

Biosensors in Environmental and Bioprocess Monitoring and Control

Amarjeet S. Bassi*, Diqing Tang, Esther Lee, J. X. Zhu and M. A. Bergougnou

Department of Chemical and Biochemical Engineering, Faculty of Engineering Science,
University of Western Ontario, London, Ontario, N6G 5A9

Received: March 11, 1996

Accepted: May 17, 1996

Summary

Many types of biosensors have now been developed and are being applied for various analyses, such as clinical, medical, environmental monitoring, industrial process control and many other fields. Advances in micro-sensor technology and genetic engineering are fuelling new developments in this field. Due to their potential in real time analysis and rapid monitoring, biosensors are particularly suited for the above mentioned applications. This review describes the recent developments and fundamental principles of biosensors for process and environmental control.

Keywords: biosensors, flow injection analysis, bioprocess monitoring, environmental applications of biosensors

Introduction

Over the last few years, there has been a great surge in research activities directed towards biosensor development and some types of biosensors have already been commercialized. The strong interest in biosensors as useful analytical devices stems from the several advantages they offer over conventional analytical techniques of measurement. These advantages include: (i) targeted specificity even in complex media; (ii) fast and continuous measurements in real time; (iii) portability; and (iv) cost-effectiveness. For these reasons, biosensors have a potential for a major impact especially in the areas of on-line bioprocess monitoring and environmental monitoring.

In the biotechnology area, biosensors can provide an inexpensive and attractive alternative in the on-line monitoring and control of non-volatile substrates and metabolites in enzyme and microbial reactors, e.g. in the production of recombinant DNA microorganisms, or monoclonal antibody production (Mabs) in hybridoma cultures. In the environmental testing market, which requires sensitive, small, portable, real-time measuring devices capable of monitoring air, water, or soil, biosensors may permit continuous pollutant monitoring at the source without the necessity of collecting samples for off-line analysis.

The field of biosensors has been previously extensively reviewed (1–7). This review is therefore limited to more recent developments in the areas of environmental and bioprocess monitoring and control and the fundamental principles of biosensors are only briefly considered.

Fundamentals of Biosensors

A biosensor may be defined as an analytical device which combines a biological sensing component with a signal transducer (Fig. 1). The biological component pro-

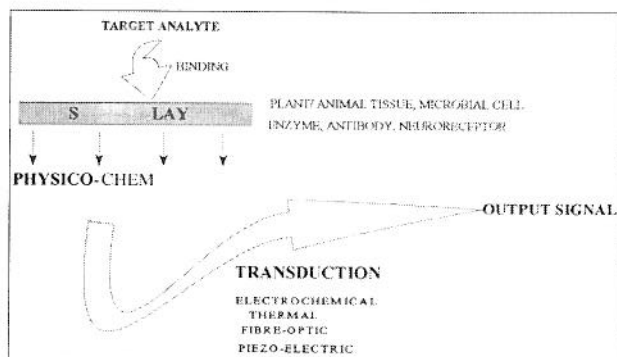


Fig. 1. Fundamental concept of a biosensor

* author to whom correspondence should be addressed

vides the targeted specificity to the device, due to its distinctive and unique interaction with the analyte of interest. Enzymes, antibodies or whole microbial cells have been frequently used to construct biosensors. More novel biological components utilized include antigens, neuro-receptors, plant/animal tissues, genetically engineered cells and antibody fragments. Based on the biological component, the majority of current biosensors can be classified as either: (a) metabolism or catalytic sensors which use enzymes or whole cells; or as (b) affinity sensors which are based on antibodies, neuro-receptors or other ligands involved in binding reactions.

Many type of signals may be produced during the interaction of the analyte with the biological element of the biosensor. For example, there may be a change in proton concentration, release or uptake of gases such as ammonia, hydrogen peroxide or oxygen, light emission, absorption or reflectance, heat emission, mass change, etc. The function of the transducer component of the biosensor is to convert this signal into a measurable response. Various type of transducers include (i) electrochemical transducers which measure changes in current, potential or conductivity; (ii) thermal transducers which measure temperature changes; (iii) fibre-optic systems to measure light signals; or (iv) piezoelectric and (v) surface acoustic wave (SAW) transducers which can measure mass changes.

A majority of the biosensors reported to-date have been based on enzymes belonging to the classes oxidoreductases, hydrolases or lyases (8). While many enzymes are very specific for their substrates, and can provide sensitivity to the biosensor, their principal limitation is poor long-term operational stability. Depending on the enzyme, its activity and thus the biosensor performance may deteriorate to unacceptable levels within a few days or weeks of operation. Much effort has been devoted to choosing appropriate immobilization strategies for putting the enzyme on the surface in order to long-term operational stability and this is considered later in this review.

While enzyme-substrate reactions are catalytic where a product or co-reactant can be measured, antibody-antigen interactions involve affinity binding. The binding effect is detected either by direct changes in the transducer output as a result of the binding event, or through indirect competitive and displacement reactions (as in immunoassays) using optical, piezoelectric or electrochemical techniques (9–14). The principle advantage of antibodies are that they can be generated against a whole host of antigens. However, antibody based biosensors require longer incubation times (15–30 min). Also, the strong binding between the antibody and the antigen makes the construction of practical, reversible antibody-based biosensors difficult. The use of genetically engineered antibody fragments with relatively weak affinities for the antigen of interest may offer a solution to this problem.

Microbial biosensors are constructed using immobilized whole cells coupled with various transducers (15,16). Microbial biosensors are inexpensive, since a single cell can contain all needed enzymes and co-factors for detection of the analyte which otherwise would

have to be provided separately. However, for the same reasons, the specificity of microbial sensors is less than pure enzyme based biosensors. If living cells are used, the microbial sensor can be easily regenerated by exposing it to fresh growth medium. Immobilization of cells for biosensor applications has been extensively reviewed (17–19).

Specific requirements of biosensors for on-line monitoring: On-line biosensor monitoring of metabolites in a bioreactor may be carried out using either an *in situ* arrangement or through flow injection analysis (FIA). A biosensor which is to be immersed into the bioreactor must be able to tolerate sterilization as well as varying environmental conditions within the bioreactor. It is difficult to design biosensors for *in situ* applications since the biological components of the sensor, i.e., enzymes, antibodies, whole cells/tissue, etc. are sensitive to the operational environment and must be maintained at the appropriate pH, temperature, ionic strength, in order to maximize the response. The problem of microbial growth on the detector surface also makes such applications difficult, thus fewer reports have appeared in this area (20–27). Flow injection analysis (FIA) is usually required when utilizing biosensors in continuous bioprocess monitoring and control (28,29). Essential requirements of a FIA system for bioprocess monitoring are shown in Fig. 2 and include: (a) a sterilizable sampling probe which can be immersed in the bioreactor and a filter; (b) a two-channel peristaltic pump module; (c) an injection valve used to load small volumes (20 to 50 μL) of filtered, diluted bioreactor sample into the carrier buffer stream at programmed intervals and (d) a biosensor and transduction system with a recorder or computer (30,31). This method offers the advantages of (i) reduced risk of contamination because the sample is not returned to the bioreactor; (ii) rapid and more frequent recalibration and washing of the biosensor; (iii) short response times, as well as (iv) requirement of small sample quantity. The signal obtained can also be multiplexed with a computer for on-line control.

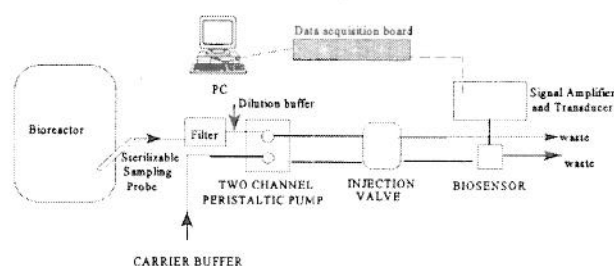


Fig. 2. Essential requirements for a flow injection analysis (FIA) system for bioprocess monitoring and control using a biosensor

Specific requirements of biosensors for environmental monitoring: Rogers (32) has discussed the various methods, including the use of biosensors, which are currently being developed for environmental monitoring. In general, a biosensor for field monitoring must be capable of repetitive, or continual monitoring, a high degree of specificity towards recognition of the analyte, and robustness. As discussed, biosensors will meet easily the first two requirements. However, the instability associated

with biological sensing component and the need to maintain conditions for optimum response, places limitations on biosensors in field monitoring.

Bioprobes with disposable test-strips or dipsticks, may prove to be as effective and less expensive than the current commercially available immuno-assay test kits. Specific areas of applications of biosensors in the environmental field include (i) monitoring of individual pollutants (33); (ii) monitoring of a certain class of pollutants (e.g., phenols (34), or xenobiotics (35), heavy metals (36), pesticides (37) etc.); (iii) measurement of toxicity (38) and (iv) biological oxygen demand (BOD) monitoring (39,40).

Novel Biological Sensing Components

Antibody Fragments and Fusion Proteins

The production of antibody fragments offers possibilities of novel biosensors. Synthesis in *Escherichia coli* of correctly folded antibody fragments that bind antigens with the same affinity as the whole antibody has been demonstrated (41). Such antibody fragments have been used successfully in immunoassays. The motivation for the use of single stranded antibody fragments encoding the variable region (sFv) as compared to whole antibodies is that they are generally easier to genetically engineer than whole Ig molecules and can be expressed as fusion partners with native bacterial proteins. These fusion proteins may be obtained as inclusion bodies within the cell, expressed on the cell surface or excreted into the medium (42). The fusion protein will then contain the antibody fragment which can retain binding affinities equivalent to the parental monoclonal antibody. The use of sFv bound to a toxin, cytokine or cytokine receptors offers possibilities for use as biosensors. The production of antibody fragments and fusion proteins may improve on the existing immunosensors due to their increased sensitivity and selectivity. As well, with increased technology and genetic manipulation, reversibility of the reaction may become a reality, fulfilling all the criteria necessary for a true biosensor.

