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BIOSENSORS: APPLICATIONS AND OVERVIEW IN INDUSTRIAL AUTOMATION

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ABSTRACT

Biosensors are analytical tools or systems consisting of an immobilized biological sensing material in close contact with a suitable transducer that convert the biochemical signals to a quantifiable electrical signal. Biosensors are now used in variety of disciplines, including medicine, food industry and environmental science. It is becoming increasingly important for researchers and scientists in these areas and other fields to have sound understanding of the different types of biosensors, which can be used, the principles behind them, and their advantages with their limitations. This review discusses the basic features of biosensors, types of biological materials used and details of most important types of biosensors currently used specifically in food industry. Online use of biosensor in processing of food materials is also discussed in detail with electronic control systems to make its use relevant as an emerging technology for food processors.

KEY WORDS: Biosensor, food application, sensitivity, transducer

I. INTRODUCTION

Biosensors are one of the leading applications of biotechnology of relevance to the food industry. These devices have the ability to provide rapid, cost effective, specific and reliable objective analytical results. Food technologists are under enormous pressure now a day to comply with legislation and satisfy consumers due to increased demands for organic food, fresh food, free from traces of chemicals and pathogenic micro-organism, which require better diagnostics methods for real-time analysis. As a consequence, food has to be chemically analyzed for a number of compounds which are indicative for the parameters mentioned above. Analyses have to be carried out upon delivery of raw material at a food-producing company, during the process of food production, and prior to delivering the product to a customer.

A typical biosensor consists of a biological component and an electronic device that converts the biological signal into a measurable output (Fig. 1). The biological part of the sensor reacts with a particular substance of interest to produce a physical or biochemical change that is detected and converted to an electrical signal by the transducer. The amplifier increases the intensity of the signal so that it can be readily measured. The bio-interface between the analyte/receptor and transducer is a key element in designing a successful biosensor. These components are usually housed often within a single portable unit. The incorporation of biological materials such as enzymes, antibodies, microorganisms, tissues and receptors, as sensing elements makes the ordinarily used transducer more selective and sensitive. The development of computer and electronics technologies provides strong support to fast, consistent signal measurement, data collection, and information analysis. This automation can result in objective, fast, consistent food quality evaluation systems, a significant advancement for food engineering and industry.

II. BIOSENSORS

The term 'biosensor' is also used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilize a biological system directly. Biosensors are an important and powerful development in analytical measurement technology as they are able to measure the presence, absence or concentration of analytes accurately and rapidly. Their advantage over existing traditional technologies is that they are capable of monitoring broad or narrow spectra of analytes continuously in real time, at the point of need (Fig. 2). The key part of a biosensor is the biological component and transducer, which makes use of a physical change accompanying the reaction. This may be

- the heat output by the reaction (calorimetric biosensors)
- the changes in distribution of charges causing the changes in electrical potential (potentiometric biosensors)
- the movement of electrons produced in a redox reaction (amperometric biosensors)

- the detection and amplification of antigen-antibody reaction (immuno biosensors)
- the light output during the reaction (optical biosensors)
- the effects due to the mass of the reactants or products (piezo-electric biosensors)

III. FEATURES OF BIOSENSOR

The success of any biosensor depends on inherent basic features:

- The biological component or biocatalyst must be highly specific, sensitive, robust and stable under normal storage conditions for large number of assays
- The reaction should be as independent of physical parameters
- The response should be accurate, precise, reproducible and linear over the useful analytical range
- The sensing should be free from different types of noises
- The probe must be flexible in size and biocompatible, having no toxic or antigenic effects
- The biosensor should be cheap, small, portable and user friendly

IV. TYPES OF BIOSENSORS

Biosensors can be classified in following two categories:

- Based on biological component of sensor
- Based on physical changes to be measured by transducers

A. Biological components of biosensors

Enzymes

Bio-catalyst basically the protein molecules recognizes a particular target substance in a similar way to a key fitting a lock. It then attaches itself to the substance and converts it to a chemically different product. This product is often something that can be detected easily, such as a substance that emits light. Like luciferase reacts with the compound luciferin in the presence of oxygen and adenosine triphosphate (ATP) to make oxyluciferin a chemically different product that emits light. This reaction is used in a number of commercially available biosensors, some of which are used to detect toxicity in soil or bacterial contamination.

The enzyme glucose oxidase acts on glucose to produce an electrochemical signal, which is proportional to the glucose concentration. This technique enables tiny amounts of analyte to be detected readily and rapidly.

Some enzymes have specific inhibitors and biosensors incorporating these enzymes can be used to determine the concentration of such inhibitors in the sample. The strength of the signal produced by the biosensor decreases as organophosphates pesticide concentration increases and provides a reliable test for pesticide contamination.

Antibodies

They are proteins produced by the immune system of living organisms in response to the presence of 'foreign' micro-organisms (bacteria and viruses). Unlike enzymes, antibodies do not catalyze reactions but recognize and bind to specific molecules. They can generally be tailored to a greater extent and can be produced for the detection of specific industrial chemicals.

