

Review Article

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Fundamental and Application of Various Types of Biosensors in Food Analysis

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ABSTRACT

A biosensor is a sensing device comprised of a combination of a specific biological element and a transducer. Microbial biosensor is an analytical device which integrates microorganisms with a physical transducer to generate a measurable signal proportional to the concentration of analytes. In recent years, a large number of microbial biosensors have been developed for environmental, food, and biomedical applications. Biosensors can essentially serve as low-cost and highly efficient devices for this purpose in addition to being used in other day-to-day applications. A “specific biological element” recognizes a specific analyte and the changes in the biomolecule are usually converted into an electrical signal by a transducer. Biosensors are an important alternative in the food industry to ensure the quality and safety of products and process controls with effective, fast and economical methods. Nowadays, a vast majority of the glucose meters are based on electrochemical biosensor technology. The use of enzymatic biosensor technology in food processing, quality control and on-line processes is promising compared to conventional analytical techniques, as it offers great advantages due to size, cost, specificity, fast response, precision and sensitivity. Enzymatic biosensors are a tool with broad application in the development of quality systems, risk analysis and critical control points, and the extent of their use in the food industry is still largely limited by the short lifetime of biosensors, in response to which the use of thermophilic enzymes has been proposed. Oxidase enzymes utilize molecular oxygen for oxidation of Substrate. In microorganisms, the enzymatic degradation of caffeine is brought about by sequential demethylation by an oxygenase, into theobromine or paraxanthine. Amount of caffeine converted by the microorganisms and the amount of oxygen consumed based on which, the amount of caffeine in the sample can be determined. Biosensor against caffeine is a new invention particularly in food Technology and other fields. Biosensors can have a variety of biomedical, industry, and military applications. In spite of this potential, however, commercial adoption has been slow because of several technological difficulties. For example, due to the presence of biomolecules along with semiconductor materials, biosensor contamination is a major issue. Potential applications within the supply chain range from testing of foodstuffs for maximum pesticide residue verification through to the routine analysis of analyte concentrations, such as, glucose, sucrose, alcohol, etc., which may be indicators of food quality/acceptability. "Biosensors market is categorized as a growth market is expected to grow from \$6.72 billion in 2009 to \$14.42 billion in 2016." Biosensor adoption is increasing every year and the number of biosensor applications is continuously growing.

Keywords

Specific biological element,
Transducer, Analyte concentrations and adoption.

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Introduction

The history of biosensors started in the year 1962 with the development of enzyme electrodes by the scientist Leland C. Clark. Since then, research communities from various fields such as VLSI, Physics, Chemistry, and Material Science have come together to develop more sophisticated, reliable and mature biosensing devices for applications in the fields of medicine, agriculture, biotechnology, as well as the military and bioterrorism detection and prevention. The first successful commercial glucose biosensor from Yellow Springs Instrument in 1975 was based on the hydrogen peroxide approach, with a cellulose acetate inner membrane and a polycarbonate outer membrane. This analyzer was almost exclusively used in clinical laboratories because of its high cost. Biosensors are powerful tools aimed at providing selective identification to toxic chemical compounds at ultra trace levels in industrial products, chemical substance, environmental sample (e.g., air, soil and water) or biological system (e.g., bacteria, virus or tissue components) for biomedical diagnosis (Albery *et al.*, 1986; Bergmeyer, 1974; Guilbault *et al.*, 1985).

The main advantages of these devices are their specificity, sensitivity and ease of sample preparation and the fact that no other reagents besides a buffer and a standard are usually required. Caffeine (1, 3, 7-trimethylxanthine) is a naturally occurring substance found in the leaves, seeds or fruits of some plant species and is a member of a group of compounds known as methylxanthines (Hall, 1986; Joachim, 1986). It is also present in many painkillers and antimigraine pharmaceuticals. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. It does not accumulate in the body over the course of time and is normally excreted within several hours of consumption. Caffeine

increases calcium excretion in the urine and so heavy caffeine usage may increase the risk of osteoporosis. In the general scheme of a biosensor, the biological recognition element responds to the target compound and the transducer converts the biological response to a detectable signal, which can be measured electrochemically, optically, acoustically, mechanically, calorimetrically, or electronically, and then correlated with the analyte concentration. Since Clark and Lyon developed the first biosensor for glucose detection in 1962, biosensors have been intensively studied and extensively utilized in various applications, ranging from public health and environmental monitoring to homeland security and food safety. Various biological recognition elements, including cofactors, enzymes, antibodies, microorganisms, organelles, tissues, and cells from higher organisms, have been used in the fabrication of biosensors. Among these biological elements, enzymes are the most widely used recognition element due to their unique specificity and sensitivity. However, the purification of enzyme is costly and time-consuming. In addition, the *in vitro* operating environment could result in a decrease of the enzyme activity.