At the present time, to our knowledge, no biosensor based on the above technology is available. However, a biosensor for analysing recombinant antibodies and phage displayed antibody libraries, (BIAcore biosensor) has been reported. Malmberg and Borrebaeck (43) have reviewed the application of the biosensor in the screening of recombinant antibody fragments, kinetic selection of phage displayed antibodies, characterization and epitope mapping of monoclonal antibodies and their fragments.

Genetically Engineered Cells

The development of genetically engineered biosensors for copper and mercury ions is described by Holmes *et al.* (44). The biosensors have been constructed by fusing the lux or light emitting genes from *Vibrio fischeri* with genetic regulating elements that respond to copper ions or mercury ions, derived respectively from *Escherichia coli* and *Serratia marcescens*. The fusions were placed into *E. coli* cells which then emitted light in re-

sponse to copper or mercury ions. The authors presented data describing the sensitivity, specificity, and dynamic range of the biosensors to their respective target metal ions. A preliminary description of experiments is provided indicating how these biosensors might be used to investigate the bioavailability of mercury and copper ions in environmental samples.

Kobatake *et al.* (45) also described the use of recombinant bacteria as biosensors. A biosensor for environmental pollutants was developed by fusing a gene of firefly luciferase to the TOL plasmid. The TOL plasmid of *Pseudomonas putida* encodes a series of enzymes for degradation of benzene and its derivatives. The expression of these enzymes is controlled with the regulating proteins xylR and xylS, whose promoters are activated in the presence of aromatic compounds. The structural gene of firefly luciferase, as a reporter enzyme, was inserted under the control of the promoter of xylS protein, and gene fusion plasmid pTSN316 was constructed. The recombinant *Escherichia coli* transformed with this plasmid was applied to the environmental biosensing of benzene derivatives. The expression of luciferase was induced in the presence of aromatic compounds and the lower detection limit for *m*-xylene was 5 μ M.

Rouch *et al.* (46) have compared the induction by the cognate metal salts of two promoters responsible for metal-resistance gene expression in bacteria. The mercuric ion resistance promoter, P-merTPAD, of transposon Tn501 and the copper resistance promoter, P-pcoE, from plasmid pRJ1004 were separately cloned to express the lacZ gene under the regulation of their normal trans-acting elements. The lux genes of *Vibrio fischeri* were also expressed from P-merTPAD. The induction of P-merTPAD gave a hypersensitive profile, where the apparent Hill coefficient was 2.6 when using β -galactosidase activity as a measure of lacZ gene expression. In contrast, the induction of P-pcoE was hyposensitive, with an apparent Hill coefficient of 0.63 for induction of β -galactosidase activity, and this may be related to the role of copper as an essential micronutrient. The authors suggest that transcriptional fusions of the P-merTPAD promoter allow the construction of strains that are suitable for detecting threshold levels of mercuric ions, but not for accurate determinations of mercuric ion concentrations across a wide range. In contrast, transcriptional fusions to the P-pcoE promoter are well suited to determination of the concentrations of copper salts. The comparison of induction profiles of P-merTPAD, using lacZ or lux reporter genes, show different stimulus-response curves, probably due to differing instrument sensitivities.

Submitochondrial Particles

A novel biosensor based on submitochondrial particles has been proposed by Argese *et al.* (47), which utilizes an *in vitro* bioassay procedure to investigate the toxic action of chlorophenols on mitochondrial respiratory parameters. The toxicity of these compounds was evaluated by determining their effects on the energy-coupled reverse electron transfer (RET) in submitochondrial particles (SMPs) from beef heart mitochondria. The bioassay procedure is based on the spectrophotometric recording of the effects of toxicants on the rate of NAD⁺

reduction, induced by ATP and succinate at the first site level of the respiratory chain. The toxicity end point was expressed as the toxicant concentration that causes 50% inhibition of NAD^+ reduction rate (EC_{50}). The EC_{50} values determined for the 14 tested chlorophenols ranged from 17 mg/L for 2-chlorophenol to 0.081 mg/L for pentachlorophenol, indicating a general trend of increasing toxicity with increasing chlorine substitution. Among chlorophenol isomers, which have the same number of chlorine atoms, a lesser toxicity was associated with ortho-substituted chlorophenols, whereas meta-substituted chlorophenols were much more toxic. The EC_{50} values were compared with the toxicity data for a variety of bioassays, by means of linear regression analysis. The authors showed that high degrees of correlation obtained with toxicity tests involving different freshwater species demonstrate the ability of SMPs to reproduce the toxic effects of the tested compounds upon aquatic organisms. This supports the assessment that the respiratory chain is the main target of this class of toxicants. The authors have found that results obtained with chlorophenols and, in previous studies, with other environmental contaminants confirm the suitability of the SMP bioassay as a pre-screening or complementary short-term test for monitoring aquatic toxicity.

Membrane Vesicles

A fibre optic biosensor based on brush border membrane vesicles purified from susceptible and resistant larvae of diamondback moth (*Plutella xylostella*) for the detection of CryIA(c) toxin from *Bacillus thuringiensis* has been described by Masson *et al.* (48). The biosensor utilized surface plasmon resonance to determine the kinetic rate constants for interactions between the toxins and the membrane vesicles. CryIA(c) association and dissociation rate constants for vesicles from susceptible larvae were determined to be $4.5 \cdot 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ and $3.2 \cdot 10^{-5} \text{ s}^{-1}$, respectively, resulting in a calculated affinity constant of 7 nM. CryIE toxin did not kill susceptible or resistant larvae and did not bind to brush border vesicles. Contrary to expectations based on previous studies of binding in resistant *P. xylostella*, the binding kinetics for CryIA(c) did not differ significantly between susceptible larvae and those that were resistant to CryIA(c). Determination of the number of CryIA(c) receptors revealed an approximately 3-fold decrease in total CryIA(c) receptor numbers for resistant vesicles. The authors suggest that factors other than binding may be altered in their resistant diamondback moth strain.

Recent Developments in Immobilization of Biological Components

Optimum biosensor design requires effective immobilization strategies which can be used to prepare a thin film of bio-molecules on the transducer surface in a reproducible manner. Three approaches being followed include the use of: (a) physical immobilization methods; (b) chemical binding, or (c) *in situ* synthesis of films or polymers on the biosensor surface (self-assembly). Previously reported physical methods of enzyme immobilization include adsorption (49) and gel entrapment (50)

which provide relatively small perturbation of the protein native structure and function. However, physical adsorption alone is not a very effective immobilization method and leads to very poor stability of the biosensor. Gel entrapment method suffers from long response times due to diffusional limitations encountered by substrates and products. Additional membrane layers may be necessary to prevent fouling and interference (51) which can further increase the diffusional resistance and leads to low sensitivity and poor lower detection limit. Recently, many reports have appeared on the use of two anion exchange polymers, Nafion, and Eastman AQ, which combine enzyme retention with separation of anionic interfering species such as ascorbic and uric acids (52). These polymers can be used to cast membranes directly on the surface providing a layer thinner than with conventional precast membranes.

An effective technique for immobilization of enzymes is the use of nanometer thin electrochemically prepared polymer films of various compounds such as pyrrole, thiophene, phenylenediamine, phenol etc. (53–55). For immobilization in such films, the enzyme is either (i) pre-deposited on the working electrode surface by adsorption or cross-linking and then placed in a monomer solution under suitable conditions or (ii) dissolved in the monomer solution. Polymerization is started by applying potential in an electrochemical cell with the surface to be coated being the working electrode in the electrochemical cell as well, in amperometric biosensors, the electrochemical polymer film can be used to incorporate the redox mediator (56–58).

Chemical methods of enzyme immobilization include covalent binding and cross-linking using various multi-functional reagents, e.g., glutaraldehyde, cyanuric chloride (59,60). The enzyme may be covalently attached or cross-linked to the biosensor surface itself or to commercially available hydrophilic membrane (e.g., Immunodyne from Pall BioSupport, New York; Immobilon from Millipore, Massachusetts, U.S.A.) which then can be placed on the sensing area of the transducer. The latter technique allows for ease of replacement of the enzyme membrane thus extending the operational life of the biosensor. Similar techniques as described for enzyme immobilization can be used for immobilization of antibodies.

Sol-Gel Techniques

Silicate glasses obtained by the sol-gel method provide a host matrix where bio-molecules can retain their functional characteristics. Dave *et al.* (61) have reviewed this method of bio-immobilization. The prepared films may be optically transparent thus allowing the use of fibre-optic transducers. The silicate matrix is usually formed by hydrolysis of an alkoxide precursor followed by condensation to yield a polymeric oxo-bridged SiO_2 network. The silicate monoliths can be used to entrap protein molecules such as enzymes. A number of applications are discussed by the authors including the determination of dissolved oxygen with haemoglobin and myoglobin, and the determination of nitric oxide, glucose, oxalate etc. Such biogel based biosensors are promising because the method of immobilization is simple

and the host matrix is chemically inert. Other advantages of this technique include ease of processing of glass materials, and facility of optical transduction. The reduced degrees of freedom for the confined bio-molecule in the matrix can also allow a one to one correspondence with the analyte.

Entrapment in Liposomes

Liposomes are formed by sonication of phospholipids or other suitable lipids in aqueous solutions (containing the biocatalyst). The lipid double layer forms closed vesicles which entrap the biocatalyst, in a soft, deformable, liquid like membrane. Several reports of biosensors based on this technique have appeared. Recently, Kim *et al.* (62) investigated a biosensor strategy for rapid amperometric detection of organisms in which a redox mediator was entrapped within liposomes. The selective release of mediator by haemolytic bacteria, followed by signal generation, confers differentiation between haemolytic and non-haemolytic species. The potential of this approach was illustrated by results for various strains of *Listeria monocytogenes*, *Listeria welshimeri* and *Escherichia coli*.

