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The Microbial Cell Based Biosensors

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1. Introduction

The typical feature of the advancement of knowledge as a whole is initiation of novel interdisciplinary trends and divisions of science. They usually appear as a result of joint creative work of specialists of different profiles, which is conditioned by widening of the scope of scientific problems, interests, objects and methods of research. One of these trends is biosensor research (biosensorics), the branch of biotechnology that originated in the second half of the 20th century at the interfaces between biology, biophysics, chemistry, physics, electronics, and informatics.

The essence of this trend may be defined as follows: biosensor studies pursue the construction of analytic systems, i.e. biosensors, the primary function of which is express analysis for sought-for substance detection. The main "character" in biosensor analyzer is biological material: it provides sensitivity of instrument to sought-for substance.

The analysis of events resulting in the development of biosensorics as a research trend shows that the author of biosensor conception and the first biosensor developer is USA biochemist L. C. Clark, Jr. In 1962, Clark and Lyons introduced the term "enzyme electrode" into practice (Turner, 1996). For manufacture of this device, a minor quantity of glucose oxidase was applied onto the surface of platinum electrode and covered with cellophane. The platinum was at positive potential to relative to silver electrode. The system did not react to dissolved oxygen but generated current in the presence of hydrogen peroxide. L. Clark showed that the electrode current quickly increased at addition of glucose to the solution and was proportional to its concentration (Clark, 1993).

The formalization and specification of terminology related to electrochemical biosensors but extendable to biosensors of other types was carried out by recommendation of the International Union of Pure and Applied Chemistry (IUPAC). It gives recommendations for researchers, editorial boards of journals, and publishers on application of concepts such as biosensor and its parameters: sensitivity, general and linear concentration measurement range, detection limit, selectivity, life time, etc. (Thevenot et al., 2001).

Quite a number of publications show the stages of development and state-of-the-art of biosensor studies. So the question of efficiency of biosensor analysis of the environment is considered in papers (Rodriguez-Mozaz et al., 2006); general problems of the analytical aspects of biosensors are presented in review (Pearson et al., 2000); specific problems of electrochemical biosensor measurement are presented in review (Mehrvar & Abdi, 2004); the aspects of the functioning of biosensors based on ion-selective field transistors are

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described in paper (Yuqing et al., 2003). The analysis of application of whole cells in biosensors can be found in reviews (Bousse, 1996; Ziegler, 2000; Bentley et al., 2001). Review (Murphy, 2006) presents the analysis of advantages of biosensors (DNA-, immunosensors, enzyme sensors with direct charge transfer, etc.) constructed with application of nanomaterials. The use of microbial cells in receptor elements of biosensors is described in works (D'Souza, 2001b; Riedel et al., 1989; Racek, 1995).
The microbial cells based biosensors are considered in the present chapter.

2. Microbial biosensors design and characteristics

2.1 Biosensor design

The first microbial biosensor as indicated in the review (Turner, 1996) was described in 1975 by Divies and was based on the use of *Acetobacter xylinum* and oxygen electrode. The work became the foundation of the investigations devoted to the development of microbial biosensors for application in biotechnology and environmental monitoring.
Taking a biomaterial type in the bioreceptor as a basis it is possible to pick out the class of cell based biosensors (Thevenot et al., 2001). They can incorporate the plant or animal isolated cells or subcellular structures as well as the microbial cells. The most of the cell based biosensors developed to date fall into the subclass of microbial biosensors (D'Souza, 2001b; Bousse, 1996). This is due to the simplicity of the microorganisms' cultivation, the rich analytical prospects of the microbial cells (Racek, 1995) and their reliability when using as the base of the immobilized biocatalysts (Cassidy et al., 1996).
The appearance of the microbial sensors was the logical extension of the enzyme electrodes' development. The signal generation mechanism is analogous in general terms for both of the microbial and enzyme biosensors. In either case the biocatalytic reaction following the enzyme kinetics statements takes place. According to the model used in the work (Ikeda et al., 1996) the microbial cell is treated as the "bag of enzymes". The representation is unconditionally primitive and do not take into the account the complexity of the cell's structural organization and homeostatic system of intracellular biochemical processes but from the standpoint of the biosensorics in its elementary form the microbial cell could really be considered as an integrated biocatalyst that is analogous to the enzyme preparation in many ways.
The scheme of the intracellular metabolic processes underlying the background of microbial biosensors could be represented as follows. The analyte enters the cell and is converted using the intracellular enzymes. As a result, the co-substrates are consumed and the reaction products that could also be electrochemically active are generated. The registration of the oxygen level, medium ionic composition and other parameters in the immobilized cells' layer can be used as the indicators of the cells' metabolic state and the background for the electrochemical determination of biologically active compounds.
The selection of the microorganism for the use in a biosensor is a milestone of the development process. The key factors of the problem are the substrate specificity and the sensitivity of detection. Thus, the study of a number of *Gluconobacter* strains allowed to predict the cells' specificity to carbohydrates, alcohols, and organic acids using the potentiometric and amperometric transducers (Reshetilov et al., 1997a).
The bioreceptor of the typical microbial biosensor is represented by a membrane or gel strip containing the immobilized microorganisms fixed onto the working area of a transducer. The immobilization of cells ensures small (~10-100 mg) biomaterial consumption, high assay

rate and allows the multiple measurements without the bioreceptor replacement. The choice of the immobilization technique is determined by the criteria of the sensor stability and measurement rate (i.e. the diffusion characteristics of a support). The immobilization methods have been improved with the time (D'Souza, 2001a); the most common of them have been considered in the manuscript (D'Souza, 2001b). In the most of cases the cells' immobilization in the bioreceptor have been carried out by the sorption or inclusion into the gel or polymer matrixes.

Generally, the immobilization techniques could be divided into active and passive. The natural adhesive ability of microorganisms can be used for their passive immobilization. On the other hand, the active immobilization supposes the use of chemical and physical methods for cell fixing. The choose of the most efficient technique is determined by the type of supposed assay and cells' features. The inventory of the microorganisms immobilization includes the same approaches as for the enzyme immobilization (Thevenot et al., 2001). These approaches are described below in short form.