Microbes (e.g., algae, bacteria, and yeast) offer an alternative in the fabrication of biosensors because they can be massively produced through cell-culturing. Also, compared to other cells from higher organisms such as plants, animals, and human beings, microbial cells are easier to be manipulated and have better viability and stability *in vitro*, which can greatly simplify the fabrication process and enhance the performance of biosensors. Microbes are analogous to a “factory” consisting of numerous enzymes and cofactors/coenzymes, endowing themselves with the ability to respond to a number of chemicals, which can be used as the signal for sensing purposes. Even though metabolisms of the

microorganisms are non-specific, highly selective microbial biosensors can be potentially achieved by blocking the undesired or inducing the desired metabolic pathway and by adapting the microorganisms to an appropriate substrate of interest (target) through selective cultivation conditions. Of particular significance is the lower detection potential for these redox species (about +0.3 V versus Ag/AgCl reference electrode), at which the oxidization of common interferences are suppressed and thus the membranes can be omitted. This redox mediator-based approach is termed as the second generation glucose biosensors. Furthermore, recent development in molecular biology offers a novel method to construct genetically engineered microorganisms (GEMs), thus providing a new direction to manipulate the selectivity and sensitivity of microbial biosensors at the DNA level. DNA can be used to identify organisms ranging from humans to bacteria and viruses. Immobilizing microorganisms on transducers plays an important role in the fabrication of microbial biosensors (Kernez et al., 1983; Kricka et al., 1986).

Traditional methods for the immobilization of microorganisms include adsorption, encapsulation, entrapment, covalent binding, and cross-linking. Besides these methods, many novel immobilization strategies have been explored in recent years in order to improve the analytical performance and storage stability of the microbial biosensor (Lowe, 1984; North et al., 1985).

The development of biosensors is described in numerous works, the majority in the areas of clinical, environmental, agricultural and biotechnological applications. Their use in the food sector is convenient to ensure the quality and safety of foods. The potential uses of biosensors in agriculture and food transformation are numerous and each

application has its own requirements in terms of the concentration of analyte to be measured, required output precision, the necessary volume of the sample, time required for the analysis, time required to prepare the biosensor or to reuse it and cleanliness requirements of the system (North, 1985; Russell et al., 1986).

A successful biosensor must possess at least some of the following beneficial features:

1. The biocatalyst must be highly specific for the purpose of the analyses and should be good stability over a large number of assays.
2. The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable.
3. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration.
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects.
5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.

The key part of a biosensor is the transducer that makes use of a physical change accompanying the reaction.

This may be:

1. The heat output (or absorbed) by the reaction (calorimetric biosensors),
2. changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors),
3. Movement of electrons produced in a redox reaction (amperometric biosensors),
4. Light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors), or

5. Effects due to the mass of the reactants or products (piezo-electric biosensors).

Types of biosensors

Biosensors are classified depending upon different criteria like bioreceptors, transducers and different types of physical and chemical interaction. Depending upon type of transducers, biosensor can be classified as:

Calorimetric biosensor

Many enzyme catalysed reactions are exothermic, generating heat which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration. This represents the most generally applicable type of biosensor.

The temperature changes are usually determined by means of thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment. Under such closely controlled conditions, up to 80% of the heat generated in the reaction may be registered as a temperature change in the sample stream. This may be simply calculated from the enthalpy change and the amount reacted. If a 1 mM reactant is completely converted to product in a reaction generating 100 kJ mole⁻¹ then each ml of solution generates 0.1 J of heat.

Potentiometric biosensor

These make use of ion-selective electrodes in order to transduce the biological reaction into an electrical signal. In the simplest terms this consists of an immobilised enzyme membrane surrounding the probe from a pH-meter where the catalysed reaction generates or absorbs hydrogen ions. The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly

from the pH-meter's display. Typical of the use of such electrodes is that the electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction.

