	–	[41]
	Peroxidase	Potentiometric	–	[41]
Caffeine	<i>Pseudomonas alcaligenes</i>	Amperometric	0.1–1 mg/mL	[85]

Starch can be determined using both enzyme and microbial biosensors. The analytical procedure in this case typically includes starch hydrolysis to glucose by amylolytic enzymes ( $\alpha$ -amylase, glucoamylase) followed by glucose determination with an amperometric sensor based on glucose oxidase or microbial cells. For estimating the total utilizable sugar content of wort being brewed, the microbial biosensor may be preferable, because the wide substrate specificity of the microorganisms makes it possible to obtain an integral estimate of the total sugar content [75]. There is a rich assortment of amperometric biosensors for glucose determination [8].

Food manufacturers extensively use sweeteners, such as D-glucose and D-xylose. The co-immobilization of glucose oxidase and xylose dehydrogenase on an electrode modified with nanocomposite films afforded a voltammetric biosensor for the simultaneous determination of D-glucose and D-xylose [76].

Researchers of Tula State University developed various biosensors for determining the ethanol, glucose, and starch contents of brewing semiproducts [77–79] and for determining the biochemical oxygen demand of alcohol production waste [80, 81]. For example, Alferov et al. [77] developed an amperometric biosensor for ethanol, glucose, and starch quantification in brewing semiproducts. They demonstrated that the biosensor involving glucose

oxidase allows the glucose concentration to be measured in the 0.5–2.5 mM range and the biosensor based on alcohol oxidase allows ethanol determination in the 0.7–12.3 mM range.

Milk protein coagulation is among the basic processes in cheese making. In this process, milk is curdled using rennet, which eventually destabilizes casein micelles. For the first time, this process was monitored by electrochemical impedance spectroscopy using a faradic impedimetric biosensor, and the curdling activity of rennet was estimated using the hexacyanoferrate(II)/(III) redox couple [82].

Lactic acid is among the most important substances to be analyzed, since it is a product of the metabolism of practically all living organisms and a native or artificial component of many foods. Lactate oxidase immobilization in a conductive polymer film on the surface of planar electrodes modified with Prussian blue made it possible to develop a lactate biosensor characterized by a high sensitivity ( $190 \pm 14$  mA/(M cm<sup>2</sup>)), a linear response range of  $5 \cdot 10^{-7}$ – $5 \cdot 10^{-4}$  M, and a high operating stability. This sensor was demonstrated to be applicable to food (kvass) quality control [83].

Owing to their balanced amino acid composition and high digestibility, casein and caseinates are widely used as filler materials in the production of sausages, bread, tinned stew, ice-cream, sauces, confectioner's frosting, etc.; at the same time, casein is among the

most potent allergens. Ha Minh Hiep et al. [19] developed a localized plasmon resonance based immunosensor for casein determination in milk. This immunosensor is easy to manufacture and maintain and is highly sensitive, having a lower casein detection limit of 10 ng/mL.

A quick method involving an amperometric glucose biosensor was suggested for testing chilled meat for freshness [84].

Quality of coffee is determined by its caffeine content. Babu et al. [85] developed an amperometric caffeine biosensor by immobilization of *Pseudomonas Alcaligenes* MTCC 5264 on a cellophane membrane.

A challenging present-day problem is that of detecting GMOs. DNA- or oligonucleotide-based sensors capable of detecting complementary segments of DNA or RNA molecules upon hybridization can be successfully used for this purpose [38]. This was demonstrated by studies on food quality assessment [86, 87]. The data of these studies indicate that these sensors provide means to selectively and specifically detect GMOs via hybridization between the gene fragment (single-stranded DNA molecule) that is immobilized on the sensor surface and is responsible for mutations and the DNA isolated from the material being examined [38].

The concentration of antioxidants is estimated as their effect on the DNA sensor signal measured in the presence of a DNA-damaging factor (Fenton's reagent, copper(II) phenanthroline complexes, ionizing

radiation, etc.). In testing an antioxidant mixture of unknown composition, such as plants, foods, and tea extracts, the antioxidant content can be expressed in units of standard antioxidant (e.g., quercetin) concentration. For example, natural flavonoids were determined as their effect on the cleavage of thermally denatured DNA by active oxygen species generated in the Cu(II)–H<sub>2</sub>O<sub>2</sub>–ascorbic acid system [88, 89].