Inclusion into a gel or polymer matrix. This method ensures the retention of cells in the spatial network formed by a polymer. The key advantage of the method is the improved (over against the adsorbed cells) sensor stability (Racek, 1991). Furthermore, it is known that in a number of cases the polysaccharide gels reduce the toxic effect of aromatics on the microbial cells (Fedorov et al., 1999) that is the important factor during the development of the sensors for environmental needs. Agar, Ca-alginate, carrageenan, gelatin and collagen gels and PVA are widely used in microbial sensors. Although the polymerization of these materials arises in the stress conditions they ensure high cells' viability and reproducibility of analysis. The polyacrylamide gel is also every so often used in microbial sensors (Wollenberger et al., 1980) in spite of its toxicity. The PVA cryogels (Philp et al., 2003) and photo-crosslinked polymers like ENT/ENTP (a composite mixture polymerizing under near UV light) or modified PVA (Fukui & Tanaka, 1984), poly(carbamoyl sulfonate) and polyurethane (Konig et al., 1998), sol-gel matrixes based on alumina or composite polymers (Jia et al., 2003), latex-type polymers based on acrylic and methacrylic acids (D'Souza, 2001a), redox hydrogels like $[Os(bpy)_2Cl]^{+/2+}$ also should be mentioned among the high-used carriers. Generally, the inclusion have been used in microbial biosensorics approximately as often as the adsorption and essential in the case of high cells' desorption from the support.

Adsorption. The common supports for adsorption include various membranes, a filter paper, carbon materials and other carriers possess the high absorbance; sometimes the cells have been adsorbed directly onto the electrode surface. The review (D'Souza, 2001b) describes the immobilization by the simultaneous adhesion of viable and unviable cells on the different materials including the glass, cotton, and polymer carriers. The specific for microorganisms technique is the flocculation - the aggregation of cells and sorption of the aggregates on the support (Cassidy et al., 1996). One of the major advantages of the adsorption is its simplicity; also, the adsorption is a "soft" method with minimal damaging action. The stability of the sensors with adsorbed cells is rather high; it have been observed that such biosensors retain the activity within several weeks and even months (Rechnitz et al., 1977; Karube et al., 1980; Matsunaga et al., 1984). These factors make the adsorption one of the most preferable approaches for microbial biosensors development.

Covalent attachment. This approach supposes the use of cross-linking agents like glutaraldehyde, carbodiimide, titanium oxide that linked to molecules exposed on the

microbial cell wall and with functional groups on the surface of the carrier or transducer. The obtained film has been fixed on the transducer. The approach is widely used with the enzyme electrodes but rarely with microbial sensors because the cross-linking in the most of cases leads to the loss of the viability or decrease of the cells' catalytic activity.
The immobilization on the membrane is not the only possible design of a bioreceptor. A number of biosensors described in works (Alkasrawi et al., 1999; Gu & Gil, 2001) used the receptors represented by reactors with displacement and continuous or pulse substrate feed. The transducer in this case have been installed at the output of the reactor that allow to control the changes of biochemical activity of the immobilized biomass under the substrate feed. The cells immobilization in a bioreactor may be carried out by means of adsorption on a granular carrier or by inclusion into a gel. In the case when the substrate conversion is accompanied by the generation of compounds that could be analyzed using a biosensor an efficient approach consists in a creation of a hybrid sensor by means of installation of a such biosensor at the output of the reactor (Damgaard et al., 2001). However, the reactor based biosensors have been utilized infrequently due to labor content of the reactor exploitation over against the membrane-based sensors as well as high consumption of the biomass for the receptor formation.
One of the reactor biosensor designs is the case when the cells are suspended in the solution of a measuring cuvette (i.e. the biosensor is represented by a transducer placed into the cuvette with the cell suspension or with the suspension of a dispersed carrier). The design is similar to a fuel cell and is known as "fuel-cell-type sensor". The essential disadvantages of the approach consist in the high consumption of a biomaterial, impossibility or difficulty of the continuous measurements. However, in spite of these reasons, the approach have been exploited from time to time in model biosensor studies (Kim et al., 2003; Chang et al., 2004; Vais et al., 1989; Guliy et al., 2003).

2.2 Comparative characteristics of microbial and enzyme biosensors.

The belonging of the electrochemical and calorimetric enzyme or microbial biosensors to the group of catalytic sensors determines their properties and, in particular, the range of detectable concentrations. For the most of models reported to date it lies within 10^{-6}-10^{-2} M. The exception are the optical microbial sensors whose signal is not based directly on catalytic transformation of the analyte; they are characterized by lower limit of detection about 10^{-9}-10^{-7} M. In spite of the similarity of the operating principles of the microbial and enzyme sensors the use of whole microbial cells have particular advantages and disadvantages in contrast to the enzyme utilization (D'Souza, 2001b; Racek, 1995; Riedel et al., 1987; Bousse, 1996). The advantages include:
a) the absence of need in obtaining and exploitation of pure enzymes, i.e. reduction of labor content and prime cost of analysis; b) some enzymes may inactivate during isolation or immobilization if these processes disarrange their molecular structure. The use of whole cells minimizes this obstacle; c) the microbial cells can be genetically modified that makes it possible to obtain recombinant organisms with determined biocatalytic properties; d) the enzymes within the cells are in naturally occurring, evolutionary optimized environment that ensures high stability of a number of microbial sensors. Also, the cells contain coenzymes and activators of biochemical pathways that eliminates the need in their addition in the medium; e) the utilization of microorganisms allows the realization of sequential biochemical processes that in the case of an enzyme sensor would be artificially designed; f) the receptor of microbial sensor can be regenerated by conditioning the cell growth.

The important reason for the utilization of the whole cells in analytical devices is the fact that only the use of living cell allow the obtaining of the functional information, i.e. determining how the factor affects the organism in vivo (Bousse, 1996). The examples supposing the obtaining of functional information include the questions:
a) does the compound affect the cell metabolism and how; b) is the compound agonist or antagonist of the particular receptor (the question is essential for the pharmaceutics and drug development); c) is the sample or substance toxic. The question is directly related to objectives of biosensorics, at the same time the influence of the environment on the organisms in a general sense is related to functional information. The microbial biosensors have been applied for the such investigation over a long period of time. The studies include the evaluation of BOD, total toxicity, genotoxicity, i.e. the parameters that are inherently related to functional state of organisms.
The most common disadvantages of microbial sensors include the decreased rate of signal generation and the low selectivity. The cell based receptor carries out the analyte conversion slower in contrast to the enzyme based one due to presence the cell wall that acts as a diffusion barrier. An efficient approach for solving the problem consists in the exploitation of permeabilized cells (D'Souza, 2001b). Another method allowing to reduce the sensor's reaction time suppose the application of genetic engineering technique in order to ensure the exposing of the particular enzymes on the outer surface of cell wall; in this case the diffusion obstacles are eliminated. Thus, in (Rainina et al., 1996; Mulchandani et al., 1998) the recombinant *Escherichia coli* strain containing the surface-expressed organophosphate hydrolase had been utilized. The cells carried out the substrate degradation with higher rate over the cells with the intracellular expression of the enzyme.