Suh *et al.* (63) described a nicotinic acetylcholine receptor (nAChR) isolated from the electric tissues of *Torpedo californica* which has been reconstituted into a vesicle comprising a bifunctional azo-ligand compound, and a liposome containing phospholipids and cholesterol (1:1, mass ratio). The liposome-mediated reconstituted receptor showed a concentration-dependent response to cholinergic drugs in a lithium ion flux assay. This liposome-mediated reconstituted nAChR was immobilized onto an electrode using various synthetic polymers which were tested for their response to the cholinergic ligands. The immobilized nAChR not only exhibited a linear response to a wide range of cholinergic ligand concentrations but also retained an operational stability which lasted for longer than 6 days. Thus, this result provides a basis for application of the immobilized nAChR-based biosensor in detecting cholinergic ligands *in vitro*.

Biosensors based on glucose oxidase (GOD) enzyme are already commercially available for the measurement of glucose in order to monitor diabetic patients. Due to limitations of enzyme saturation kinetics, the measurement of clinically relevant glucose concentrations is restricted to 0 to 25 mM. Recently Taylor *et al.* (64) have described a liposomal enzyme electrode whereby glucose oxidase was encapsulated within liposomes so that the lipid bilayer was the diffusion limiting membrane. It was shown that the electrode response was defined by the lipid constituents of the liposome, and that a linear response to glucose could be achieved up to 40 mM. Improved immobilization of liposomes is demonstrated by the authors with the use of poly-L-lysine solution. The variation in electrode response with respect to the amount of glucose oxidase liposomally encapsulated is reported. The authors suggested that the new method allows a greater number of sensors to be produced from a single batch of liposomes. One of the problems in this system is the bio-fouling effects of the lipid constituents of ruptured liposomes on the response of the electrode to glucose over time.

Photo-polymerization

Photo-assisted polymerization is another useful technique of bio-component immobilization on the biosensor surface. Quinn *et al.* (65) have described a glucose biosensor constructed by photo-polymerization of an immobilizing film on the biosensor surface. This technique is useful for producing biocompatible coatings on biosensors used for *in vivo* applications. A copolymer containing 88% 2-hydroxyethyl methacrylate (HEMA), 9% poly(ethylene glycol) ($M_r = 18500$) tetra-acrylate and 3% ethylene dimethacrylate was prepared and evaluated for use as a biocompatible interface between glucose biosensors and tissue in the rat. The glucose sensor utilizes glucose oxidase that is electrically 'wired' to a gold current collector by a reduction-oxidation polymer. Coatings of the copolymer were cross-linked *in situ* on the sensors using long wavelength ultraviolet light and 2,2-dimethoxy-2-phenyl-acetophenone as the initiator. The effect of these films on the current response to glucose was also measured. Copolymer-treated and control electrodes were implanted in the intrascapular subcutaneous tissue of male Sprague-Dawley rats for three days. The explanted samples were evaluated using scanning electron microscopy. The control electrodes were highly encapsulated with fibrous material, while the copolymer-treated electrodes induced much less encapsulation. The authors suggested that this co-polymer is a suitable candidate for a biocompatible coating for electrically wired oxidoreductase-based subcutaneous biosensors.

Lipid Films

The incorporation of antibody in lipid films has also been investigated for biosensor preparation (66). IgG was incorporated into lipid monolayers prepared from 7:3 mixtures of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidic acid. The antibody was found to penetrate the fluid region of the mixed-phase system when monolayers were held at low pressure. It was found that there is an »exclusion pressure« observed in pressure-area curves that are collected for monolayers that contain antibody. This term refers to a specific threshold of lateral pressure (which is reached by monolayer compression) that can cause expulsion of antibody from the interior of a membrane. Microscopic images of monolayers containing the fluorescent phospholipid nitrobenzoxadiazole dipalmitoyl phosphatidylethanolamine (NBD-PE), or antibody labelled with tetramethylrhodamine isothiocyanate (TRITC), were used to determine the structure of membranes, and the location of effects on structure caused by IgG. Ellipsometric measurements of lipid monolayers that were cast onto silicon wafers by the Langmuir-Blodgett method were used to study the thickness of monolayers and to investigate the structural changes that occurred at the »exclusion pressure.« Both the use of fluorescent antigen and ellipsometry indicated that antibody binding activity was present and was dependent on compression pressure. The effects of pH and ionic strength of sub-phase, antibody concentration, incubation time, and lateral pressure have been examined. The authors suggested that results may indicate the conditions that can be used

to improve the incorporation of active IgG for preparation of biosensors that are based on lipid membranes.

Applications of Biosensors in On-line Monitoring

Biosensors Based on Conductometric Transducers

Conductometric sensors utilize changes in conductivity which can occur due to the interactions with the analyte. A disadvantage of such biosensors is the poor signal-to-noise ratio at low levels of the analyte. Previously, some types of conductometric biosensors have been reported (1,4). Recently, the quantification of ethanol in alcoholic beverages was performed by Korpan *et al.* (67) using a yeast cell-based conductometric biosensor. A membrane with yeast cells immobilized in 2% Ca-alginate gel was attached on gold planar electrodes. Changes in conductivity due to the specific consumption of ethanol by yeast cells were registered by the computer-controlled sensor system. The response time of the constructed microbial sensor was less than 5 min, linearity (in a logarithmic scale) was observed in the range of 5–100 mM alcohol concentration. It was established that pH value in the region from 5 to 8 did not influence the level of initial signal. The increase of a buffer capacity in the sample results in the decrease of the biosensor output. The minimal detectable level of ethanol was 1 mM and the relative standard deviation appeared to be 10–12% for 15 repeated assays. When the system was operated and stored at 20–25 °C, the biosensor response was stable for only 3 days. However, when the microbial sensor was stored at 4 °C, the system was stable up to 12 days. Good correlation between the results obtained by a conductometric cell-biosensor and gas chromatograph was observed.

Biosensors Based on Potentiometric Transducers

A potentiometric biosensor is an ion-selective or gas-sensitive electrode, e.g., pH, ammonium, fluoride, CO₂ electrode, coated usually with an immobilized enzyme layer. The enzyme catalyzed reaction with the substrate generates a potential change which can be logarithmically related to the analyte concentration. Ion-selective field effect transistors (ISFETs) are an improvement in potentiometric biosensor technology. The enzyme based field effect transistor (ENFET), which consists of an enzyme layer coated on an ion-selective FET, displays several advantages over the ion-selective electrode. It has a small compact size and requires only minute quantity of enzyme. The immobilized enzyme layer thickness can be more easily controlled and no retaining membranes are required (68). In another type of FET, the pF-FET, 4-fluoroaniline and oxidases (e.g. glucose oxidase) are combined to form a new type of biosensor. H₂O₂ produced by oxidases during the enzymatic conversion reacts with 4-fluoroaniline to generate F⁻ ions which are detected (69,70).

Several potentiometric biosensor systems have been reported for on-line bioprocess monitoring (71–73). More recently, Park *et al.* (74) described a potentiometric microbial sensor for the determination of carbohydrates.

Cells of *Zymomonas mobilis* possessing glucose-fructose oxidoreductase and gluconolactonase were permeabilized with toluene and co-immobilized with invertase within a gelatin membrane. This membrane was coated over a pH electrode, and the resulting biosensor was placed in a flow-through cell to develop a flow injection analysis (FIA) system for the specific determination of glucose, fructose, and sucrose. Peak height resulting from the production of hydrogen ion was correlated with sugar concentration, and the effect of operating variables on the response characteristics of the FIA system was investigated on the basis of theoretical and experimental analyses. Under the optimized conditions, the calibration curves for glucose, fructose, and sucrose were linear up to 8, 80, and 60 g/L, respectively. The FIA system was applied to the on-line monitoring of glucose production in the enzymatic hydrolysis of cellulose, and the glucose concentrations determined using the FIA system coincided well with those determined by the conventional enzymatic methods.

Biosensor Based on Capacitance Changes

Another novel concept is the development of a biosensor based on the measurement of capacitance changes (75). This biosensor was constructed by coating a silicon based three layer structure, the electrolyte insulator semiconductor (EIS) chip, with penicillinase, urease or glucose oxidase. Either pH sensitive (Si/SiO₂/Si₃N₄/Ta₂O₅) or pF sensitive (Si/SiO₂/Si₃N₄/LaF₃) layer structures were prepared which could be used for the selective detection of either H⁺ or F⁻ ions. Due to a change in the surface potential on the ion sensitive (Ta₂O₅ or LaF₃) layers by influence of H⁺ or F⁻ ions a C/V shift resulted which was proportional to the detected ion concentration. These sensors were used in a FIA set-up for the continual and on-line detection of the appropriate substances. The authors reported that the fluorine based biosensors offered the advantage of independence with regard to buffer capacity of the solution.

Biosensors Based on Amperometric Transducers

A large majority of biosensors based on the amperometric enzyme electrodes have been developed. The popularity of these types of biosensors is due to the availability of a large number of oxido-reductase enzymes which can act on fatty acids, sugars, amino acids, aldehydes, phenols, etc. The first type of amperometric biosensors developed were based on the detection of hydrogen peroxide production or oxygen consumption during the enzyme catalysis of the analyte at the electrode surface. The disadvantages associated with these biosensors were dissolved oxygen fluctuations, and interferences from the non-specific electrochemical oxidation of many compounds, e.g., ascorbate, uric acid, glutathione at the potentials used. These disadvantages were overcome in chemically modified electrodes with low molecular weight mediators such as tetrathiafulvalene (TTF), tetracyanoquinodimethane (TCNQ), hexacyanoferrate (III), organic dyes such as methylene blue, ferrocene (*bis*(h⁵-cyclopentadienyl)iron), quinones, *N*-methylphenazin-5-ium (NMP⁺) acting as electron shuttles at the electrode surface (76). Such mediators can be

Table 1. Environmental pollutants of concern

Name	Concern
Non-metals: arsenic, selenium	Carcinogen, mutagen
Metals: barium, cadmium, chromium, lead, mercury, silver	Toxic to highly toxic depending on exposure, long term and short term toxicities
Organics: benzene, ethyl benzene, toluene	Carcinogenic, toxic
Halogenated compounds: chloro-benzene, chloroethane, dichloro-methane, tetrachloroethene	Toxic to extremely toxic, carcinogens
Pesticides: Endrin, Lindane, Methoxychlor, Toxaphene, Silvex	Toxic by inhalation, skin absorption or ingestion
Other chemicals: phenolics, ammonia, formaldehyde	Toxic
Polychlorinated biphenyls (PCBs)	Toxic, carcinogens
Pentachlorophenol (PCP)	Toxic, carcinogen
Toxins, pathogens, viruses	Infectious agents
Nitrite/ Nitrates	Carcinogens

incorporated into the electrode in various ways: (a) chemisorption of mediator using a mediator-organic solvent solution which is allowed to evaporate (1); (b) entrapment in or behind a polymer film at the electrode surface (58); (c) covalent binding to monomers or polymers which can be deposited on the electrode surface (56,77); (d) mixing in the paste in carbon paste electrodes which have a paste of graphite and mineral oil (78) or (e) in polymer chains at the electrode surface (79,80).