The analytical potential of the above biosensors is not limited to the examples presented here. Many systems have been developed and tested in recent years, and some of them have found wide application in environmental and analytical monitoring, medicine, biotechnologies, and food quality control. However, although there have been numerous publications dealing with biosensors for food analysis, only a few types of biosensors are now on sale [41]. Unfortunately, most biosensors have been tested only in distilled water or a buffer solution, and only since very recently biosensors have increasingly been tested on real objects. In the food industry, biosensors can be of use in solving a number of specific problems: food aging, estimating the age of wine and distilled beverages, disclosing food falsifications, and the GMO problem. Obviously promising is the wide introduction of relatively cheap, portable biosensors into analytical practice, since they would make it possible to markedly shorten the analysis time, enhance the quality of analysis, and detect and quantify biological compounds in environmental objects, foods, and biological fluids.

## REFERENCES

- Babkina, S.S., Ulakhovich, N.A., Zyavkina, Yu.I., Moiseeva, I.N., Filyushina, E.E., Nikitin, E.V., and Zagumennov, V.A., *Khimiya i khimicheskaya tekhnologiya (Chemistry and chemical engineering)*, 2005, vol. 48, no. 1, pp. 22–25.
- Liu, J., Brown, A.K., Meng, X., Crokek, D.M., Istok, J.D., Watson, D.B., Lu, Y., and Liu, J., *Proceedings of the National Academy of Sciences of the United States of America*, 2007, vol. 104, no. 7, pp. 2056–2061.
- Evyugin, G.A., Budnikov, G.K., and Porfir'eva, A.V., *Rossiiskii khimicheskii zhurnal (Russian chemical journal)*, 2008, vol. 52, no. 2, pp. 66–79.
- Karyakin, A.A., Ulasova, E.A., Vagin, M.Yu., and Karyakina, E.E., *Sensor (Sensor)*, 2002, no. 1, pp. 16–24.
- Korotkaya, E.V., *Biosensory na osnove kollodievnykh plenok: Monografiya (Biosensors Based on Collodion Films: A Monograph)*, Kemerovo: Kemerov. Tekhnol. Inst. Pishchevoi Prom–sti., 2011.
- Evyugin, G.A., Budnikov, G.K., and Nikol'skaya, E.B., *Russian Chemical Reviews*, 1999, vol. 68, no. 12, pp. 1041–1064.
- Budnikov, G.K., *Sorosovskii obrazovatel'nyi zhurnal (Soros educational journal)*, 1996, no. 12, pp. 26–32.
- Eggibs, B., *Chemical Sensors and Biosensors*, New York: Wiley, 2002.
- Biosensors: Fundamentals and Applications*, Turner, A., Karube, I., and Wilson, G., Eds., Oxford: Oxford Univ. Press, 1987.
- Rastislav Monošíka, Miroslav Stred'anskýb, and Ernest Šturdík, *Acta Chimica Slovaca*, 2012, vol. 5, no. 1, pp. 109–120. doi 10.2478/v10188-012-0017-z
- Cavalcanti, A., Shirinzadeh, B., Zhang, M., and Kretly, L.C., *Sensors*, 2008, vol. 8, pp. 2932–2958.
- Lojou, É. and Bianco, P., *Journal of Electroceramics*, 2006, vol. 16, no. 1, pp. 79–91.
- Kitova, A.E., *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Saratov: Institute of Biochemistry and Physiology of Plants and Microorganisms, 2009.
- Chunhui Dai and Seokheun Choi, *Open Journal of Applied Biosensor*, 2013, vol. 2, pp. 83–93.
- Mohanty, S.P. and Kougiannos, E., *IEEE Potentials*, 2006, vol. 25, no. 2, pp. 35–40. doi 10.1109/MP.2006.1649009
- Lei, Y., Chen, W., and Mulchandani, A., *Analytica Chimica Acta*, 2006, vol. 568, nos. 1–2, pp. 200–210. doi 10.1016/j.aca.2005.11.065
- D'Souza, S.F., *Biosensor and Bioelectronics*, 2001, vol. 16, no. 6, pp. 337–353. doi 10.1016/S0956-5663(01)00125-7.
- Lud, S.Q., Nikolaidis, M.G., Haase, I., Fischer, M., and Bausch, A.R., *ChemPhysChem*, 2006, vol. 7, no. 2, pp. 379–384.
- Ha Minh Hiep, Tatsuro Endo, Kagan Kerman, Miyuki Chikae, Do-Kyun Kim, Shohei Yamamura, Yuzuru Takamura, and Eiichi Tamiya, *Science and Technology of Advanced Materials*, 2007, vol. 8, pp. 331–338.
- Kitova, A.E. Alferov, V.A., and Ponamoreva, O.N., in *Mikrobnnye biokatalizatory i perspektivy razvitiya fermentnykh tekhnologii v pererabatyvayushchikh otraslyakh APK (Microbial Biocatalysts and Outlooks for the Development of Enzyme Technologies in Processing Sectors of Agribusiness)*, Moscow: Pishchepromizdat, 2004, pp. 255–262.