2.3 Hybrid biosensors

The bioreceptor of hybrid biosensors contain two or more different biocatalysts. As applied to the microbial sensors the term "hybrid" usually supposes that the sensor contain a mixed culture of two or more strains or "cells + enzyme" type composite. Both of the biomaterials must catalyze the coupled reactions. In hybrid biosensors' design, two major schemes of the bioreceptor have been utilized - a) the biocatalysts are separated from each other by a membrane allowing the substrate diffusion or b) the biocatalysts are mixed together. The hybrid biosensors with coupling the membrane and reactor bioreceptors have also been reported.
The major advantages of hybrid biosensors are the enhanced selectivity and the possibility to analyze compounds that could not be determined by single-component sensors. On the other hand, the labor content of hybrid biosensors' development and exploitation higher in contrast to the single-component ones. Maybe this explains the small amount of hybrid microbial sensors described to date. The hybrid sensor containing *Bacillus subtilis* cells in combination with the glucoamylase for the evaluation of α-amylase activity was reported in (Renneberg et al., 1984). The hybrid biosensor analyzer using the combination of the enzymes and bacterial cells and the column-type reactor with the membrane receptor was presented in publication (Reiss et al., 1998). The authors utilized two reactors containing α-amylase and amyloglucosidase coupled with a commercial BOD biosensor (Prüfgerätewerk Medingen) based on the immobilized *Trichosporon cutaneum* cells. The results of BOD evaluation in starch-containing wastes agreed well to the data of conventional BOD assay.
Hybrid microbial biosensors based on Clark type electrode and containing *Gluconobacter oxydans* in combination with *Saccharomyces cerevisiae* or *G. oxydans* combining with the

permeabilized *Kluyveromyces marxianus* cells were used for determination of sucrose and lactose, respectively (Svitel et al., 1998). The approach based on the attachment of glucose oxidase to microbial cell surface by concanavalin A or polyethyleneimine was applied for the development of sensors for sucrose and lactose detection; the carrier cells contained induced invertase and β-galactosidase (D'Souza, 1989).
The hybrid biosensors coupling different microorganisms in the bioreceptor have been widely used for BOD index evaluation. Thus, the simultaneous immobilization of *T. cutaneum* and *Bacillus licheniformis* expanded the analyzer's substrate specificity due to differences in metabolic activity of the cultures (Suriyawattanakul et al., 2002). Simultaneous immobilization of yeast strain *T. cutaneum* and bacterial culture *B. subtilis* (Jia et al., 2003) made it possible the creation of a BOD sensor with enhanced long-term stability.

2.4 Substrate specificity of microbial sensors and its improvement

The term "selectivity" usually means the sensor's ability to generate signals in response to the analyte appearance along with the minimal sensitivity to other compounds in the sample. It can be quantitative assessed using two ways. The first one expresses the selectivity as the ratio of the analyte-induced response to responses to the analogous concentration of the interfering substances. The second approach consists in addition of the interfering substances in the medium that already contains the analyte; the selectivity in this case is expressed as the percentage of the signal increment after the interfering compound addition. The second way is more simple but allows to evaluate the selectivity more particularly (Thevenot et al., 2001).
The broad substrate specificity (low selectivity) of microbial biosensors is due to the variety of the intracellular enzyme systems. There is rather small amount of microbial sensor models possessing the sensitivity only to analytes - as a rule, due to the good choice of the strain and/or detection principle or measurement mode optimization. Most of the microbial sensors are characterized by sensitivity to a wide number of substances that can significantly hamper the analysis of complex samples (Riedel et al., 1990b). A number of approaches directed to the improving of microbial sensors' selectivity have been reported. These approaches will be considered below.
In the review (Racek, 1995) several ways of the sensors' selectivity improving have been described. Thus, it has been noted that one of the efficient methods consists in isolating the bioreceptor or transducer from the medium using an additional membrane impermeable for the interfering compounds. For example, the use of cellulose acetate membrane in a biosensor based on the *G. oxydans* cells made it possible the 60-fold increase of the response to ethanol in contrast with the response to glucose. In addition, the authors revealed the effect of culture age on the selectivity. Bacteria collected after 10 and 16 hours of cultivation varied in the ethanol to glucose sensitivity ratio approximately by a factor of 3 (Tkac et al., 2003). In the case when the analyte is volatile it is appropriate to use a gas-permeable membrane isolating the electrode from the medium. The approach is simple and reliable; at the other hand, the covering membrane may be an additional diffusion barrier for the analyte and decrease the rate of analysis.
Another approach that could be related to this section consists in the sample pretreatment in order to eliminate the interfering compounds. The particular case of such pretreatment is the introducing of the enzyme solution into the measuring cell or addition of a membrane containing the immobilized enzyme that carries out the conversion of the interfering substances into inactive products (Park et al., 1991). The approach has been utilized in a

hybrid sensor for sucrose detection. The sensor contained *Zymomonas mobilis* bacteria and invertase. The elimination of glucose initially presented in a sample has been carried out by glucose oxidase.

One of the simplest ways to increase the microbial sensor selectivity is based on the choice of microorganism able to use only the analyte as the carbon and/or energy source. In particular, this approach was used in a works directed to the development of a methane sensor (Karube et al., 1982b). However, it should be noted that this method is not an universal tool for enhancing the selectivity. Even in the case when the organism is able to utilize only one substrate it's metabolic pathway for the most part includes several steps and the pathway intermediates are also expected to induce the biosensor signal. Besides, the inability of the cells to utilize the substrate as the source of carbon does not exclude the sensitivity of sensor to this compound. Thus, *G. oxydans* bacterium can not to utilize glucose as the carbon source due to metabolic peculiarities but the sensors based on the culture possess high sensitivity to glucose due to ability of the cells to convert glucose into ketogluconic acid accompanied by oxygen consumption and acidification of the medium.

In a number of cases a choice of a transducer or measurement mode is also important for the selectivity. The time of response to interfering compounds can differ from the time of response to the analyte due to variation in the rate of their diffusion through cell wall or metabolic processes. If the difference is significant it could be used for the selectivity improving (Racek, 1995). Thus, the use of *G. suboxidans* in a mediator sensor demonstrated a steady level of signal within 30 s and 15 min for ethanol and glucose, respectively (Ikeda et al., 1992). The effect can be used for selective determination of the substances in a mixed sample.