Several amperometric biosensors for bioprocess monitoring have been reported which are based on oxygen depletion, hydrogen peroxide production or on mediated electrodes (81–96). One of the limitations of biosensors is the lack of a suitable on-line calibration procedure since enzyme activity decays with time on-stream. Kyrolainen *et al.* (97) describe an on-line calibration procedure of a biosensor applicable to continuous

monitoring systems. Control of the calibration value and recalibration on-line during monitoring is possible without having to disrupt the sample withdrawal. The calibration procedure has been applied and evaluated in a continuous biosensor system based on the detection of oxygen depletion during enzymatic substrate conversion by immobilized oxidases. The sensor evaluation also included on-line calibration during continuous measurements of glucose and lactate in bovine blood samples. Calibration of the complete system consisting of a sampling device, a sample handling step, a biocatalytic step, a detection step, and a data processing unit is performed by the on-line addition of a calibration solution to a blank sample which is fed through the system. The calibration cycle is completed within 5.5 min. When recalibration is carried out during monitoring, the calibration solution is added to the sample, instead of to a blank sample, and the increase in outlet signal is registered. The major advantage of this internal standard principle is that the calibration solution is fed through the whole system according to the same path as the sample solution and thus takes into account all parameters influencing the sample.

A regenerable glucose biosensor for *in situ* applications is described by Phelps *et al.* (98). The biosensor is based on the reversible immobilization of glucose oxidase (GOD) using cellulose binding domain (CBD) technology. GOD, chemically linked to CBD, is immobilized by binding to a cellulose matrix on the sensor-indicating electrode. Enzyme immobilization can be reversed by perfusing the cellulose matrix with a suitable eluting solution. An autoclavable sensor membrane system is employed which is shown to be practical for use in real microbial fermentations. The prototype glucose biosensor was used without failure or deterioration during fed-batch fermentations of *Escherichia coli* up to a maximum cell density of 85 g (dry weight)/L. Medium glucose concentration based on sensor output correlated closely with off-line glucose analysis and was controlled manually at (0.44 ± 0.2) g/L for 2 h based on glucose sensor output. The sensor enzyme component could be eluted and replaced without interrupting the fermentation.

Table 2. Commercially available biosensors and their manufacturers

Manufacturer	Analyte measured	Biosensor type	Response time/min	Stability
Omron Tateisi, Japan	Lactate	Amperometric, enzyme based, hydrogen peroxide electrode	1–2	ND
Provesta Corp., U.S.A.	Glucose, Lactate, Lactose, Alcohol	Amperometric, enzyme based, oxygen electrode	4–5	ND
Seres, France	Glucose, Lactate, Lysine	Amperometric, enzyme based, oxygen electrode	2	ND
Setric, France	L-Lactate	Amperometric, enzyme based, mediated electrode	1–2	up to 200 assays
TOA Electronics, Japan	Glucose	Amperometric, enzyme based, hydrogen peroxide electrode	6	ND
Universal Sensors, U.S.A.	Glucose	Amperometric, enzyme based, hydrogen peroxide electrode	1–2	up to 500 assays
YSI, U.S.A.	Glucose, Sucrose, Alcohol, L-Lactate, Lactose	Amperometric, enzyme based, hydrogen peroxide electrode	1–3	up to 3 weeks
ZWG, Germany	Glucose, Uric acid	Amperometric, enzyme based	1–2	1000 assays

ND = not determined

Luong *et al.* (99) have described the on-line monitoring of glucose oxidase enzyme production using an amperometric mediated biosensor based on a water-soluble 1,1'-dimethylferrocene (DMF):2-hydroxypropyl- β -cyclodextrin complex (DMF⁺). This mediator was superior to other soluble ferrocenes, notably carboxyferrocene, in terms of both solubility (110 mM *vs.* 0.5 mM) and oxidation potential (150 mV *vs.* 300 mV against Ag/AgCl). Although the cyclic voltammogram of DMF⁺ was electrochemically equivalent to DMF, the use of the former resulted in a significantly lower background current (10 nA *vs.* 30 nA). Because of its higher solubility, concentrated stock solutions of DMF⁺ can be prepared and supplied to the electrode. A linear response of current *versus* units of glucose oxidase (GOD) was obtained up to 0.5 unit/mL. The detection limit was estimated to be 0.03 unit/mL and the response time was 2.5 min or less. The amperometric system was used successfully to follow the GOD activity during the growth of *Aspergillus niger*. The results obtained correlated well with a standard absorbance-based assay using dichlorophenol-indophenol (DCPIP).

Another report describes the on-line monitoring of D-lactic acid production by *Lactobacillus delbrueckii* ATCC 9649. The biosensor was based on a D-lactate dehydrogenase modified carbon paste electrode in a flow injection system integrated with a filtration sampling device (100). The time delay between sampling and detection was approximately 6 min. The use of an electro-polymerized *o*-phenylenediamine membrane on the electrode resulted in a very selective sensor response with acceptable stability and sensitivity. The D-lactate concentrations determined on-line agreed well with those determined by a standard method, suggesting that this sensor system is suitable for on-line monitoring of fermentation processes.

Amperometric biosensors based on rhodinated carbon electrodes have been reported for monitoring of glucose, glutamine and glutamate (101). The sensors were incorporated into a three cell parallel FIA system and used to monitor the three analytes on-line during two mammalian cell perfusion cultures. All measurements were made simultaneously from undiluted media sample. Use of the FIA system enabled easy and rapid exchange of the sensors, during cultivation. The inclusion of a calibration step, regularly for all sensors, helped to maintain the accuracy of all measurements. Comparison with off-line measurements indicated that all three biosensors operated successfully, providing accurate information.

Biosensors Based on Thermal Transducers

Thermal enzyme sensors are based on the principle that the heat evolved in an enzymatic reaction can be utilized to calorimetrically determine the amount of substrate reacted (102,103). Thus thermometric indicators or transducers require only a single reaction step producing sufficient or measurable heat. Molar enthalpies of enzyme catalyzed reactions range from < 5 to 100 kJ/mol. In thermal enzyme sensors, the enzyme is directly attached to the temperature transducer, a thermistor, either by cross-linking or by entrapping the enzyme in a membrane enclosing the thermistor (104). Alternatively, the

enzyme is placed in a column at controlled temperatures and a thermistor is placed at the outlet of the column. As the sample flows through the column of immobilized enzyme, the heat of reaction is measured by the increase in temperature between the inlet and outlet of the column. In such systems, temperature changes down to 10⁻⁴ °C can be measured. The main disadvantage of such systems are (a) non-specific thermal effects and (b) a drift in baseline due to heating up of the unit. In order to overcome the problem of non-specific thermal effects, in most cases the sample flow is split with one part being pumped through an enzyme-free column with thermistors. The relevant signal from the enzyme-free column is subtracted from that obtained from the channel containing the enzyme column. The problem of drift is overcome by installing the complete unit in a thermostatically-controlled aluminum block. These additional requirements increase the cost of the system. Thermal biosensors for on-line process monitoring have been reported by several investigators (105–109). Recently, Rank *et al.* (110) describe a prototype of a split-flow modified thermal biosensor to monitor industrial fermentations. Acetaldehyde and glycerol in the extracellular broth were monitored over the first 48 h of fed-batch fermentations. The aim was to determine the usefulness of these secondary metabolites for on-line monitoring and control. When fermentation of the 13–16 g/L batch sugar was monitored, using immobilized aldehyde dehydrogenase, the acetaldehyde reached a peak value of 0.3 g/L. With immobilized alcohol oxidase a much larger peak of 3.5 g/L ethanol was seen immediately after the acetaldehyde peak. When glycerokinase was used a delayed peak of 1 g/L glycerol was monitored. Of the three metabolites monitored, the ethanol proved the most valuable indicator of suitable timing for the start of the feeding phase and later for controlling and preventing overfeed using the on-line biosensor system.

Biosensors Based on Fibre-Optics

In biosensors based on optical methods, the change in optical properties such as UV/VIS absorption, bio- and chemi-luminescence, reflectance and fluorescence brought by the interaction of the biocatalyst with the target analyte is monitored optically. Fibre-optic based biosensors offer advantages of compactness, flexibility, resistance to electrical noise, and a small probe size. Several applications of these biosensors for on-line process monitoring have been described (111–121). Shear *et al.* (122) have demonstrated a new biosensor system based on the response of living cells that can detect specific components of a complex mixture fractionated by a micro column separation technique. This system uses ligand-receptor binding and signal-transduction pathways to biochemically amplify the presence of an analyte after electrophoretic separation. The transduced signal was measured by means of two approaches: (i) fluorescence determination of intracellular calcium concentrations in one or more rat PC-1 2 cells and (ii) measurement of trans-membrane current in a *Xenopus laevis* oocyte micro injected with messenger RNA that encodes a specific receptor. This analysis system has the potential to identify biologically active ligands present in a complex mixture with exceptional sensitivity and selectivity.

Biosensors Based on Surface Acoustic Wave/Piezoelectric Transducers

Piezoelectric transducers provide the advantages of conceptual simplicity, chemical inertness, ruggedness and low cost. Since the sensitivity of piezoelectric transducers is based on mass per unit area, this readily permits miniaturization without loss in sensitivity. Piezoelectric sensors and biosensors involve the modification of the transducer with a coating which interacts with the analyte in a specific way. The most common format has been the use of the quartz crystal microbalance (QCM). The earliest examples included the detection of hydrocarbons using QCMs coated with chromatographic substrates, instruments for moisture analysis and particle detection. Detection systems for gaseous pollutants (e.g., ammonia, formaldehyde, hydrogen sulfide, ozone, sulfur dioxide and mercury) were also developed.