21. Reshetilov, A.N., Kitova, A.E. Alferov, V.A., and Ponamoreva, O.N., *Biotekhnologiya-2003: Tezisy dokladov seminara-prezentatsii innovatsionnykh nauchno-tekhnicheskikh proektov* (Biotechnology 2003: Workshop and Presentation of Innovative Research and Development Projects), Pushchino, Moscow oblast, 2003, pp. 64–65.
22. Shevtsova, O.A., *Zbirnik naukovikh prats' Kharkivs'kogo universitetu povitryanikh sil* (Transactions of the Kharkiv university of air forces), 2010, issue 1 (23), pp. 201–205.
23. Spiridonova, V.A., *Biochemistry* (Moscow) *Supplement Series A: Membrane and Cell Biology*, 2010, vol. 4, no. 2, pp. 138–149.
24. Juan Zhang, Lihua Wang, Dun Pan, Shiping Song, Freddy Y.C. Boey, Hua Zhang, and Chunhai Fan, *Small*, 2008, vol. 4, no. 8, pp. 1196–1200.
25. Lui, C., Cady, N.C., and Batt, C.A., *Sensors*, 2009, vol. 9, pp. 3713–3744.
26. Reshetilov, A.N., Ponamoreva, O.N., Arlyapov, V.A., Alferov, V.A., Rogova, G.V., Blokhin, I.V., and Chepkova, I.F., *Voda: khimiya i ekologiya* (Water: chemistry and ecology), 2008, no. 3, pp. 23–30.
27. Perov, S.N., Kalach, A.V., and Meshcheryakova, O.L., *Sorbtsionnye i khromatograficheskie protsessy* (Sorption and chromatographic processes), 2008, vol. 8, no. 6, pp. 1008–1012.
28. Reshetilov, A.N., *Applied Biochemistry and Microbiology*, 2005, vol. 41, no. 5, pp. 442–449.
29. Deryabin, D.G. and Aleshina, E.S., *Applied Biochemistry and Microbiology*, 2008, vol. 44, no. 3, pp. 292–296.
30. Deryabin, D.G. and Aleshina, E.S., *Gigiena i sanitariya* (Hygiene and sanitation), 2008, no. 4, pp. 83–86.
31. Lavrinenko, I.A. and Lavrinenko, V.A., *Vestnik Novosibirskogo gosudarstvennogo universiteta, seriya: Biologiya, klinicheskaya meditsina* (Bulletin of Novosibirsk State University, series: Biology, clinical medicine), 2007, vol. 5, no. 1, pp. 118–126.
32. Zubov, V.P., Ivanov, A.E., Zhigis, L.S., Rapoport, E.M., Markvicheva, E.A., Lukin, Yu.V., and Zaitsev, S.Yu., *Bioorganicheskaya khimiya* (Bioorganic chemistry), 1999, vol. 25, no. 11, pp. 868–880.
33. Koval'chuk, M.V., Klechkovskaya, V.V., and Feigin, L.A., *Priroda* (Nature), 2003, no. 12, pp. 1–12.
34. Madou, M., *Fundamentals of Microfabrication: The Science of Miniaturization*, Boca Raton, Fla.: CRC, 2002, pp. 62–63.
35. Kaifer, A. and Gómez-Kaifer, M., *Supramolecular Electrochemistry*, Weinheim: Wiley-VCH, 1999, pp. 191–193.
36. Schramm, L., *Dictionary of Nanotechnology, Colloid and Interface Science*, Weinheim: Wiley-VCH, 2008.
37. Gol'dfarb, O.E., *Extended Abstract of Cand. Sci. (Chem.) Dissertation*, Kazan: Kazan State University, 2005.
38. Ermolaeva, T.N., Kalmykova, E.N., and Shashkanova, O.Yu., *Rossiiskii khimicheskii zhurnal* (Russian chemical journal), 2008, vol. 52, no. 2, pp. 17–29.
39. *Analytical Chemistry: A Modern Approach to Analytical Science*, Kellner, R., Mermet, J.-M., Otto, M., Valcarcel, M., and Widmer, H., Eds., Weinheim: Wiley-VCH, 2004.