One of the most common ways of the microbial sensor selectivity improving consists in the cultivation of the biomass at a later stage used in a bioreceptor with the expected analyte as a sole source of carbon and energy or as a co-substrate (Simonian et al., 1992; Renneberg et al., 1984). The approach does not eliminate the ability of a sensor to respond to a wide spectrum of substances. It only allows to selectively increase (Renneberg et al., 1984) the sensor sensitivity to the analyte as a result of the induction and expression of the enzymes responsible for its conversion. The popular modification of the approach is the case when the biosensor or biomass after cultivation has been incubated with the analyte (Racek & Musil, 1987a; Riedel et al., 1990b). It is obvious that the efficiency of the approach is extremely high when the enzymes of the analyte metabolism are inducible and the analyte acts as the inductor. The metabolic activation of cells were utilized in a number of studies directed to the development of sensors for determination of carbohydrates, organic acids, sterols, amino acids, phenol etc. Thus, the application of the approach to biomass cultivation allowed to change the selectivity of the sensor based on yeast cells *Pichia angusta* VKM Y-2518. By means of growing the cells on various substrate it was possible to shift the cells' selectivity to ethanol or methanol several times (Voronova et al., 2008).

The similar technique is related to metabolic inhibition of undesirable pathways or their individual steps during the biomass cultivation or after it (Riedel & Scheller, 1987). In contrast to previous, this approach do not affect on the absolute value of the sensitivity to analyte but allows the elimination or reducing the signals to interfering compounds. For the further selectivity improving, it can be combined with the metabolic induction of analyte conversion enzymes. The common drawback of these approaches in the possibility of reducing and even total disappearance of the induction or inhibition effects after long-term

downtime or operating of the sensor under the presence of interfering compounds (Racek & Musil, 1987a). The attempts to solve the problem by means of incubation of the sensor between measurements in the solution of inductor or inhibitor are known (Kobos et al., 1979; Suzuki et al., 1992) but this can cause a contamination of the culture and altering the analytical characteristics of the sensor.

A promising approach is related to genetic manipulations allowing to enhance the expression of genes responsible to an analyte conversion or eliminate or block the genes of interfering compounds metabolism (Korpan et al., 1993; Mulchandani et al., 1998). The modification ensures actually the same result as in the case of metabolic activation or inhibition but eliminates the possibility of reverting the initial activity of enzymes after inductor or inhibitor removal. Besides, the biochemical altering of the cells' catalytic activity may affect on a number of enzymes while the genetic approach modifies the particular locus, i.e. is more specific. Also, the application of genetic engineering tools makes it possible to impart the principally new abilities to the cells – for example, such modification are often used for insertion of luciferase loci in the operon encoding the enzymes metabolizing the analyte in order to develop a bioluminescent sensor (Kurittu et al., 2000; Thouand et al., 2003).

Thus, the biosensor based on recombinant *Hansenula polymorpha* strains are described (Korpan et al., 1993). This yeast utilizes methanol to CO_2 and water through formaldehyde and formate. One of the strains was formate dehydrogenase-deficient that led to formate accumulation and acidification of medium during the methanol transformation. In the second case the recombinant strain did not contain two enzymes - alcohol oxidase and formate dehydrogenase. The sensor based on this strain was insensitive to methanol and formate and made it possible for selective determination of formaldehyde.

A special case of cell's genetic modification is the transformation or elimination of a plasmid harboring reporter genes or genes encoding the enzymes of analyte metabolism. The use of plasmids allows to obtain a pair of strains for differential detection system (D'Souza, 2001b). Thus, the high selectivity of biosensor for sulphoaromatic compounds detection was ensured using the strain *Comamonas testosteroni* BS1310 harboring the arylsulphonate degradation plasmid pBS1010. The sensitivity of the eliminant strain based sensor to *p*-toluene sulphonate was reduced 10-fold, in addition this sensor possess the decreased sensitivity to catechol, benzene sulphonate, sulphobenzoate. The results obtained supposed the possibility of selectivity improving using differential measurement principle (Makarenko et al., 1999).

The hybrid biosensor creation is also related to a number of methods of selectivity improving (Renneberg et al., 1984). In this case only one of simultaneously immobilized biocatalysts carries out the reaction registered by a transducer; actually, it's selectivity determines the total selectivity of a system. The second biocatalyst serves for the conversion of an initial analyte to compound recognizable by the detecting component and so ensures the system's sensitivity to analyte.

Another way to improve selectivity is based on the development of differential analyzers and sensor arrays (Racek, 1991; Held et al., 2002). The such analyzer includes two or more sensors that are differs only by the sensitivity to analyte(s). The differential analyzer based on *H. anomala* cells was developed for analysis of glucose in urine (Racek, 1991); the reference electrode contained cells with thermally inactivated glycolysis enzymes. Another example of a differential biosensor system is represented by the sensor of genotoxicity

described in (Karube et al., 1982a) and based on the use of two *E. coli* or *Salmonella* strains one of which lacks the SOS reparation system.
The result of this approach development is the case when the sensor array includes a number of low-selective sensors measuring different parameters. The application of chemometrics for processing of the array signals makes it possible to carry out the selective quantitative assay of components in a complex sample. Thus, the differences in the maximal rate of signal changes in response to various substrates allowed to analyze components of mixtures of organic acids with the measurement error less than 10% (Slama et al., 1996; Plegge et al., 2000).
The possibility of selective analysis of a substrate mixture by a system including various combinations of sensors was studied in a number of works (Reshetilov et al., 1998; Lobanov et al., 2001). The system containing an enzyme sensor for glucose determination and a microbial (*Gluconobacter* cell based) sensor sensitive to glucose and ethanol was used for selective ethanol quantification in presence of glucose (Reshetilov et al., 1998). At the next step the authors exploited a system containing microbial sensors based on *G. oxydans* and *P. methanolica* cells (Lobanov et al., 2001). The possibility of selective analysis of both ethanol and glucose in the substrate mixture have been demonstrated. In another work the possibility of identification of glucose, xylose and ethanol in their mixture was shown by means of three microbial sensors based on *G. oxydans*, *H. polymorpha* and *E. coli* cells. The data were processed using cluster analysis and artificial neural network.
None of the above approach of the selectivity improving is universal. However, their individual or combined application in the most of cases allows to reach the acceptable analysis parameters. To top it all, there are situations when high selectivity is not necessary or even undesirable. These situations, in particular, take place in the field of environmentally oriented sensors. Thus, the analysis of wastes supposes not so much that the determination of particular pollutant as the assessment of a group of compounds or total content of organics indirectly determined through the BOD index (Chee et al., 1999; Kim & Park, 2001). In these conditions the use of sensors sensitive to a wide range of substances is essentially efficient (Lehmann et al., 1999; Suriyawattanakul et al., 2002).