Microorganisms

The microorganisms used within biosensors are typically bacteria or yeast cells. This type of biosensor generally employs one of three possible mechanisms:

- The target analyte acts as 'food' for the microorganism. The most widely used micro-organism-based biosensors are those that detect biodegradable organic compounds. The microorganisms are immobilized onto electrochemical transducers to allow the rate of metabolism of the organic compounds to be measured.
- Broad range of toxic compounds can reduce the activity of the microorganisms used within biosensors. These biosensors are therefore particularly suitable for general toxicity screening. The respiration of the microorganisms can be detected by optical or electrochemical transducers.
- Genetically modified microorganisms can recognize the presence of particular substances. The presence of toxic compounds inhibits the enzyme reaction and the reduction in the light signal is used as an indication of sample toxicity.

B. Physical changes to be measure by transducers

Amperometric biosensors

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. This generates a current (I), which is carried between the electrodes by means of a saturated solution of KCl. This electrode compartment is separated from the biocatalyst (glucose oxidase) by a thin plastic membrane, permeable only to oxygen (Teflon, polytetrafluoroethylene). The analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate and product. This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilizing dip-coated membranes. The following reactions occur:

Ag anode $4Ag^0 + 4Cl^- \rightarrow 4AgCl + 4e^-$

Pt cathode $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be effectively zero. The rate of this electrochemical reduction therefore depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration. It is clear that this process consumes a small but significant proportion of the oxygen present in the bulk; the oxygen electrode measuring the rate of a process that is far from equilibrium, whereas ion-selective electrodes are used close to equilibrium conditions. This causes the oxygen electrode to be much more sensitive to changes in the temperature than potentiometric sensors. Determination of glucose concentrations requires an immobilized glucose oxidase membrane. The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes.

Calorimetric biosensors

Many enzyme catalyzed 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 immobilized 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. This may be simply calculated from the enthalpy change and the amount reacted. If a 1mM reactant is completely converted to product in a reaction generating 100kJ $mole^{-1}$ then each ml of solution generates 0.1J of heat. At 80% efficiency, this will cause a change in temperature of the solution amounting to approximately 0.02°C. This is about the temperature change commonly encountered and necessitates a temperature resolution of 0.0001°C for the biosensor to be generally useful.

Immunosensors

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 immobilised 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 utilising enzyme-catalysed 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.

Recently ELISA techniques have been combined with biosensors, to form immunosensors, in order to increase their range, speed and sensitivity. A simple immunosensor configuration is a tube coated with (immobilised) antigen. An excess of specific antibody-enzyme conjugate is placed in the tube and allowed to bind. After a suitable period any unbound material is washed off. The analyte antigen solution is passed into the tube, binding and releasing some of the antibody-enzyme conjugate dependent upon the antigen's concentration. The amount of antibody-enzyme conjugate released is determined by the response from the biosensor.

Optical biosensors

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 common use is for blood glucose monitoring in diabetes.

Piezo-electric biosensors

Piezo-electric crystals vibrate under the influence of an alternating electrical field. The frequency of this oscillation (*f*) depends on their thickness. Each crystal is having a characteristic resonant frequency. This resonant frequency changes as molecules adsorb or desorbs from the surface of the crystal, obeying the relationships

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).

A simple use is as formaldehyde biosensor, utilising a formaldehyde dehydrogenase coating immobilised to a quartz crystal and sensitive to gaseous formaldehyde. The major drawback of these devices is the interference from atmospheric humidity and the difficulty in using them for the determination of material in solution. However, they are inexpensive, small, robust and capable of giving a rapid response.

Potentiometric biosensors

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 immobilized enzyme membrane surrounding the probe from a pH-meter, where the catalyzed 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.

V. INDUSTRIAL APPLICATIONS

Biosensor technology is having an increasing impact on manufacturing industry with a significant opportunity for its expansion. The application in areas where rapid detection, high sensitivity and specificity are important should provide a continuing driver for scientific development as well as commercialization. A number of potential industrial applications for biosensor technology are outlined as:

- All manufacturing sectors for a cost-effective, rapid means of monitoring industrial effluent
- Water quality assessment in chemical, food and pharmaceutical industries
- Analytical quality control and monitoring, especially for quantitative and differential analysis of gas mixtures from chemical processes and products
- The analysis of volatile organic compounds in petrochemical industries
- Monitoring of bleaching processes and in detection of toxic substances in paper and pulp industry
- In-situ operation in the characterization, remediation and post-closure monitoring of contaminated land and hazardous waste sites
- As Glucose Sensor, Lactate Sensor, Alcohol Sensor, Biochemical Oxygen Demand Sensor, Sensor for detection of microbes, Biosensors to detect E. coli in food,
- Sensors for the detection of fish, meat and poultry flesh and product quality assessments

VI. CONCLUSION

Biosensor technology is a rapidly expanding area, as evidenced by the quantity of research papers and patents published during the last five years. Although broad range of biosensors are available for food analytes, such as sugars, amino acids, organic acids, proteins and bacteria biosensors but those are rarely used in food industry. Production at high quantities biosensors with low cost will definitely have an impact in various processing and manufacturing industry for high level of automation. The main barriers to market acceptance are resistance to change from conventional analytical techniques and a lack of awareness of the advantages of biosensor technology. Analysis of food can be a complex issue due to the diversity of food matrices but time and cost savings using biosensors may greatly increase the potential of this technology for food and allied industry.

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