40. Shlyapnikov, Yu.M., *Extended Abstract of Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State University, 2010.
41. <http://www.intechopen.com/books/state-of-the-art-in-biosensors-environmental-and-medical-applications/biosensors-for-contaminants-monitoring-in-food-and-environment-for-human-and-environmental-health>
42. Vaefolomeev, S.D., *Sorosovskii obrazovatel'nyi zhurnal* (Soros educational journal), 1997, no. 1, pp. 45–49.
43. Luong, J.H.T., Groom, C.A., and Male, K.B., *Biosensors and Bioelectronics*, 1991, vol. 6, pp. 547–554.
44. Kopa, Yu. and Momynaliev, K., *Nanoindustriya* (Nanoindustry), 2007, no. 1, pp. 28–33.
45. Rumayor V.G., Iglesias, E.G., Galán, O.R., and Cabezas, L.G., *Aplicaciones de biosensores en la industria agroalimentaria*, Madrid: Comunidad de Madrid y la Universidad Complutense de Madrid, 2005.
46. Valimaa, A., Kivisto, A.T., Leskinen, P.I., and Karp, M.T., *Journal of Microbiological Methods*, 2010, vol. 80, no. 1, pp. 44–48.
47. Kalmykova, E.N., Melikhova, E.V., Dergunova, E.S., Eremin, S.A., and Ermolaeva, T.N., *Sorbtsionnye i khromatograficheskie protsessy* (Sorption and chromatographic processes), 2004, vol. 4, no. 5, pp. 597–605.
48. Pohanka, M., Pavlis, O., and Skladal P., *Sensors*, 2007, vol. 7, pp. 341–353.
49. Kalmykova, E.N. and Ermolaeva, T.N., *Izvestiya vysshikh uchebnykh zavedenii, seriya: Khimiya i khimicheskaya tekhnologiya* (Bulletin of higher education institutions, series: Chemistry and chemical engineering), 2005, vol. 48, no. 12, pp. 76–80.
50. Ermolaeva, T.N. and Kalmykova, E.N., *P'ezokvartsevye sensory: Analiticheskie vozmozhnosti i perspektivy* (Piezoelectric Quartz Crystal Sensors: Analytical Potential and Prospects), Lipetsk: Lipetsk. Gos. Tekh. Univ., 2007.
51. Lebedev, M.Yu., Eremin, S.A., Skladal, P., *Analytical Letters*, 2004, vol. 36, pp. 2443–2457.
52. Nartova, Yu.V., Eremin, S.A., and Ermolaeva, T.N., *Sorbtsionnye i khromatograficheskie protsessy* (Sorption and chromatographic processes), 2006, vol. 6, no. 5, pp. 764–772.
53. Nartova, Yu.V., Eremin, S.A., and Ermolaeva, T.N., *Rossiiskaya shkola-konferentsiya molodykh uchenykh "Ekotoksikologiya: Sovremennye bioanaliticheskie sistemy, metody i tekhnologii"* ("Ecotoxicology: Advanced Bionalytical Systems, Methods, and Technologies," Russian Young Scientists' School and Conference), Pushchino, Moscow oblast, 2006, pp. 68–70.
54. Nartova, Yu.V., *Mezhdunarodnaya konferentsiya molodykh uchenykh po fundamental'nym naukam "Lomonosov-2007", Khimiya* ("Lomonosov 2007," International Young Scientists' Conference on Fundamental Sciences, Section: Chemistry), Moscow, 2007, vol. 1, p. 39.
55. Dergunova, E.S. and Ermolaeva, T.N., *Vserossiiskaya studencheskaya nauchno-tekhnicheskaya shkola-konferentsiya "Imzhenernye nauki — zashchite okruzhayushchei sredy"* ("Engineering Science for Environmental Protection," Russian National Students' Science & Technology School and Conference), Tula, 2006, pp. 136–140.