Electrochemical biosensor

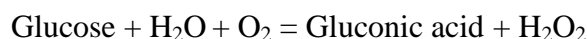
This biosensor is usually based on potentiometry and amperometry. Amperometric biosensors function by the production of a current when a potential is applied between two electrodes. They generally have response times, dynamic ranges and sensitivities similar to the potentiometric biosensors. The simplest amperometric biosensors in common usage involve the Clark oxygen electrode. This consists of a platinum cathode at which oxygen is reduced and a silver/silver chloride reference electrode. When a potential of -0.6 V, relative to the Ag/AgCl electrode is applied to the platinum cathode, a current proportional to the oxygen concentration is produced. Normally both electrodes are bathed in a solution of saturated potassium chloride and separated from the bulk solution by an oxygen-permeable plastic membrane (e.g. Teflon, polytetrafluoroethylene). The following reactions occur:



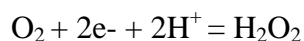
Glucose biosensor

Among all the biosensors, the most studied and developed biosensor application is glucose biosensor. In 1962 the American scientist Leland C. Clark first developed glucose biosensor. The basic operation of glucose biosensor is based on the fact that the enzyme *glucose oxidase* (GOD) catalyses the oxidation of glucose to gluconic acid. Here

the enzyme acts as a biorecognition element, which recognizes glucose molecules. These enzyme molecules are located on an electrode surface, which acts as a transducer. As soon as the enzyme recognizes the glucose molecules, it acts as a catalyst to produce gluconic acid and hydrogen peroxide from glucose and oxygen from the air. The electrode easily recognizes the number of electron transfer due to hydrogen peroxide/oxygen coupling. This electron flow is proportional to the number of glucose molecule present in blood. The glucose oxidation, catalyzed by GOD is



At the electrode:



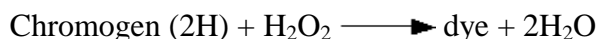
A voltage of -0.7 V is applied between the platinum cathode and the silver anode and this voltage is sufficient to reduce the oxygen. The cell current is proportional to the oxygen concentration and the current is measured (amperometric method of detection has been employed). The concentration of glucose is then proportional to the decrease in current (oxygen concentration).

Optical biosensor

This biosensor detects changes in absorbance or fluorescence of an appropriate and changes in the refractive index. There are two main areas of development in optical biosensors. These involve determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process. The former usually involve the widely established, if rather low technology, use of colorimetric test strips. These are disposable single-use cellulose pads impregnated with enzyme and reagents. The most common use of this technology is for whole-blood monitoring in diabetes control.

In this case, the strips include glucose oxidase, horseradish peroxidase (EC 1.11.1.7) and a chromogen (e.g. *o*-toluidine or 3, 3', 5, 5'-tetramethylbenzidine). The hydrogen peroxide, produced by the aerobic oxidation of glucose, oxidising the weakly coloured chromogen to a highly coloured dye.

Peroxidase



Piezo-electric biosensor

This biosensor is based on an alternating potential and produce a standing wave in the crystal at a characteristic frequency. This frequency is highly sensitive to the surface properties of the crystal such that, if a crystal is coated with a biological recognition element, the binding of the target analyte to receptors will produce a change in the resonant frequency. Piezo-electric crystals (e.g. quartz) vibrate under the influence of an electric field. The frequency of this oscillation (f) depends on their thickness and cut, each crystal having a characteristic resonant frequency. This resonant frequency changes as molecules adsorb or desorb from the surface of the crystal, obeying the relationships.

$$\Delta f = Kf^2 \Delta m/A$$

Where:- Δf is the change in resonant frequency (Hz), Δm is the change in mass of adsorbed material (g), K is a constant for the particular crystal dependent on such factors as its density and cut, and A is the adsorbing surface area (cm^2).

Immunosensor

These Biosensors may be used in conjunction with enzyme-linked immunosorbent assays (ELISA). ELISA is used to detect and amplify an antigen-antibody reaction; the amount of

enzyme-linked antigen bound to the immobilized antibody being determined by the relative concentration of the free and conjugated antigen and quantified by the rate of enzymic reaction. Enzymes with high turnover numbers are used in order to achieve rapid response. The sensitivity of such assays may be further enhanced by utilizing enzyme-catalyzed reactions which give intrinsically greater response; for instance, those giving rise to highly coloured, fluorescent or bioluminescent products. Assay kits using this technique are now available for a vast range of analyses.

Immobilization of enzyme

An immobilized enzyme is an enzyme that is attached to an inert, insoluble material such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalyzed reactions. An alternative to enzyme immobilization is whole cell immobilization.

There are three different ways by which one can immobilize an enzyme, which are the following, listed in order of effectiveness:

Adsorption on glass, alginate beads or matrix

Enzyme is attached to the outside of an inert material. In general, this method is the slowest among those listed here. As adsorption is not a chemical reaction, the active site of the immobilized enzyme may be blocked by the matrix or bead, greatly reducing the activity of the enzyme.

