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# Biosensors in food industry

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In modern food processing plants, the in-line monitoring of processed food has become very important part of factory operations. Sensor technology currently attracts increasing attention as a successor to conventional analytical techniques in the food industry. Food analysis is thus a very challenging field, especially when dealing with the detection of minor components, unwanted toxic heavy metals from soil, herbicides or pesticide residues etc. Detection of such chemicals is very difficult to analyze because these methods are most unfriendly with surroundings and time-consuming while sensor technology is a rapid method. A biosensor is usually a compact analytical device that detects and transmits information pertaining to biochemical reactions. Two primary components consist of a bioreceptor that recognizes a target analyte and a transducer that converts biochemical signals into a quantifiable electrical response. The bioreceptor is a biological or organic material such as an antigen, enzyme, microbe, or nucleic acid. The transducer may assume many forms (such as optical, amperometric etc.) depending on the parameter being measured. Clearly, the term biosensor is used in diverse ways, but generally, a biosensor should respond selectively, continuously, rapidly, specifically, and ideally without added reagent to biological events. A huge variety of biosensors have been invented for food analysis like immunosensor, microbial and DNA biosensors have been reported for analysis of different components in food. Biosensor technology can offer the food industry a new, rapid monitoring and measuring devices whose speed, sensitivity and ease of operation exceed the current methodologies. Potential use of biosensor technologies in coming years in food industries may include proximate analysis, nutritional labeling, pesticide residues, toxins and antinutrients, processing changes, microbial contamination and BOD of biowastes. There are some limitations such as life of bioreceptor that affect the market of biosensor. Therefore, further research is needed for improved performance of biosensors.

**Key Words** : Biosensore, Cross-linking, Sensitivity

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Sensor is a device that measures a parameter related to the product quality. It acquaints with the state or performance of equipment or a production process. Sensors thus include laboratory instruments as well as on-line and in-line devices. According to Richter (1993) biosensor is a self-containing analytical system that respond directly and selectively to biologically important species. Biosensors comprise of (1) biologically derived molecules or bioreceptor such as enzymes, antibodies and microbial cells, which react with chemical species or organisms for which the bio-molecules have specificity (2) physical transducers (transforms signal resulting from interaction of analyte with biological element into another signal that can be more easily measured and quantified) and (3) suitable electronics for signal amplification and display.

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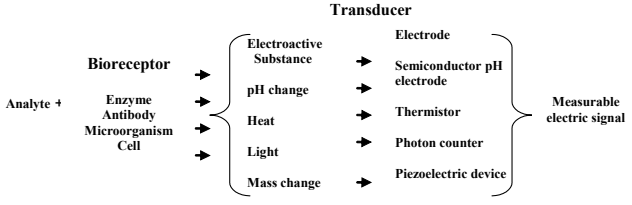
**Principle of biosensor:**

A bioreceptor recognizes and detects specific molecules or chemical reactions rapidly. This produces a chemical change (*e.g.* production of chemical entities such as hydrogen peroxide) or produce physical effect (*e.g.* changes in temperature, mass etc.) by the biological element that can be monitored and



converted by one or more of the physical transducers into forms which are electronically amplified, stored and displayed (Turner and Newman, 1998).

The biocomponent may be entrapped or immobilized onto a membrane of selective permeability. The membrane is assembled in a close contact with transducer which in turn is in intimate proximity with the electronic component(s). Biomolecule is immobilized or coupled onto transducer by four basic methods as given below.

*Physical adsorption:*

Active charcoal, silica gel, clay etc. are used to adsorb the biomolecule by physical forces such as hydrogen bonding or hydrophobic interactions (Narayanaswamy, 2006).

*Entrapment/ Encapsulation:*

A water soluble polymer such as polyacrylamide type gel and naturally derived gels *e.g.* cellulose triacetate, agar, gelatin, carrageenan, alginate etc. are used to entrap biomolecule by electrostatic attraction between ionic groups (Narayanaswamy, 2006).

*Covalent bonding:*

Agarose, cellulose, resins etc. forms stable covalent bond with biomolecule under a broad range of pH, ionic strength and other variable conditions.

*Cross-linking:*

Biomaterial is chemical bonded to solid support or to cross-linking agent such as gluteraldehyde, diazonium etc.

**Basic characteristics of biosensors:**

*Sensitivity:*

It means steady-state changes in the magnitude of biosensor output with respect to change in concentration of the analyte.

*Linearity:*

It should be high for the detection of high substrate concentration.

*Detection limit:*

It means concentration of analyte being measured which gives a minimum detectable difference signal.

*Specificity:*

The response of biosensor should be specific to only change in concentration of the target analyte and not be influenced by the presence of other chemical species.

*Dynamic response:*

The physical properties and relative size of biosensor determine how quickly it responds to a change in concentration of the target analyte.

*Life-time:*

The biological materials used in biosensor are generally the least stable component of the system.

**Classification of biosensors:**

They have been categorized according to their transduction element and biological element (Corcuere and Cavalieri, 2003).