3. Transducers in microbial biosensors

3.1 Amperometric transducers

Among the advances of biosensorics, three generations of amperometric bioelectrodes have been developed (Albery & Craston, 1987). Each of the generations acts according the principles of transformation of the biochemical reaction into the electric current.
The background of the first generarion bioelectrodes operating is the registration of consumption of a co-substrate (usually oxygen) during the analyte oxidation or generation of reaction product. The most typical design is based on the oxygen Clark-type electrode and bears a name "respiratory electrode" (Racek, 1995). As the major part of aerobes' metabolism is accompanied by the oxygen consumption the approach can be used for development of biosensors for determination of a wide number of compounds (Held et al., 2002; Svitel et al., 1998; Karube et al., 1980; Beyersdorf-Radeck et al., 1998). Biosensors of this type are also used for determination of compounds suppressing the microbial activity (Campanella et al., 2001; Okochi et al., 2004) or possessing antibacterial or antimicotic activity. In this case the registering parameter is represented by the decreasing of background respiratory activity of biomass.

The amperometric first generation electrodes registering the products of biochemical reactions (for example, hydrogen peroxide), are widely used in enzyme biosensors but rather rarely in microbial ones (Gonchar et al., 1998). So, the first generation electrodes among the microbial sensors are almost solely represented by the Clark type electrode.
In the second generation amperometric biosensors the registration of the biochemical activity has been carried out using the electron transfer mediators. The phenolic and quinoid compounds, ferricyanide, NAD^+/NADH, tetrathiafulvalen (TTF), the derivatives of ferrocene, pyridine, imidazol, complex metal-containing polymers and other substances have been used as mediators in enzyme biosensors (Chaubey & Malhotra, 2002). Sometimes the carrier for biomass immobilization (Tkac et al., 2007; Timur et al., 2007) or the electrode material (McNeil et al., 1992) possess mediatory characteristics - such electrodes belong to third generation able to intercept the electrons from redox enzymatic reaction directly (Shleev et al., 2005).
The major advantage of the 2nd and 3rd generation electrodes is the elimination of the dependence of the sensor signal on a dissolved oxygen concentration. Also, the transducer of mediator sensors is often represented by screen-printed electrodes that allows to reduce the assay cost and facilitates the unification of the sensors' characteristics. At the same time, this approach requires additional expenses for the mediator obtaining. In relation to microbial sensors it can be realized only if the analyte conversion involves the easily accessible cell surface-localized enzymes or if the cell wall and membrane are permeable for mediator in both directions. To date, all types of charge transfer are reported for cell based biosensors including the utilization of various mediators (Katrlik et al., 2007; Tkac et al., 2002; Bhatia et al., 2003), direct charge transfer in biofuel cells (Chaudhuri & Lovley, 2003), and also the immobilization using the conducting carriers (Vostiar et al., 2004; Timur et al., 2007).
The coupling of enzyme and electrochemical reactions on the conducting materials made it possible for the development of a large number of microbial biosensors for carbohydrates and alcohols detection (Ikeda et al., 2004). The amperometric microbial sensors can also be used for determination of a wide spectrum of pollutants; the backgrounds of the method are described in the review (Paitan et al., 2003).

3.2 Potentiometric transducers

In the cases when the biochemical reaction leads to alteration of ionic composition of the medium it can be registered using potentiometric ion-selective electrodes (ISE). If ISE is placed into the medium containing the expected ion an electric potential appears on the electrode's membrane. The ISE most often used in biosensorics is the pH-electrode. Protonation/deprotonation of a bioreceptor due to biochemical reaction results in a potential generation on the electrode membrane.
The development of ion-selective field effect transistors (ISFETs) were started in 70s by P. Bergveld team (University of Twente, The Netherlands). In general, the developments were directed to the creation of pH-selective ISFETs that soon after became compete with glass pH-electrodes (Bergveld, 2003).
The detailed description of ISFETs related studies and their application in biosensorics is presented in a number of reviews (Domansky et al., 1993; Bergveld, 2003; Yuqing et al., 2003). To present, the ISFETs for evaluation of various ions as well as concentration of oxygen and carbon dioxide have been reported. At the same time, the amount of publications devoted to microbial sensors based on ISE is rather small (Mulchandani et al., 1998; Rechnitz & Ho, 1990).

Seeking for the improving the quality of biosensoric analysis is a motivation of new transducer types development. The light-addressable potentiometric sensors (LAPS) was created as a result of the development of the concept related to control of the processes in semiconductor material using applied external electrical field. One of the important characteristics of LAPS is the fact that the photo-generated current reflects the chemical processes at the irradiation point. This lays the background for development of multichannel sensors by means of successive irradiation of the points.
Two major fields of LAPS application are the development of affine biosensors and microphysiometers. The latter's purpose is the evaluation of intracellular metabolic processes in vivo. The majority of to-date reported microphysiometers were used for the studies of metabolism of homoeothermic animal cells. The first paper related to this subject was published in 1989 (Parce et al., 1989). The LAPS based device ensured the possibility of detection the receptor-ligand interaction, assessment of the sensitivity of tumor cells to drugs and evaluation of the toxic action of various factors on the cells (Bousse, 1996). The study of bacterial cells metabolism using LAPS has been presented in paper (Baxter et al., 1994) that is related to evaluation of cells' sensitivity to antibacterial agents.
Thus, it should be conclude that the most important characteristics of LAPS as a biosensor transducer are high reliability and simple design, as well as the possibility to evaluate the metabolic processes in microbial and animal cells in vivo. The LAPS-based multichannel sensors could easily be designed without complex procedures that are necessary during development a multichannel FET-based device.

3.3 Conductometric sensors

Conductometric biosensors register the bioreceptor activity by the changes of the solution conductivity caused by biochemical reactions. The typical design of a conductometric sensor includes interpenetrating electrodes on a dielectric support covered by a biomaterial film. The enzyme conductometric sensors for detection of ethanol, urea, penicillin etc. have been described. An important drawback of conductometric sensors is the susceptibility to unspecific factors associated with biochemical reactions, so the measurements are usually carried out by a pair of sensors for error minimization. The application of conductometry for development of microbial sensors is limited.
Several examples of toxic compounds determination using a conductometric sensor have been reported. Microbial biosensor based on whole *Rhodococcus ruber* cells has been created for express analysis of acrylonitrile (Roach et al., 2003). A sensor based on *Chlorella vulgaris* cells and conductometric transducer has been developed for evaluation of activity of intracellular alkaline phosphatase and cadmium and zinc ions. The cells were immobilized in bovine serum albumin by cross-linking with glutaraldehyde. (Chouteau et al., 2004).
The conductometric microbial sensors for alcohol analysis are also known. Detection of ethanol in beverages has been carried out using a conductometric yeast-based biosensor; the results of analysis correlated well to the gas chromatography data (Korpan et al., 1994).