Entrapment

The enzyme is trapped in insoluble beads or microspheres, such as calcium alginate beads. However, this insoluble substance hinders the arrival of the substrate, and the exit of products.

Cross-linkage

The enzyme is covalently bonded to a matrix through a chemical reaction. This method is by far the most effective method among those listed here. As the chemical reaction ensures that the binding site does not cover the enzyme's active site, the activity of the enzyme is only affected by immobility. However, the inflexibility of the covalent bonds precludes the self-healing properties exhibited by chemoadsorbed self-assembled monolayers. Use of a spacer molecule like poly (ethylene glycol) helps reduce the steric hindrance by the substrate in this case. The operating stability and the stability in storage can be significantly improved by the additional incorporation of gelatin in the polymer matrices. Gelatin prevents enzyme inactivation as a result of enzyme modification by the free-radical oxidation products of phenolic compounds.

Advantages of immobilization

1. Immobilization provides cell or enzyme reuse.
2. Immobilization improves genetic stability. For some cells, protection against shear damage.
3. Immobilization may also provide favorable micro-environmental conditions. (e.g., cell-cell contact, nutrient-product gradients, pH gradient) resulting in better performance of the biocatalysts. (e.g., higher product yields and rates).

Limitations may be such as control of micro-environmental condition is difficult. With living cells, growth and gas evolution present significant problems in some system and can lead to significant mechanical disruption of the immobilizing matrix.

Surface attachment of biological elements

An important part in a biosensor is to attach the biological elements (small molecules/protein/cells) to the surface of the sensor (be it metal, polymer or glass). The simplest way is to functionalize the surface in order to coat it with the biological elements. This can be done by polylysine, aminosilane, epoxysilane or nitrocellulose in the case of silicon chips/silica glass. Subsequently the bound biological agent may be for example fixed by Layer by layer deposition of alternatively charged polymer coatings. Alternatively three dimensional lattices (hydrogel/xerogel) can be used to chemically or physically entrap these (where by chemically entrapped it is meant that the biological element is kept in place by a strong bond, while physically they are kept in place being unable to pass through the pores of the gel matrix). The most commonly used hydrogel is sol-gel, glassy silica generated by polymerization of silicate monomers (added as tetra alkyl orthosilicates, such as TMOS or TEOS) in the presence of the biological elements (along with other stabilizing polymers, such as PEG) in the case of physical entrapment.

Application of biosensors

There are many potential applications of biosensors of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based

techniques in some situations. Some examples are given below:

- Glucose monitoring in diabetes patients ←historical market driver
- Other medical health related targets.
- Environmental applications e.g. the detection of pesticides and river water contaminants.
- Remote sensing of airborne bacteria e.g. in counter-bioterrorist activities.
- Detection of pathogens.
- Determining levels of toxic substances before and after bioremediation.
- Detection and determining of organophosphate.
- Routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay.
- Determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey.
- Drug discovery and evaluation of biological activity of new compounds.
- Protein engineering in biosensors.
- Detection of toxic metabolites such as mycotoxins.

There are also disadvantages to be dealt with

- Heat sterilization is not possible as this would denature the biological part of the biosensor.
- The membrane that separates the reactor media from the immobilized cells of the sensor can become fouled by deposits.
- The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane
- Changes in the reactor broth (i.e., pH) can put chemical and mechanical stress on the biosensor that might eventually impair it.
- They can easily be set off and break down.

Table.1 Most important biosensors applied to evaluate food quality

Analyte	Matrix Recognition enzyme	Transduction system
Glucose	Grape juice, wine, juice, honey, milk and yogurt	Amperometric
Fructose	Juice, honey, milk, gelatin and artificial edulcorants	Amperometric
Lactose	Milk	Amperometric
Lactate	Cider and wine	Amperometric
Lactulose	Milk	Amperometric
L-amino acids	Milk and fruit juices	Amperometric
L-glutamate	Soya sauce and condiments	Amperometric
L-lysine	Milk, pasta and fermentation samples	Amperometric
L-Maltate	Wine, cider and juices	Amperometric
Ethanol	Beer, wine and other alcohol drinks	Amperometric
Glycerol	Wine	Amperometric
Catechol	Beer	Amperometric
Cholesterol	Butter, lard and egg	Amperometric
Citric Acid	Juice and athletic drinks	Amperometric
Lecithin	Egg yolk, flour and soya sauce	Electrochemical

Biosensors for food analysis

There are several applications of biosensors in food analysis. In food industry optic coated with antibodies are commonly used to detect pathogens and food toxins. The light system in these biosensors has been fluorescence, since this type of optical measurement can greatly amplify the signal. A range of immuno- and ligand-binding assays for the detection and measurement of small molecules such as water-soluble vitamins and chemical contaminants (drug residues) such as sulfonamide and Beta-agonists have been developed for use on SPR based sensor systems, often adapted from existing ELISA or other immunological assay. These are in widespread use across the food industry (Table 1).