Yao *et al.* (123) describe a new biosensor system based on a conductance-surface acoustic wave (SAW) frequency response for the determination of glutamine. A SAW resonator oscillating at 61 MHz and a pair of parallel electrodes were used in this system. Glutaminase from porcine kidney or *Escherichia coli*form was used as a biocatalyst for the hydrolysis of glutamine. There was an excellent linear relationship between the frequency shift response and the glutamine concentration in the range of $6.8 \cdot 10^{-4}$ M to $6.8 \cdot 10^{-2}$ M. The SAW frequency responses obtained from the proposed biosensor system were compared with the conductance response.

Biosensors for Environmental Pollutants

The broad range of environmental pollutants of concern, shown in Table 1, represent many unique opportunities as well as technical obstacles for the development of novel and effective biosensors. Most types of biosensors for environmental pollutants including toxicity sensors are based on a proportional reversible inhibition of activity on exposure to the analyte. If the inhibition is irreversible, renewal of the biological sensing layer will be required. Other biosensors are based on changes in respiration rates of immobilized bacteria, or on light emission by recombinant photo-bacteria. Some recent reports on biosensors for pesticides, xenobiotics, phenolics, toxins and heavy metals are discussed below.

Pesticide Monitoring

Imato and Ishibashi (124) describe a highly sensitive potentiometric butyrylcholine (BuCh) sensor based on plasticized poly(vinyl chloride) membrane fabricated with (3,5-bis(2-methoxy-hexafluoro-methyl) phenyl) borate (HFPB) as a cation exchanger. The sensor showed a Nernstian response to BuCh in the 10^{-1} M to 10^{-6} M range. The higher hydrophobicity of the HFPB cation exchanger provided improved potential stability, lifetime and detection limit to the BuCh-sensor. The sensor was not affected by interference from choline, a product of the enzymatic reaction. Determinations of organophosphate pesticides, 2,2-dichloro-vinyl dimethyl phosphate and *O*-(4-bromo-2-chlorophenyl) *O*-ethyl-S-propyl-phos-

phothiolate) were conducted by measuring inhibition of enzyme activity. The proportional decrease in the enzymatic reaction was related to the concentration of pesticides. The authors reported that pesticides were successfully determined at micromol and subnanomolar levels by the BuCh-sensor.

Ivnickii and Rishpon (125) have developed a solid-state acetylcholinesterase (AChE) sensor on the basis of AChE acetylthiocholine-hexacyanoferrate(III) reaction. Various properties of the sensor were investigated and possibilities for its practical use in the detection and assay of pesticides are suggested. The response of the sensor was induced by two phenomena: the Donnan effect and the redox potential. Unlike pH-sensitive sensors, this type of sensor is operated at high ionic strength and a high buffer capacity. The ability of the AChE sensor to assay pesticides was demonstrated by the characterization and quantitative determination of dichlorvos based on its inhibition of AChE. The kinetics of inhibition by dichlorvos of the reaction with AChE were studied by potentiometric and photometric methods. The bimolecular reaction constant was $7.07 \cdot 10^5$ M⁻¹ min⁻¹ and the affinity constant was $4.76 \cdot 10^6$ M. The detection limits for determination of the pesticide dichlorvos were 1 μM for a flow-injection mode and 0.2 μM for stationary measurement. The AChE acetylthiocholine-hexacyanoferrate(III) system opens up possibilities for the detection and assay of toxic chemicals by the use not only potentiometric but also of other types of sensors.

Xenobiotics

The detection of polychlorinated biphenyls (PCBs) has been carried out using microbial sensors based on *Alcaligenes eutrohus* H 850, *Corynebacterium* NB 1 and *Pseudomonas putida* LB 400, for details see (35). All the microbial sensors were sensitive to more than one PCB. The cells were immobilized in a non-polar membrane. Changes in the metabolic respiration rates were related to PCB concentrations. The dissolution of the aromatics in a suitable organic solvent led to an improved response of the respiratory biosensor to non-polar substrates.

Phenolic Compounds

Biocomposite (graphite-epoxy) electrodes whose surfaces can be renewed by polishing are useful where irreversible inactivation of the biosensor occurs. The characterization and analytical performance of a tyrosinase graphite-epoxy electrode for the detection of phenolic compounds are described by Oennerfjord *et al.* (126). The biocomposite configuration is based on the entrapment of commercially available tyrosinase in a graphite-epoxy matrix, and the mixing of the resulting conductive epoxy resin with a hardener. The enzyme electrode is mounted as a working electrode in an amperometric flow cell of the confined wall-jet type and studied in the flow injection mode. The bio-probe is electrochemically characterized by hydrodynamic and cyclic voltammetry for catechol and phenol. An applied potential of -100 mV *vs.* Ag/AgCl is found to be optimal for electrochemical reduction of the enzyme products (quinone forms) for the biocomposite electrode. The dependence of the response of the biocomposite on the flow

rate, the amount of loaded enzyme, the buffer composition, pH, and oxygen is investigated. The response of the biosensor to different phenolic compounds is also evaluated. The limits of detection for phenol and catechol have been reported by the authors to be 1.0 μM and 0.04 μM , respectively. No loss in response could be detected after 100 injections of catechol. Stability of the biocomposite was found to depend on storage conditions.

Riedel *et al.* (127) describe an amperometric biosensor for the determination of phenol and chlorophenols using *Trichosporon beigeli* (*cutaneum*). This sensor is more sensitive to chlorophenols, especially to mono- and dichlorinated phenols, than to phenol and is insensitive to benzoate. A linear relationship between the current range and the concentration of 4-chlorophenol is observed up to 40 μM . The detection limit for all substrates studied is 2 μM . The current difference is reproducible within 5.5% when the test solution contains 0.40 μmol 4-chlorophenol.

Ethanol Vapor

The release of volatiles into the environment is another area of significant concern. Biosensors are required for measuring concentrations in the gaseous phase. Park *et al.* (128) describe an amperometric biosensor fabricated on a flexible polyester substrate using thick-film technology. This biosensor was developed for the measurement of breath alcohol, however the technology may be adapted for other volatiles as well. Conventional screen-printing technology was employed to apply alcohol dehydrogenase to the carbon working electrode. For the activation, the sensor was either exposed to a humidified gas or prehydrated by dipping in a buffer solution. The latter method was found to be more effective than the former. A good linear response to ethanol vapor has been obtained. It was demonstrated that the amperometric thick-film biosensor can satisfactorily be used for the disposable determination of breath alcohol.

Heavy Metals

Kong *et al.* (129) have reviewed the general area of heavy metal toxicity testing in environmental samples. The toxicity of heavy metals in the environment depends on a number of physico-chemical and biological factors. Bioassays based on toxicity to selected species of fish and daphnia are commonly used for regulatory testing. However, they are time consuming (48 h or more) and pose problems for rapid screening of samples. Microbial and biochemical assays based on the inhibition of bioluminescence, enzyme activity, enzyme biosynthesis, growth, respiration, and heat production are typically faster and less expensive than the traditional fish bioassays. Some of these tests approach or equal the sensitivity of daphnias to heavy metals. Since the soil acts as a sink for airborne and waste-applied metals, the uptake of metals by plants and the associated toxic impacts are important. Growth inhibition, enzyme induction, and production of stress proteins have been considered as toxicity end points. Enzymatic tests have been developed that are specific for heavy metal toxicity. Such tests can facilitate toxicity reduction evaluations. Detection of individual metals in the environment may eventually be

possible using biosensors consisting of genetically engineered microorganisms. Direct solid-phase tests for soil, sediment, or sludge toxicity, using bacterial bioluminescence or enzyme activity as end points, have been developed. Such tests may complement traditional solid-phase toxicity tests using nematodes or earthworms as indicator organisms. Biosensors, using genetically engineered microorganisms, offer an elegant means of detecting the presence of specific heavy metals in environmental samples. Biosensors have been developed which are based on the fusion of genes induced by metals to a reporter system, the *lux* operon of *Vibrio fischeri* (130). Bacterial luciferases produce light through the oxidation of fatty aldehydes. The gene fusions are useful for the determination of heavy metal concentrations in the environment.

Collard *et al.* (131) describe the use of *Alcaligenes eutrophus* CH34 bacteria which have a potential for such applications. This microorganism is the main representative of a group of mostly facultative chemolithotrophs that are well adapted to environments containing high levels of heavy metals. It harbors the mega-plasmids pMOL28 and pMOL30 which carry resistance determinants to Co^{2+} , Ni^{2+} , CrO_4^{2-} , Hg^{2+} , Tl^+ , Cd^{2+} , Cu^{2+} and Zn^{2+} . Among the best characterized determinants are the *cnr* operon (resistance to Co, Ni) on pMOL28 and the *czc* operon on pMOL30 (resistance to Co, Cd and Zn). Although the two systems reveal a significant degree of amino acid similarity in the structural genes, the regulation of the operons is different. The resistance mechanism in both cases is based on efflux. The efflux mechanism leads to a pH increase outside of the cytoplasmic membrane. Metals are sequestered from the external medium through the bio-precipitation of metal carbonates formed in the saturated zone around the cell. The bacteria can be immobilized on composite membranes in a continuous tubular membrane reactor (CTMR). The effluent continuously circulates through the inter-tubular space, while the external surface of the tubes is in contact with the growth medium. Metal crystals are eventually removed by the effluent stream and collected on a glass bead column. The system has been applied to effluents containing Cd, Zn, Co, Ni and Cu. By introducing catabolic plasmids involved in the aerobic degradation of PCBs and 2,4-D into metal-resistant *A. eutrophus* strains, the application range can be widened to include effluents polluted with both organic and inorganic substances.