3.4 Microbial calorimetric sensors

Microcalorimetry is one of the conventional techniques of the cell metabolism assessment. The majority of enzyme reaction have been accompanied by heat emission; their molar enthalpies are in the range of 25-100 kJ/mole (Turner et al., 1987). The heat measurement is the operating principle of the calorimetric or thermal biosensors. The first enzyme thermal

sensor also known as "enzyme thermistor" was developed in early 1970s (Mosbach & Danielsson, 1974). The typical calorimetric sensor includes a receptor designed as a reactor heat-insulated from the environment. The temperature measurements have been carried out at the input and output of the reactor. To improve the measurement precision and minimize errors a two-channel differential measurement system has been utilized.
The thermal biosensors advantages include the possibility of continuous measurements, high long-term stability, insensitivity to electrical or optical interferences, the absence of interfering action of the reaction products, high reproducibility and rapid responses. At the other hand, the restrictions are related to necessity of some samples pretreatment and the possibility of the system contamination as a result of continuous measurement of the untreated samples (Ramanathan et al., 1999).
One of the first microbial thermistors has been described in (Mattiasson et al., 1977). The authors mentioned that the sensor could be used for the analysis of a wide spectrum of compounds. However, to date only a small amount of the microbial thermal sensor related studies have been reported in spite of the fact that the heat emission during the microbial conversion of a substrate is similar to that during the enzyme oxidation. The author of review (Schugerl, 2001) notes that the microcalorimetric approach could be used for real-time monitoring of the biomass cultivation processes.

3.5 Optical microbial sensors

The important requirements specified for the environmentally oriented analyzers intended for evaluation of the pollutants in situ include their operational efficiency and specificity. The development of bioluminescent microbial sensors based on the optical transducers allows to obtain analytical devices satisfying all the wants. The detailed review of this subject can be found in reviews (D'Souza, 2001b; Hansen & Sorensen, 2001). It is possible to select two characteristic versions of this approach. The realization of the first one involved the fusion of reporter genes to the operon controlled by the analyte-inducible promoter; the common reporters include bacterial and firefly luciferase components as well as green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* or sea pen *Renilla reniformis* (Daunert et al., 2000). Thus, the appearance of the analyte results in the bioluminescence enhancement that is registered by a transducer; the approach could potentially be realized for detection of almost any compound involved into the cell metabolism. The special case is the technique that is consisted in the insertion of a reporter to one of loci responsible to DNA reparation; thus, any DNA damaging factor induces the biosensor signal that makes it possible the total genotoxicity assessment (Afanassiev et al., 2000; Mitchell & Gu, 2004).
The second version of the bioluminescent sensors related approach supposes the employment of recombinant or wild-type luminescent strains for control of non-specific factors reducing the microorganisms viability. In this case the reporter genes are controlled by constitutive promoters and the sensor's operating principle is based on the registration of background luminescence decrease due to reduction of the living cells amount caused by the effect of an analyte (Philp et al., 2003; Ritchie et al., 2001). This version of the bioluminescent sensor has been realized in the total toxicity evaluation system MICROTOX™ (Ribo & Kaiser, 1987).
The bioluminescence related approach ensures high selectivity of analysis and in a number of cases allows significant (in several orders) enhancement of the detection sensitivity over the biocatalytic sensors (Billard & DuBow, 1998). Its drawbacks include the low rate of analysis and increased labor content due to the need in genetic manipulations. To present, at

least several hundreds of bioluminescent microbial sensors tailored to determination of antimicrobial drugs, pollutants, genotoxicity and total toxicity have been reported (Anko et al., 2002; Applegate et al., 1998; Gu & Chang, 2001; Min et al., 2000).
The original optical microbial sensor based on the employment of surface plasmon resonance device reported in (Kononov et al., 2007). The authors demonstrated the possibility of obtaining the signal reflecting the metabolic state of microorganisms in real time, in spite of high thickness of the cells. The results obtained indicate that the cyclic generation of gas due to metabolic processes leads to reversible alteration of bacterial cells properties in the immobilized cells monolayer.

4. Analytes and application of microbial biosensors

4.1 Determination of carbohydrates.

Carbohydrates are the most common analytes of enzyme and microbial sensors. It suffice to say that the first microbial sensor was tailored to glucose determination and in spite of a plenty of investigations carried out within several dozens of years the determination of carbohydrates and their employment as the model substrates remains a widely used direction of biosensorics. As related to microbial sensors, this circumstance is due to the high bioavailability of carbohydrates and also by practical significance of their analysis for biotechnology, food industry and medicine. The most often reported "carbohydrate" sensors are glucose and lactose analyzers; at the other hand, a large number of microbial biosensor models for detection of other mono- and disaccharides and even polymeric carbohydrates have been described. Thus, in one of characteristic studies a microbial sensor for glucose determination based on *Pseudomonas fluorescens* and oxygen electrode has been developed (Karube et al., 1979). The similar work described three models of microbial sensors for detection of glucose, sucrose and lactose based on *G. oxydans*, co-immobilized *G. oxydans* and *S. cerevisiae* and co-immobilized *G. oxydans* and *K. marxianus* cells, respectively (Svitel et al., 1998). In the publication (Held et al., 2002) a sensor array including a number of oxygen electrodes with immobilized *E. coli* strains lacking the transfer systems of various mono- and disaccharides has been created. Several models of low-selective sensors able to determine the total carbohydrate content are known; they include, for example, the mediator biosensor based on *G. oxydans* cells and tailored to determination of total carbohydrate content in lignocellulose hydrolyzate (Tkac et al., 2000).
It should be noted that among the microbial carbohydrate analyzers there is rather large amount of mediator sensors. Besides of the sensor mentioned in (Tkac et al., 2000) the examples include the sensor based on *G. oxydans* and carbon paste electrode (Takayama et al., 1993), the glucose sensor based on *E. coli* cells and carbon paste electrode modified by benzoquinone and PQQ (Ito et al., 2002) and the glucose biosensor based on *Aspergillus niger* and carbon paste electrode. The ferricyanide and ferrocene were utilized as the mediators. The sensor was exploited for glucose monitoring during biotechnological processes (Katrlik et al., 1997).
The hybrid sensors are also often used for carbohydrate detection. The approach is especially appropriate for di- and polysaccharides analysis; in this case, one of the biocatalysts carries out the glycoside bond hydrolysis while the other determines the generated monomers. Two hybride carbohydrate sensors were mentioned above (Svitel et al., 1998); besides them the hybrid sensors for sucrose determination based on invertase and *Z. mobilis* cells (Park et al., 1991) and for detection of lactose in dairy products based on