56. Ermolaeva, T.N., Dergunova, E.S., Kalmykova, E.N., and Eremin, S.A., *Journal of Analytical Chemistry*, 2006, vol. 61, no. 6, pp. 609–613.
57. Kalmykova, E.N., Melikhova, E.V., Eremin, S.A., and Ermolaeva, T.N., *Antibiotiki i khimioterapiya (Antibiotics and chemotherapy)*, 2004, vol. 49, no. 1, pp. 8–13.
58. Melikhova, E.V., Kalmykova, E.N., Eremin, S.A., and Ermolaeva, T.N., *Journal of Analytical Chemistry*, 2006, vol. 61, no. 7, pp. 687–693.
59. Yüce, M., Nazir, H., and Dönmez, G., *Biosensors and Bioelectronics*, 2010, vol. 26, no. 2, pp. 321–326.
60. Yüce, M., Nazir, H., and Dönmez, G., *Bioresource Technology*, 2010, vol. 101, no. 19, pp. 7551–7555.
61. Yüce, M., Nazir, H., and Dönmez, G., *New Biotechnology*, 2011, vol. 28, no. 4, pp. 356–361.
62. Alpat, S., Aplat, S.K., Cadirci, B.H., Yasa, I., and Telefoncu, A., *Sensor and Actuators B: Chemical*, 2008, vol. 134, no. 1, pp. 175–181.
63. Yüce, M., Nazir, H., and Dönmez, G., *Bioelectrochemistry*, 2010, vol. 79, no. 1, pp. 66–70.
64. Ravikumar, S., Ganesh, I., Yoo, I., and Hong, S., *Process Biochemistry*, 2012, vol. 47, no. 5, pp. 758–765.
65. Liu, P., Huang, Q., and Chen, W., *Environmental Pollution*, 2012, vol. 164, pp. 66–72.
66. Barrocas, P.R.G., Landing, W.M., and Hudson, R.J.M., *Journal of Environmental Sciences*, 2012, vol. 22, no. 8, pp. 1137–1143.
67. Singh, J. and Mittal, S.K., *Sensors and Actuators B: Chemical*, 2012, vol. 165, no. 1, pp. 48–52.
68. Reshetilov, A.N. and Kitova, A.E. <http://cbio.ru/page/43/id/4791/>.
69. Park, Je-K., Yee, H.-J., Lee, K. S., Lee, W.-Y., Shin, M.-C., Kim, T.-H., and Kim, S.-R., *Analytica Chimica Acta*, 1999, vol. 390, pp. 83–91.
70. Morozova, N.O., Ashin, V.V., Trotsenko, Yu.A., and Reshetilov, A.N., *Applied Biochemistry and Microbiology*, 1999, vol. 35, no. 5, pp. 537–541.
71. Akyilmaz, E. and Dincaya, E., *Biosensors and Bioelectronics*, 2005, vol. 20, no. 7, pp. 1263–1269.
72. Valach, M., Katrik, J., Sturdik, E., and Gemeiner, P., *Sensors and Actuators B: Chemical*, 2009, vol. 138, no. 2, pp. 581–586.
73. Hammerle, M., Hilgert, K., Marcus, A.H., and Ralf, M., *Sensors and Actuators B: Chemical*, 2011, vol. 158, no. 1, pp. 313–318.
74. Wen, G.M., Shuang, S.M., Dong, C., and Choi, M.F., *Chinese Chemical Letters*, 2012, vol. 23, no. 4, pp. 481–483.
75. Reshetilov, A.N., Kitova, A.E., Alferov, V.A., Ponamoreva, O.N., Kuzmichev, A. V., and Ezhkov, A.A., *Biotehnologiya-2003: Tezisy dokladov seminar-prezentatsii innovatsionnykh nauchno-tehnicheskikh proektov (Biotechnology 2003: Workshop and Presentation of Innovative Research and Development Projects)*, Pushchino, Moscow oblast, 2003, pp. 64–65.