Toxins

Some biosensors to monitor toxins have been reported (132). Type E botulinum neurotoxin *Clostridium botulinum* was detected using a fibre optic-based biosensor by complexing the toxin with its binding protein. The neurotoxin binding protein, $M_r = 118000$, helps protect the neurotoxin from adverse pH, temperature, and proteolytic conditions. The interaction was favoured at low pH as indicated by an initial binding rate of 8.4 min^{-1} at pH = 5.7 compared to 4.0 min^{-1} at pH = 7.5 as determined.

A novel cyanide biosensor has been developed using cyanide-degrading microorganisms immobilized in membrane on an oxygen electrode (133). Immobilized

Pseudomonas fluorescens NCIMB 11764 specifically oxidized cyanide thereby consuming oxygen without reacting with other toxic materials (like Cr^{3+} , Cd^{2+} , Pb^{2+}). A change in oxygen concentration was detected using a Clark oxygen electrode. Cyanide could thus be determined at concentrations between 0.1 and 1 ppm in 50 mM phosphate buffer (pH = 8). The optimum working conditions of the sensor were pH = 8 at 30 °C, resulting in a response time of less than 2 min. This sensor was unaffected by chloride and heavy-metal ions. The relative standard deviation was estimated to be about 8%. With its specificity, rapid response time and ease of operation, this cyanide sensor has a potential commercial application for the monitoring and sensing of cyanide in industrial wastes.

A new, portable fibre-optic biosensor to detect staphylococcal enterotoxin B (SEE), a causative agent of food poisoning, has been described by Tempelman *et al.* (134). The toxin can be detected at levels as low as 0.5 ng/mL in buffer and quantitated in other relevant media: human serum, urine, and aqueous extract of ham. The level of toxin, from 5 to 200 ng/mL, can be accurately predicted in these media by calibrating each fibre and by comparing results to a single standard curve based on toxin in buffer. The quantitative fluorescent sandwich immunoassay provides results in 45 min; while qualitative results are provided in 15–20 min. Using a blender and a benchtop centrifuge, fast, simple aqueous extracts of contaminated ham samples were prepared and tested. Ham spiked with 5 or 40 µg SEE per 100 g food resulted in biosensor readings indicative of 11 or 69% recovery of the toxin, respectively. Finally, the SEE assay is highly specific; this specific, sensitive assay for SEE on the portable fibre-optic biosensor could be used to monitor clinical samples or for on-site analysis of suspect food samples.

Drug Residues

The use of antibiotics and chemotherapeutics in animal husbandry has led to the occurrence of veterinary drug residues in all types of food of animal origin. Due to the specification of toxicologically based maximum residue levels for a large number of substances, existing control strategies need even faster and more sensitive methods to meet new and more rigorous regulations (135). The applicability of an immuno-sensor device for biospecific interaction analysis was investigated by Sternesjo *et al.* (136) and the development of an assay for analysis of sulfamethazine (SMZ) in milk is described. SMZ was covalently immobilized to a carboxymethyl-dextran-modified gold film. Spiked samples with known concentrations of SMZ were prepared in HBS buffer and skim and raw milk for construction of standard curves. Polyclonal antibodies against SMZ were added to the sample and the immobilized surface was used to determine the amount of free antibodies by surface plasmon resonance detection. After each measurement the surface was regenerated by NaOH and HCl. In milk, the mean relative standard deviation of the assay was approximately 2% and the limit of detection less than 1 ppb. By introduction of a secondary sheep anti-rabbit antibody, the use of specific antibody could be reduced. Milk samples from the individual cow, herd, and tanker levels

were analyzed and the relative standard deviations within each sample category were 4.4, 2.4, and 2.2%, respectively. The effect of some potential interferences, e.g., high somatic cells, bacterial contamination, and preservatives, was investigated. The results were not influenced in such a way that the risk for so-called false-positive findings was obvious.

Pathogens

A fibre optic biosensor was developed which was based on a 200-mer fragment of the *flaA* gene from *Listeria monocytogenes*. This fragment was amplified using the polymerase chain reaction (PCR) incorporating biotin- and fluorescein amidite (FAM-)labelled primers. Methods are described for isolating the single stranded FAM-labelled 200-mer. A central portion of this 200-mer was successfully hybridized onto a complementary sequence coated onto a fibre optic biosensor (137). Advantages in the specificity and speed of this approach compared to standard agarose gel electrophoresis and probing methods are discussed.

Cao *et al.* (138) have described a fibre optic biosensor that was used to detect the fraction 1 (F1) antigen from *Yersinia pestis*, the etiologic agent of plague. The instrument employs an argon ion laser (514 nm) to launch light into a long-clad fibre and measures the fluorescence produced by an immunofluorescent complex formed in the evanescent wave region. This sensing area is a short section (12.5 cm) at the end of the optical fibre from which the cladding has been removed and in which the silica core has been tapered. Capture antibodies, which bind to F1 antigen, were immobilized on the core surface to form the basis of the sandwich fluoro immunoassay. The ability to detect bound F1 antigen was provided by adding tetramethylrhodamine-labelled anti-plague antibody to form fluorescent complexes. The evanescent wave has a limited penetration depth, which restricts detection of the fluorescent complexes bound to the fibre's surface. The direct correlation between the F1 antigen concentration and the signal provided an effective method for sample quantization. This method achieved a high level of accuracy for determining F1 antigen concentrations from 50 to 400 ng/mL in phosphate-buffered saline, serum, plasma, and whole blood, with a 5 ng/mL limit of detection. Subsequent blind studies, which included serum samples from patients, yielded results in good agreement with measurements by enzyme-linked immuno-sorbent assay. A major advantage of the fibre optic biosensor is that results can be generated within minutes while isolating the user from hazardous samples. These factors favour development of this biosensor into a facile and rapid diagnostic device.

Gatto-Menking *et al.* (139) describe an optical sensor for the measurement of biotoxoids and bacterial spores. Extremely sensitive detection of various biotoxoids and bacterial spores using the commercial ORIGEN analyzer was achieved by capture on antibody-conjugated micron sized magnetic beads (MBs) followed by binding of ruthenium-(II)-trisbipyridal chelate labelled reporter antibodies. Immuno-magnetically captured target materials were collected on a magnet. Electrochemiluminescence (ECL) was evoked from the reporter antibodies by application of an electrical potential. Femtogram sensitivity

levels were obtained for all biotoxoids tested including botulinus A, cholera β subunit, ricin and staphylococcal enterotoxoid B by this immuno-magnetic (IM)-ECL approach. An IM-ECL assay for *Bacillus anthracis* spores yielded a detection limit of at least 100 spores. The ECL signal was a function of analyte quantity over several orders of magnitude, but the immunological 'hook' effect at high antigen loads made quantization impossible over a broader range. All assays were performed with a maximum combined incubation and assay time of approximately 40 min. This work demonstrates the extreme sensitivity of the IM-ECL approach for soluble and particulate antigens.

A biosensor to monitor the efficiency of a biological containment system has been described by Tedin *et al.* (140) who show that the lysis genes of different phage origin function in a variety of bacteria. They may therefore be suited for conditional suicide cassettes. The authors investigated whether the *Escherichia coli* *rrnB* P1 promoter could function as an environmentally responsive element sensing poor growth conditions expected after an accidental release of *E. coli* production strains from a bioreactor. Mimicking poor nutrient conditions by production of the alarmone guanosine tetraphosphate (ppGpp) with a plasmid encoded ppGpp synthetase 1, the *rrnB* P1 promoter activity was completely turned off. These experiments suggested that the *rrnB* P1 promoter may be used as an efficient biosensor for altered growth conditions. A concept for a conditional suicide system employing the *rrnB* P1 promoter and phage-derived lysis genes as key components is discussed by the authors.

Conclusions and Future Prospects

As the above review indicates, biosensor research today is filled with many challenges. Researchers must not lose sight of the «raison d'être» of the biosensor which is to provide something unique in its performance that is of benefit to the user. Table 2 indicates that mostly amperometric biosensors have been commercialized to-date. The lack of biosensors on the market is not attributable to the absence of fundamental techniques but to lack of technology to manufacture devices at an effective market cost. Two areas where biosensors can fill a niche are on-line, on-the spot monitoring for use in food, environmental and other industries, as well as in health care for near patient monitoring. Bioprocess monitoring and control applications require suitable integration of biosensors to bioreactors as accomplished using FIA. There is a strong need to develop control software for bioprocess optimization using biosensors. While the commercialization of biosensors for on-line process analysis and control is just starting, the day is not far, when biosensors will become as common as other currently available analytical devices and immuno-assay kits.