glucose oxidase and *E. coli* cells (Svorc et al., 1990) have also been reported. The same approach was used for determination of α-amylase activity using co-immobilized *B. subtilis* and glucoamylase (Renneberg et al., 1984). Another manuscript describes an automated system based on a reactor containing the immobilized cells of *Rhodococcus erythropolis* and *Isaatchenkia orientalis* and screen printed oxygen electrodes (Heim et al., 1999).
In work (Nandakumar & Mattiasson, 1999) the microbial sensor for glucose detection based on psychrophilic bacteria *Deinococcus radiodurans* and oxygen electrode. Other carbohydrates could interfere with the analysis. Sensor was able to determine the analyte under 5 ^{o}C, the receptor remained 90% from the initial activity after 45 days from the start of exploitation.
Starch detection is actual for biotechnology and food industry. In (Kitova et al., 2004) microbial and enzyme sensors for detection of the glucose generated by the amylase have been considered. The high correlation of biosensor analysis results with the polarimetric starch determination in wheat and rye flour was obtained.

4.2 Detection of alcohols and organic acids.

Biosensoric determination of alcohols and organic acids is also reported rather often by virtue of the same reason as for the carbohydrate detection. The methodical ware, and in a number of cases the microorganisms employed are also similar to ones utilized in carbohydrate sensors. It should be noted that the microbial cells possessing high selectivity are often used in biosensors for alcohol detection along with the enzymes. The methylotrophic yeast belonging to *Hansenula*, *Pichia*, *Candida* genera that are characterized by high intracellular content of alcohol oxidase and substrate specificity alterable by means of biochemical or genetic manipulations seem to be especially promising for this purpose (Voronova et al., 2008; Korpan et al., 1993). Thus, in manuscript (Gonchar et al., 1998) two microbial alcohol sensors based on recombinant *H. polymorpha* cells have been described. The first of the sensors utilized the cells with highly active alcohol oxidase immobilized on an oxygen electrode while the second one was based on catalase-deficient cells and involved a peroxide electrode as a transducer. The sensors were highly stable and insensitive to glucose and glycerol.
The original development consisted in the creation of an alcohol sensor based on *Agaricus bisporus* fungus tissue homogenate. The biomass immobilized in gelatin and cross-linked by glutaraldehyde was fixed on a Clark type electrode. The electrode current linearly depended on the ethanol concentration within the range 0.2 – 20 mM (Akyilmaz & Dinckaya, 2000).
The analysis of lactate in blood plasma and whole blood was carried out by the sensor based on *H. anomala* cells. The sensor possess high reproducibility, stability and rate of analysis. The analysis results agreed with the data obtained using conventional spectrophotometric technique and lactate dehydrogenase based enzyme sensor (Racek & Musil, 1987b). The similare work was intended to the development of a microbial sensor for fatty acids detection based on an oxygen electrode and *Arthrobacter nicotianae* cells immobilized into Ca-alginate gel (Schmidt et al., 1996). In the framework of another study a sensor for detection of tannic acid (a mixture of polygalloyl–glucose esters) from *Rhus chinensis* based on *Aspergillus ustus* cells and oxygen electrode. The analyte detection range made up about 10^{-4} – 10^{-3} M. An evaluation of temperature and pH effects on the sensor response has been carried out (Zhao et al., 1998).
As in the case of carbohydrate analysis the oxygen electrode is not the only transducer employed in the microbial sensors for alcohols and organic acids determination. A microbial ethanol biosensor based on ISFET and *Acetobacter aceti* cells was described (Kitagawa et al.,

1987). A conductometric microbial sensor for ethanol based on alginate-immobilized yeast cells has also been described (Korpan et al., 1994).
PQQ-dependent dehydrogenases of *Gluconobacter* bacteria are characterized by a wide spectrum of substrates. In this connection the enzymes as well as whole *Gluconobacter* cells represent a promising base for biosensor development. In publication (Tkac et al., 2003) a microbial sensor for ethanol detection based on *G. oxydans* cells and glassy carbon electrode modified by ferricyanide was described. The selectivity of the sensor was higher over enzyme sensors due to the application of a membrane barrier for glucose. The biosensor was employed for real-time ethanol monitoring during periodical fermentation of glucose by immobilized yeast.
An original approach was used in the study related to development of a biosensor based on *Chlorella* cells and oxygen electrode for detection of volatile compound vapors. The characteristic feature of the approach was the registration of the oxygen produced by the cells has been employed. Methanol was used as the model analyte. The sensor retained 50% of initial activity after 10 days from the start of operating (Naessens & Tran-Minh, 1998a).

4.3 Environmental application of biosensors - common state

The analysis of pollutants makes specific demands for the characteristics of developed biosensors. One of these demands is high detection sensitivity, which is determined by low MPC values of most xenobiotics. Catalytic sensors can provide the lower limit of target compound detection at a level of 10^{-7} M at best, which in most cases is insufficient. Therefore, among microbial sensors for the analysis of toxic compounds and pollutants there is a lot of bioluminescent sensors providing highly selective detection at a level of 10^{-9} – 10^{-7} M (in some cases, up to 10^{-12} M). Nevertheless, biocatalytic sensors are also used for detection of compounds with relatively low MPC values (phenol, naphthalene, SAS, some ions, etc.) and BOD. The characteristics of some biosensors potentially fit for environmental monitoring and similar nature-conservative measures will be considered in this subsection.

4.4 BOD detection

The methods and equipment for quick and sensitive assessment of the degree of water source pollution are relevant for providing high-performance measures of environmental control and decontamination. One of the most widely used indices for aquatic environment control is biological/biochemical oxygen demand (BOD). At present, the routine BOD analysis is performed mainly by the method of BOD_5 taking five days for completing. The quicker methods of BOD detection are associated with biosensor analyzers usually based on microorganisms that can metabolize the wide range of organic compounds. Although BOD values obtained by these methods are not identical to the values of BOD_5, in most cases it is possible to achieve an acceptable correlation between biosensor readings and the values of conventional methods.
The first work on construction of such sensor was published in 1977 (Karube et al., 1977); microorganisms from the active sludge of water treatment facilities were used as biomaterial. By now, quite a number of laboratory models and several commercial BOD biosensor analyzers are known. Such analyzers provide for BOD detection in the mean range of 5-300 mg/l for about a few minutes.
The sensor similar to the first BOD sensor model is described in (Liu et al., 2000). Its bioreceptor also included active sludge microorganisms. Sensor readings had satisfactory

reproducibility and correlated well with BOD_5 values. BOD sensors based on mixed cultures are also reported in (Jia et al., 2003; Li et al., 1994; Suriyawattanakul et al., 2002). The BOD sensor based on a biofuel cell (BFC) containing active sludge is described in the work (Chang et al., 2004).