76. Li, L., Liang, B., Li, F., Shi, J., Mascini, M., Lang, Q., and Liu, A., *Biosensors and Bioelectronics*, 2013, vol. 42, no. 12, pp. 156–162.
77. Alferov, V.A., Arlyapov, V.A., and Kamanin, S.S., *Izvestiya Tul'skogo gosudarstvennogo universiteta, seriya: Estestvennye nauki (Bulletin of Tula State University, series: Natural sciences)*, 2010, no. 2, pp. 247–255.
78. Il'nitskii, M.Yu. and Zaitsev, M.G., *III Obshcherossiiskaya studentsheskaya elektronnyaya nauchnaya konferentsiya "Studentskii nauchnyi forum" ("Students' Scientific Forum," III Russian National Students' e-Conference)*, 2011. <http://www.rae.ru/forum2011/133/>
79. Zaitsev, M.G., *Extended Abstract of Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State University of Fine Chemical Technologies, 2013.
80. Khitrova, Yu.S. and Indzhigiya, E.Yu., *III Obshcherossiiskaya studentsheskaya elektronnyaya nauchnaya konferentsiya "Studentskii nauchnyi forum" ("Students' Scientific Forum," III Russian National Students' e-Conference)*, 2011. <http://www.rae.ru/forum2011/133/>
81. Sibirtsev, I.A., Kamanin, S.S., and Arlyapov, V.A., *III Obshcherossiiskaya studentsheskaya elektronnyaya nauchnaya konferentsiya "Studentskii nauchnyi forum" ("Students' Scientific Forum," III Russian National Students' e-Conference)*, 2011. <http://www.rae.ru/forum2011/133/>
82. Panagopoulou Maria, A., Stergiou Dimitrios, V., and Roussis Ioannis, G., *Analytical Chemistry*, 2010, vol. 82, pp. 8629–8636
83. Korneyeva, L.Kh., Borisova, A.V., Yashina, E.I., Karyakina, E.E., Voronin, O.G., Kosnier, S. and Karyakin, A.A., *Moscow University Chemistry Bulletin*, 2010, vol. 51, no. 1, pp. 49–56.
84. Kolesnikov, P.S., *Extended Abstract of Cand. Sci. (Vet.) Dissertation*, Moscow: Russian Academy of Agricultural Sciences, 2005.
85. Babu, V.R.S., Patra, S., Karanth, N.G., Kumar, M.A., and Thakur, M.S., *Analytica Chimica Acta*, 2007, vol. 582, no. 2, pp. 329–334.
86. Minunni, M., Tombelli, S., Mariotti, E., and Mascini, M., *Fresenius Journal of Analytical Chemistry*, 2001, vol. 369, no. 7/8, pp. 589–593.
87. Minunni, M., Tombelli, S., Pratesi, S., and Mascini, M., *Analytical Letters*, 2001, vol. 34, no. 6, pp. 825–840.
88. Korbout, O., Bučková, M., Labuda, J., and Gründler, P., *Sensors*, 2003, vol. 3, pp. 1–10.
89. Labuda, J., Buckova, M., Heilerova, L., Silhar, S., and Stepanek, I., *Analytical and Bioanalytical Chemistry*, 2003, vol. 376, pp. 168–173.