References

1. A. E. G. Cass: *Biosensors: A Practical Approach*, IRL Press, Oxford (1990).
2. J. V. Twork, A. M. Yacynch: *Sensors in Bioprocess Control*, Marcel-Dekker, New York (1990).
3. P. G. Edelman, J. Wang: *Biosensors and Chemical Sensors*, ACS Symposium ser. No. 487, Am. Chem. Soc. Washington, D.C. (1991).
4. E. A. H. Hall: *Biosensors*, Prentice-Hall, New Jersey (1991).
5. L. J. Blum, P. R. Coulet: *Biosensor Principles and Applications*, Marcel-Dekker, New York (1991).
6. I. Scheller, F. Schubert: *Biosensors*, Tech. Instrum. Anal. Chem. V. 11, Elsevier, New York (1992).
7. C. Tran-Minh: *Biosensors*, Chapman & Hall, London (1993).
8. G. G. Guilbault: *Analytical Uses of Immobilized Enzymes*, Marcel-Dekker, New York (1984).
9. J. R. North, *Trends Biotechnol.* 3 (1985) 180.
10. M. Aizawa, *Phil. Trans. R. Soc. Lon.* B316 (1987) 121.
11. M. J. Green, *Phil. Trans. R. Soc. Lon.* B316 (1987) 135.
12. R. F. Taylor, I. G., Marenchic, R. H. Spencer, *Anal. Chim. Acta*, 249 (1991) 67.
13. M. Nilsson, H. Hakanson, B. Mattiasson, *Anal. Chim. Acta*, 249 (1991) 163.
14. B. Mattiasson, H. Hakanson *Adv. Biochem. Eng. Biotechnol.* 46 (1992) 81.
15. K. Riedel, R. Renneberg, U. Wollenberg, G. Kaiser, F. W. Scheller, *J. Chem. Technol. Biotechnol.* 44 (1989) 85.
16. I. Karube, *J. Biotechnol.* 15 (1990) 255.
17. J. F. Kennedy, J. M. S. Cabral, *Appl. Biochem. Bioeng.* 4 (1983) 189.
18. H. W. Blanch, *Annu. Rep.* 7 (1984) 81.
19. I. Karube, K. Nakanishi, *IEEE Eng. Med. Biol.* June/July (1994) 364.
20. S.-O. Enfors, H. Nilsson, *Enzyme. Microb. Technol.* 1 (1979) 260.
21. R. Kok, P. Hogan, *Biosensors*, 3 (1987) 89.
22. N. Cleland, S.-O. Enfors, *Eur. J. Appl. Microbiol. Biotechnol.* 18 (1983) 141.
23. N. Cleland, S.-O. Enfors, *Anal. Chem.* 56 (1984) 1880.
24. J. Bradley, P. A. Anderson, A. M. Dear, R. E. Ashby, A. P. F. Turner: a. Glucose biosensors for the study and control of baker's yeast fermentation. In: *Computer applications in Fermentation Technology: Modelling and Control of Biotechnical Processes*, N. M. Fish, R. I. Fox, N. F. Thornhill (Eds.), Elsevier, London (1989) pp. 47–51.
25. J. Bradley, R. E. Ashby, A. P. F. Turner, R. D. Schmid: On-line fermentation monitoring with an amperometric glucose biosensor. In: *Dechema Biotechnology Conferences*, Vol. 3, VCH, Weinheim (1989) pp. 759–762.
26. J. Bradley, A. P. F. Turner, R. D. Schmid: An *in situ* fermenter probe for baker's yeast propagation monitoring. In: *GBF Monogr.: Biosensors Applications in Medicine, Environmental protection and process control*, Vol. 13, R. D. Schmid, F. Scheller (Eds.), VCH, Weinheim (1989) pp. 85–88.
27. C. Filippini, B. Sonnleitner, A. Fiechter, J. Bradley, R. D. Schmid, *J. Biotechnol.* 18 (1991) 153.
28. C. Riley, B. F. Rocks, R. A. Sherwood, *Talanta*, 31 (1984) 879.
29. J. Ruzicka, F. H. Hansen: *Flow Injection Analysis*, Wiley, New York, 2nd ed. (1989).
30. C. F. Mandenius, B. Danielsson, B. Mattiasson, *Anal. Chim. Acta*, 163 (1984) 135.
31. S.-O. Enfors, N. Cleland, *Methods Enzymol.* 137 (1988) 298.
32. K. R. Rogers, *Biosensors Bioelectronics*, 10 (1995) 533.
33. I. Karube, Y. Nomura, Y. Arikawa, *Trends Anal. Chem.* 14 (1995) 295.
34. A. I. Yaropolov, A. N. Kharbin, J. Emneus, G. Marko-Varga, L. Gorton, *Anal. Chim. Acta*, 308 (1995) 137.
35. B. Beyersdorf-Radeck, K. Riedel, B. Neumann, F. Scheller, R. D. Schmid, *GBF Monogr.: Gesellschaft für Biotechnologische Forschung mbH Monographs*, Vol. 17, F. Scheller, R. D. Schmid (Eds.), VCH, Weinheim, Germany (1992) p. 55.

36. D. H. Liu, A. F. Yin, K. Chen, K. Ge, L. H. Nie, S. Z. Yao, *Anal. Lett.* 28 (1995) 1323.
37. C. La Rosa, F. Pariente, L. Hernandez, E. Lorenzo, *Anal. Chim. Acta*, 308 (1995) 129.
38. I. Campanella, G. Favero, M. Tomassetti, *Science Tot. Environ.* 171 (1995) 227.
39. T. C. Tan, F. Li, K. G. Neoh, *Sens. Act. B10* (1993) 137.
40. C. K. Hyun, T. Eiichi, I. K. Toshifumi, K. Isao, *Biotechnol. Bioeng.* 41 (1993) 1107.
41. A. Pluckthum, *Biotechnology*, 9 (1991) 545.
42. J. Buchner, R. Rudolph, *Biotechnology*, 9 (1991) 157.
43. A. C. Malmberg, C. A. K. Borrebaeck, *J. Immunol. Methods*, 183 (1995) 7–13.
44. D. S. Holmes, S. K. Dubey, S. Gangolli, *Environ. Geochem. Health*, 16 (1995).
45. E. Kobatake, T. Niimi, T. Haruyama, Y. Ikariyama, M. Aizawa, *Biosensors Bioelectronics*, 10 (1995) 601.
46. D. A. Rouch, J. Parkhill, N. L. Brown, *J. Ind. Microbiol.* 14 (1995) 349.
47. E. Argese, C. Bettiol, A. Ghelli, R. Todeschini, P. Miana, *Environ. Toxicol. Chem.* 14 (1995) 363.
48. L. Masson, A. Mazza, R. Brousseau, B. Tabashnik, *J. Biol. Chem.* 270 (1995) 11887.
49. R. A. Messing, *Methods Enzymol.* 44 (1976) 148.
50. M. P. J. Kierstan, M. P. Coughlan: Immobilization of cells and enzymes by gel entrapment. In: *Immobilized Cells and Enzymes*, J. Woodward (Ed.), IRL Press, Oxford (1985) pp. 39–48.
51. P. W. Stoecker, A. M. Yacynych, *Selective Electrode Rev.* 12 (1990) 137.
52. L. Gorton, E. Csoregi, E. Dominguez, J. Emneus, G. Jonsson-Pettersson, G. Marko-Varga, B. Persson, *Anal. Chim. Acta*, 250 (1991) 203.
53. N. C. Foulds, C. R. Lowe, *J. Chem. Soc. Faraday Trans. 1*, 82 (1986) 1259.
54. P. N. Bartlett, P. Tebbutt, C. H. Tyrrell, *Anal. Chem.* 64 (1992) 138.
55. P. N. Bartlett, J. M. Cooper, *J. Electroanal. Chem.* 362 (1993) 1.
56. N. C. Foulds, C. R. Lowe, *Anal. Chem.* 60 (1988) 2473.
57. Y. Kajiya, H. Sugai, C. Iwakura, H. Yoneyama, *Anal. Chem.* 63 (1991) 49.
58. E. R. Reynolds, R. J. Geise, A. M. Yacynych: Electropolymerized films for the construction of ultramicrobiosensors and electron-mediated amperometric biosensors. In: *ACS Symp. Ser. No. 487*, Am. Chem. Soc. Washington D.C. (1992) pp. 186–200.
59. P. W. Carr, L. D. Bowers: *Immobilized Enzymes in Analytical Chemistry: Fundamentals and Applications*, Wiley and Sons, N.Y. (1980).
60. H. H. Weetall, *Appl. Biochem. Biotechnol.* 41 (1993) 157.
61. B. C. Dave, B. DuRouch, J. S. Valentine, J. I. Zink, *Anal. Chem.* 66 (1995) 1120A, 49.
62. H. J. Kim, H. P. Bennetto, M. A. Halablab, *Biotechnol. Tech.* 9 (1995) 389.
63. J. I. Suh, B. H. Paik, S. Z. Oh, J. H. Suh, K. S. Cho, Y. K. Paik, *J. Biochem. Mol. Biol.* 28 (1995) 155.
64. M. A. Taylor, M. N. Jones, P. M. Vadgama, S. P. J. Higson, *Biosensors Bioelectronics*, 10 (1995) 251.
65. C. P. Quinn, C. P. Pathak, A. Heller, J. A. Hubbell, *Biomaterials*, 16 (1995) 389.
66. H. Wang, J. D. Brennan, A. Gene, U. J. Krull, *Appl. Biochem. Biotechnol.* 53 (1995) 163.
67. Ya. I. Korpan, S. V. Dzyadevich, V. P. Zharova, A. V. El'skaya, *Ukrainskii Biokhimicheskii Zhurnal*, 66 (1994) 78.
68. U. Brand, L. Brandes, V. Koch, T. Kullik, B. Reinhardt, F. Ruther, T. Scheper, K. Schugerl, S. Wang, X. Wu, R. Ferretti, S. Prasad, D. Wilhelm, *Appl. Microbiol. Biotechnol.* 36 (1991) 167.
69. R. Hintsche, I. Dransfeld, F. Scheller, M. T. Pham, W. Hoffmann, J. Hueller, W. Moritz, *Biosensors Bioelectronics*, 5 (1990) 327.
70. M. H. Ho, *Sens. Act.* 15 (1988) 445.
71. M. E. Meyerhoff, M. Trojanowicz, B. O. Palsson, *Biotechnol. Bioeng.* 41 (1993) 961.
72. S. Mizutani, S. Iijima, M. Morikawa, K. Shimizu, M. Matsumoto, Y. Ogawa, R. Izumi, K. Matsumoto, T. Kobayashi, *J. Ferment. Technol.* 65 (1987) 325.
73. R. F. Taylor, I. G. Marenchic, R. H. Spencer, *Anal. Chim. Acta*, 249 (1991) 67.
74. J. K. Park, M. C. Shin, S. G. Lee, H. S. Kim, *Biotechnol. Prog.* 11 (1995) 58.
75. M. Beyer, C. Menzel, R. Quack, T. Scheper, K. Schugerl, W. Treichel, H. Voigt, M. Ullrich, R. Ferretti, *Biosensors Bioelectronics*, 9 (1994) 17.
76. S. A. Wring, J. P. Hart, *Analyst*, 117 (1992) 1215.
77. A. Heller, *J. Phys. Chem.* 96 (1992) 3579.
78. J. Wang, L. H. Wu, Z. Lu, R. Li, J. Sanchez, *Anal. Chim. Acta*, 228 (1990) 251.
79. W. Schuhmann, H. L. Schmidt, *Adv. Biosensors*, 2 (1992) 79.
80. W. Schuhmann, *Biosensors Bioelectronics*, 8 (1993) 191.
81. J. Rishpon, Y. Shabtai, I. Rosen, Y. Zibenberg, R. Tor, A. Freeman, *Biotechnol. Bioeng.* 35 (1990) 103.
82. S-O. Enfors, *Enzyme Microb. Technol.* 3 (1981) 29.
83. S-O. Enfors, *U.S. Patent* 4 374 013 (1983).
84. J. L. Romette, C. L. Cooney, *Anal. Lett.* 20 (1987) 1069.
85. K. Schugerl, L. Brandes, T. Dullau, K. Holzhauer-Rieger, U. Hubner, X. Wu, W. Zhou, *Anal. Chim. Acta*, 249 (1991) 87.
86. M. Hikuma, H. Obana, T. Yasuda, I. Karube, S. Suzuki, *Enzyme Microb. Technol.* 2 (1980) 234.
87. I. Karube, *J. Biotechnol.* 15 (1990) 255.
88. I. Queinnec, C. Destruhaut, J. B. Pourciel, G. Goma, *World J. Microbiol. Biotechnol.* 8 (1992) 7.
89. G. W. Luli, S. M. Schlasner, D. E. Ordaz, D. M. Mason, W. R. Strohl, *Biotechnol. Tech.* 1 (1987) 225.
90. O. Holst, H. Hakanson, A. Miyabayashi, B. Mattiasson, *Appl. Microbiol. Biotechnol.* 28 (1988) 32.
91. R. Renneberg, G. Trott-Kriegeskorte, M. Leitz, V. Jager, M. Pawlowa, G. Kaiser, U. Wollenberger, F. Schubert, R. Wagner, R. D. Schmid, F. W. Scheller, *J. Biotechnol.* 21 (1991) 173.
92. M. V. Cattaneo, J. H. T. Luong, *Biotechnol. Bioeng.* 41 (1993) 659.
93. K. B. Male, J. H. T. Luong, R. Tom, S. Mercille, *Enzyme Microb. Technol.* 15 (1993) 26.
94. M. E. Meyerhoff, M. Trojanowicz, B. O. Palsson, *Biotechnol. Bioeng.* 41 (1993) 961.
95. S. L. Brooks, R. E. Ashby, A. P. F. Turner, M. R. Calder, D. J. Clarke, *Biosensors*, 3 (1987/88) 45.
96. J. Bradley, R. D. Schmid, *Biosensors Bioelectronics*, 6 (1991) 669.
97. M. Kyrolainen, H. Hakanson, B. Mattiasson, *Biotechnol. Bioeng.* 45 (1995) 122.
98. M. R. Phelps, J. B. Hobbs, D. G. Kilburn, R. F. Turner, *Biotechnol. Bioeng.* 46 (1995) 514.
99. J. H. T. Luong, C. Masson, R. S. Brown, K. B. Male, A. Nguyen, *Biosensors Bioelectronics*, 9 (1994) 577.
100. H. C. Shu, L. Gorton, B. Persson, B. Mattiasson, *Biotechnol. Bioeng.* 46 (1995) 280.