Biosensors as biotechnology tools

In the field of medicine, industry, agriculture,

environment monitoring and biotechnology research, routine analyses using physical instruments are conducted for estimation and monitoring the levels of certain analytes (an analyte is a compound or molecule, whose presence and concentration needs to be determined and monitored). Conventional physical methods for this routine analysis do not involve the use of any living organisms or molecules of biological origin. However, for this purpose, biological molecules or living cells have been used to develop sensitive devices that are described as biosensors. The biosensors have been considered to be superior and more sensitive, in comparison to physical instruments (Scheller *et al.*, 1985; Turner, 1987).

Biosensor: opportunities and challenges

Biosensors are a class of electrical biosensors that show promise for point-of-care and other applications due to low cost, ease of

miniaturization, and label-free operation. Unlabeled DNA and protein targets can be detected by monitoring changes in surface impedance when a target molecule binds to an immobilized probe. The affinity capture step leads to challenges shared by all label-free affinity biosensors. Electrode size impacts the required measurement frequency range, and measurement accuracy depends on measurement frequency and instrumentation design. Optimizing electrode size may allow smaller impedance changes to be reliably detected, which may lower the detection limit. Future research in the area of label-free affinity biosensors should be targeted towards applications that leverage the techniques' advantages (low cost, small size, low power, simplified sample preparation, and moderate multiplexing capability) without requiring exquisite sensitivity. There has been no systematic improvement in reported detection limits during the past 15 years of label-free affinity biosensor research. On-going fundamental studies on mediated and direct electron-transfer electrochemistry, on new sensing principles, and on enzyme stabilization, coupled to extensive commercial efforts, should have a tremendous impact on point-of-care clinical testing, and upon biomedicine, in general.

In conclusions the food industry is benefitting from major advances in the development of enzymatic biosensors with different transduction systems that can be applied in the areas of food safety, quality and process control; studies are focused mainly on determining composition, contamination of primary materials and processed foods. In the area of food safety, enzymatic biosensors allow for identifying the presence of highly toxic organic contaminants and the presence of anti-nutritional elements that affect the food chain, either accidentally or by intention. This early detection protects the environment from contaminants and consumers from chronic illnesses and allergies. Equally,

enzymatic biosensors are being used in the food industry to determine the freshness of products given that it is possible to detect enzymes and compounds of aroma and flavor that originate from the senescence stage of products (Turner *et al.*, 1987; DSouza, 2001).

Biosensors have proven to be especially useful in the control of fermentative processes in follow-up of the consumption of the substrate by microorganisms, control of acidity and assessing the thermal profile. While the use of biosensors in the food industry is on a mass scale, there are still obstacles to be overcome, such as the high cost of purifying the enzymes that are used as detecting elements, the low specificity and low response time that are obtained when complete cells or tissue are used, the lack of reliable responses low concentrations, interference reactions, the need to calibrate the devices and the stability of the enzymes. This last factor is the most limiting for the lifetime of enzymatic biosensors. If these limiting factors can be overcome, it will be possible to develop enzymatic biosensors that are more rapid, versatile, reliable, long lasting and cost-effective. A high level overview of different types of biosensors is also given. Working principles, constructions, advantages, and applications of many biosensors are presented. There are various technical difficulties for which some solutions exist, but still more research efforts are needed in order to find better alternatives. Such as (a) contamination: bioelements and chemicals used in the biosensors need to be prevented from leaking out of the biosensor over time, (b) immobilization of biomolecules: to avoid contamination, biomolecules are attached to the transducer, (c) sterilization: if a sterilized probe is used some sensor's biomolecules may be destroyed whereas if non-sterile probes are used some compromises are needed, (d) uniformity of biomolecule preparation: fabrication of biosensors that can

reproduce results need such uniformity, (e) selectivity and detection range: should be more selective and the detection range should be large, (f) cost: research should be focused on the development of low-cost biosensors. At present, with the threat of bioterrorism omnipresent, the development of faster, reliable, accurate, portable and low-cost biosensors has become more important than ever.

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