Although mixed cultures broaden the spectrum of biodegradable compounds (and, consequently, provide more profound BOD detection), the wrong side of this approach in most cases is lower stability and reproducibility of results associated with the ratio dynamics of cultures in bioreceptor. Therefore, pure bacterial and fungal cultures with broad substrate specificity are used in BOD sensors in parallel with active sludge and other microbial mixtures. The examples of such sensors are descirbed in the works (Yang et al., 1997; Kim & Park, 2001; Chee et al., 1999) etc. The list of the microorganisms most frequently used in BOD sensors includes *T. cutaneum*, *Arxula adeninovorans*, *E. coli*, *Bacillus* and *Pseudomonas* species and other microorganisms with broad specificity. Thus, different authors have published no less than ten works on construction of *T. cutaneum*-based BOD sensors; besides, it is used in some of the commercial BOD analyzers. The example of such sensor is described in the work (Yang et al., 1996).

Most of the BOD sensors are based on amperometric oxygen transducers (as a rule, Clark-type electrodes), but this is not the only possible way of constructing BOD sensors. The paper (Trosok et al., 2001) describes a mediator BOD sensor based on yeast cells and a glass-carbon electrode. The authors investigated the possibility of using 10 mediators for BOD detection; among them, ferricyanide was the most effective for detection of glucose-glutamate mixture. The sensor detected BOD in the range of 2-100 mg/l.

The new type of oxygen microsensor measures the level of oxygen using organically modified silicon and oxygen-sensitive dye tris(4,7-diphenyl-1,10-phenanthroline)ruthenium (II) chloride. The bacterial culture of *Stenotrophomonas maltophilia* was used. Measurement time was 20 min. Satisfactory coincidence with the data of standard BOD_5 method was obtained (Pang et al., 2007).

The work (Chee et al., 2000) describes the BOD sensor based on an oxygen optrode and *Pseudomonas putida* cells. The sensor detected BOD in the range of 1-10 mg/l and was insensitive to chlorides and heavy metal ions. Sensor readings correlated well with the BOD_5 data. The sensor was used for the analysis of river water samples.

Bioluminescent BOD sensor was based on the recombinant *E. coli* strain bearing fragments of *V. fisheri lux*-operon (Sakaguchi et al., 2003). Sensor readings correlated well with the BOD_5 data. The sensor was used for assessment of organic pollution in different wastewater samples.

One more approach to BOD detection is based on registration of temperature changes caused by microbiological destruction of organic compounds using calorimetric transducers; the biosensor based on such transducer is described in paper (Mattiasson et al., 1977).

In most cases, the BOD index is supposed to display the total quantity of organic compounds in a sample; accordingly, BOD sensors are constructed, as has been mentioned above, on the basis of microorganisms with broad substrate specificity. However, in some cases, BOD measurement is associated with the presence of specific compounds in the medium, which compels one to correct the choice of biocatalyst. So, the work (Konig et al., 1999) describes the sensor for detection of BOD at nitrification (N-BOD) and the degree of nitrification inhibition in wastewater. The bioreceptor of this sensor included a mixed culture of nitrifying microorganisms isolated from water treatment facilities. Nitrification inhibition was assessed in the presence of allylthiourea. The sensor had a high correlation

with the standard method of N-BOD detection and was used for the analysis of wastewater samples.

One more example of biosensor detection of BOD determined by the presence of particular compounds in wastewater samples is described in paper (Reiss et al., 1998). In this case, the sensor was designed for BOD detection in starch-polluted waters. The device was based on a commercial biosensor BOD analyzer (Prüfgerätewerk Medingen) including the cells of *T. cutaneum*. The sensor was additionally equipped with two enzyme reactors containing immobilized α-amylase and amyloglucosidase for starch hydrolysis. Sensor readings were close to the standard BOD_5 data.

The functioning of BOD sensors may be influenced by various factors, including the chemical composition of analyzed samples. In particular, the presence of compounds toxic for cells may partially or completely inactivate the sensor or reduce its lifetime. The work (Qian & Tan, 1999) pursued the study of the effect of heavy metal ions on the BOD sensor based on *B. subtilis* cells killed by heating. This sensor was characterized by rather high sensitivity and stability of readings, in spite of nonviability of the biomaterial. The sensor allowed to estimate the effect of various metal ions on BOD determination.

It should be noted that the biosensor approach usually accounts only for easily utilized organic phase in measured samples and its results may not always correspond to the BOD_5 test. The correlation between biosensoric and conventional BOD measurements was assessed in the work (Liu et al., 2003). It was shown that the high correlation of biosensor and BOD_5 results was observed only for the samples that contained no organic polymers; in the presence of the latter, biosensor estimates were lower. The new approach to enhancement of the correlation between the biosensor method of measurement and traditional BOD_5 is presented in papers (Chee et al., 2005; Chee et al., 2001). The authors have proposed the method of photocatalytic pretreatment of water samples by means of their irradiation with the UV light for decomposition of large organic molecules into smaller ones. It has been shown that such scheme of analysis enhances biosensor sensitivity and increases the degree of correlation between the biosensor data and BOD_5. The analogous work on BOD measurement in river water was carried out at a stopped-flow plant using ozone treatment. After ozone treatment, the biosensor signal increased 1.6-fold (Chee et al., 2007).

One of the problems of BOD biosensors operation is the short time of their functioning because of membrane pollution. Besides, a pure culture based BOD sensor not always has sufficiently broad substrate specificity that would allow more complete estimation of BOD. The application of mediator-less biofuel cell (BFC) was a new approach to creation of analytical system for BOD assessment. In (Kim et al., 2003), the authors presented the experience of 5-year application of mediator-less BFC for BOD monitoring. The main parameters of this system, providing its high practical value, are stability and long (5 years) period of functioning without maintenance and the high correlation of results with the BOD_5 index.

The prevalence and importance of BOD biosensor related studies have naturally resulted in commercialization and industrial production of a number of the most promising models. The first commecrial BOD biosensor analyzer was manufactured by Nisshin Denki (Electric) Co. Ltd. in 1983. Later on, similar analyzers were manufactured by some European companies (Riedel, 1998). At present, a number of biosensor systems for the control of biological oxygen demand are produced by European, Japanese and USA manufacturers.

Thus, biosensor BOD detection is a rather well-developed trend of analytical biotechnology. Biosensor BOD analyzers are reliable, simple and inexpensive analytical instruments, which