101. S. F. White, A. P. F. Turner, U. Biltewski, J. Bradley, R. D. Schmid, *Biosensors Bioelectronics*, 10 (1995) 543.
102. B. Mattiasson, B. Danielsson, C. F. Mandenius, F. Winquist, *Ann. N.Y. Acad. Sci.* 369 (1981) 295.
103. C. F. Mandenius, B. Danielsson, *Methods Enzymol.* 137 (1988) 307.
104. G. G. Guilbault, B. Danielsson, C. F. Mandenius, K. Mosbach, *Anal. Chem.* 54 (1983) 1582.
105. B. Danielsson, B. Mattiasson, R. Karlsson, F. Winqvist, *Biotechnol. Bioeng.* 21 (1979) 1749.
106. B. Danielsson: Enzyme Thermistor Devices. In: *Biosensor Principles and Applications*, L. J. Blum, P. R. Coulet (Eds.), Marcel-Dekker, New York (1991) pp. 83–106.
107. H.-G. Hundeck, A. Sauerbrei, U. Hubner, T. Scheper, K. Schugerl, R. Koch, G. Antranikian, *Anal. Chim. Acta*, 238 (1990) 211.
108. M. Rank, J. Gram, B. Danielsson, *Anal. Chim. Acta*, 281 (1993) 521.
109. P. Bataillard, E. Steffgen, S. Haemmerli, A. Manz, H. M. Widmer, *Biosensors Bioelectronics*, 8 (1993) 89.
110. M. Rank, J. Gram, K. S. Nielsen, B. Danielsson, *Appl. Microbiol. Biotechnol.* 42 (1995) 813.
111. T. Scheper, C. Muller, K. D. Anders, F. Eberhardt, F. Plotz, C. Schelp, O. Thordsen, K. Schugerl, *Biosensors Bioelectronics*, 9 (1994) 73.
112. B. A. A. Dremmel, S.-Y. Li, R. D. Schmid, *Biosensors Bioelectronics*, 7 (1992) 133.
113. Y. L. Huang, S.-Y. Li, B. A. A. Dremmel, U. Bilitewski, R. D. Schmid, *J. Biotechnol.* 18 (1991) 161.
114. I. Ogbomo, R. Kittsteiner-Eberle, U. Englbrecht, U. Prinzing, J. Danzer, H.-L. Schmidt, *Anal. Chim. Acta*, 249 (1991) 137.
115. R. Polzius, F. F. Bier, V. Bilitewski, V. Jager, R. D. Schmid, *Biotechnol. Bioeng.* 42 (1993) 1287.
116. M. V. Cattaneo, J. H. T. Luong, *Biotechnol. Bioeng.* 41 (1993) 659.
117. R. D. Schmid, W. Kunnecke, *J. Biotechnol.* 14 (1990) 3.
118. T. Scheper, W. Brandes, C. Grau, H. G. Hundeck, B. Reinhardt, F. Ruther, F. Plota, C. Schelp, K. Schugerl, K. H. Schneider, B. Rehr, H. Sahm, *Anal. Chim. Acta*, 249 (1991) 25.
119. O. Thordsen, T. Scheper, B. Rehr, H. Sahm, *Biotechnol. Bioeng.* 42 (1993) 387.
120. M. Nilsson, H. Hakanson, B. Mattiasson, *Anal. Chim. Acta*, 249 (1991) 163.
121. S. M. Gautier, L. J. Blum, P. R. Coulet, *Biolumin. Chemilumin.* 5 (1990) 57.
122. J. B. Shear, H. A. Fishman, N. L. Allbritton, D. Garigan, R. N. Zare, R. Scheller, *Science*, 267 (5194) (1995) 74.
123. S. Yao, D. Liu, K. Ge, K. Chen, L. Nie, *Enzyme Microb. Technol.* 17 (1995) 413.
124. T. Imato, N. Ishibashi, *Biosensors Bioelectronics*, 10 (1995) 435.
125. D. M. Ivnikskii, J. Rishpon, *Biosensors Bioelectronics*, 9 (1994) 569.
126. P. Oennerfjord, J. Emneus, G. Marko-Varga, L. Gorton, F. Ortega, E. Dominguez, *Biosensors Bioelectronics*, 10 (1995) 607.
127. K. Riedel, B. Beyersdorf-Radeck, B. Neumann, F. Schaller, *Appl. Microbiol. Biotechnol.* 43 (1995) 7.
128. J. K. Park, H. J. Yee, S. T. Kim, *Biosensors Bioelectronics*, 10 (1995) 587.
129. I. C. Kong, G. Bitton, B. Koopman, K. H. Jung, *Rev. Environ. Contam. Toxicol.* 142 (1995) 119.
130. I. Karube, K. Nakanishi, *Curr. Opin. Biotechnol.* 5 (1994) 54.
131. J. M. Collard, P. Corbisier, L. Diels, Q. Dong, C. Jeanthon, M. Mergeay, S. Taghavi, D. van der Lelie, A. Willemotte, S. Wuertz, *FEMS Microbiol. Rev.* 14 (1994) 405.
132. B. R. Singh, J. Foley, C. Lafontaine, *J. Protein Chem.* 14 (1995) 7.
133. J. I. Lee, I. Karube, *Anal. Chim. Acta*, 313 (1995).
134. L. A. Tempelman, K. D. King, G. P. Anderson, F. S. Ligler, *Anal. Biochem.* 233 (1996) 570.
135. G. W. Harwood, C. W. Pouton, *Adv. Drug Del. Rev.* 18 (1996) 163.
136. A. Sternesjo, C. Mellgren, L. Bjorck, *Anal. Biochem.* 226 (1995) 175.
137. N. J. C. Strachan, D. I. Gray, *Lett. Appl. Microbiol.* 21 (1995) 5.
138. L. K. Cao, G. P. Anderson, F. S. Ligler, J. Ezzell, *J. Clin. Microbiol.* 33 (1995) 336.
139. D. L. Gatto-Menking, H. Yu, J. G. Bruno, M. T. Goode, M. Miller, A. W. Zulich, *Biosensors Bioelectronics*, 10 (1995) 501.
140. K. Tedin, A. Witte, G. Reisinger, W. Lubitz, U. Blaes, *J. Biotechnol.* 39 (1995) 137.

Biosenzori za motrenje okoliša te motrenje i vođenje bioprocasa

Sažetak

Do sada su razvijeni mnogi tipovi biosenzora koji se primjenjuju pri različitim analizama, kao što su motrenje kliničkih, medicinskih i ekoloških postupaka, u motrenju i vođenju industrijskih procesa i u drugim područjima. Napredak u mikrosenzorskoj tehnologiji i genetičkom inženjerstvu potiču novi razvoj na tom području. Zbog njihovih osobina omogućen je kratkotrajan postupak analize i brzo praćenje procesa pa su biosenzori osobito prikladni za spomenute primjene. U ovom su pregledu prikazani najnoviji razvoj i osnovna načela ustrojstva biosenzora za motrenje okoliša te motrenje i vođenje bioprocasa.