**Biosensors based on type of transduction system:**

*Optical biosensors:*

Light may be generated by fluorescent or luminescent/ chemiluminescent reactions associated with enzymes immobilized at the tip of the fibre optic bundle which in turn is transmitted through an optical fibre to a detector. They are suitable where an enzyme consumes or generates a fluorescent cofactor such as NAD(P)H (French and Cardosi, 2007). Such

**Table 1.** Enzymes used in biosensors

| Analyte | Biocomponent | Transducer | Detection range | Application |
|---|---|---|---|---|
| Glucose | Glucose oxidase | Amperometric | 50-500 mM | Soft drink, juices and milk |
| Glucose | Glucose oxidase | Amperometric | $1x10^{-5} – 8x10^{-4}$mol/l | Musts and wine |
| Glucose and lactose | Glucose oxidase | Amperometric | 100-10000 ppm | Biscuit, juices and milk |
| Glucose | Glucose oxidase | Amperometric | 0.5-10mM | Juice and honey |
| Glucose and glutamate | Glucose oxidase | Amperometric | 10µM-3mM (glu)<br>3µM-.05mM (glutamate) | Beverages |
| Glucose, ascorbic acid and citric acid | Glucose oxidase, peroxidase and urease | Amperometric | 1-10mM (glucose)<br>0.25-2mM (Vitamin C)<br>5-100mM (citric acid) | Fruit drinks |
| Glucose | Glucose oxidase | Thermal | 0.2-30 mM | Fruit juices and coca-cola |
| Glucose and galactose | Glucose oxidase, peroxidase and galactose oxidase | Amperometric | 250-4000mg/l | Yoghurt and milk |

biosensors are mostly based on Surface Plasma Resonance (SPR) phenomenon that occurs when light is reflected off thin metal films, which is deposited on the reflecting surface of glass prism and may be used to measure the interaction of biomolecule on the surface.

*Potentiometric biosensors:*

In this acumulación of aprópiate ion causes a change in voltage with very little current flow (French and Cardosi, 2007). They may be suitable when the reaction generate or consumes $H^+$, $NH_4^+$, or $CO_2$ by means of ion selective electrode.

*Amperometric biosensors:*

It measures the current generated between a test electrode and a reference electrode, when the potential between them is maintained at a constant level by means of potentiostat. Oxidation or reduction of redox-active molecule at the electrode surface generates a current, which is measured. This transduction is ideal where a reaction generates/consumes a redox-active substance such as $O_2$, $H_2O_2$, NAD(P)H etc. (French and Cardosi, 2007).

*Piezoelectric biosensors:*

It is based on linear relationship between the changes in the oscillating frequency of a piezoelectric crystal when on its surface mass is varied due to interaction of analyte and bioreceptor. Detection is based on the change in mechanical properties of the surface film as its mass increases and that is measured by the changes in its vibration frequency when an electrical potential is applied (French and Cardosi, 2007).

*Thermal or Calorimetric biosensors:*

They measure thermal energy released or absorbed in biochemical reaction (Wang *et al.,* 2008). It is based on the use of a thermistor to detect tiny amount of heat released by an enzyme-catalyzed reaction. The bioreceptor is immobilized in the thermistor. Thermal detection of enzyme reactions is possible with any class of enzyme, provided that the heat released by the reaction can be measured with sufficient sensitivity.

**Biosensors based on type of biological element or bioreceptor:**

*Enzyme biosensors:*

This depends on ability of an enzyme to generate a signal that can be detected and converted into an electrical response. Depending on the analyte and the physical transducer to be employed either a single-enzyme or multienzyme system is used. These types of biosensors are given in Table 1 (Lakhanpal *et al.,* 2011).

*Immunosensor or Bioaffinity sensors:*

In this the immune response of certain biological species (usually bacteria) to contaminants produces antibodies, which in turn can be measured. They are divided into two groups namely Labeled type (involves a labeling agent such as enzymes, nanoparticles and fluorescent or electrochemiluminescent probe to quantify the amount of antibodies or analyte) and Label-free type (detect the analyte and the antibodies on a transducer surface without any label) as given in Table 2 (Jiang *et al.,* 2008).

**Table 2.** Immunosensor with labels and labels free to determine residual pesticide

| With label | | | | Label free | | |
|---|---|---|---|---|---|---|
| Analyte | Detector | Label | Detection range | Analyte | Detector | Detection range |
| Chlorsulfuron | Amperometric | Glucose oxidase | 0.01-1 ng/ml | 2,4-D | SPR | 0.1 nmol/l |
| Glyphosate | Fluorescent | Glyphosate-peroxidase | 0.021 ng/ml | Atrazine | Piezoeelectric | 1.5ng/ml (direct)<br>0.025ng/ml (competitive) |
| Simazine | Potentiometric | Peroxidase | 3 ng/ml | Trifluralin | Piezoeelectric | 100ng/ml (direct)<br>$2x10^{-7}$ng/ml (competitive) |
| Isoproturon | Fluorescent | Flurescein | 0.1 ng/ml | DDT | Nanomechanical | < nM range |

**Table 3.** Microbes in biosensors

| Analyte | Microorganism | Transducer | Detection Range |
|---|---|---|---|
| Alcohal | *Candida vini* | Oxygen electrode (porous acetyle cellulose filter) | $2x10^{-2}$ - $2x10^{-1}$ mM |
| Short-chain fatty acids in milk | *A. nicotianae* | Oxygen electrode (Ca-alginate) | 9.5-165.5 mM |
| $CO_2$ | *Pseudomonas* | Oxygen electrode (cellulose nitrate membrane) | 0.2-5 mM |
| Vitamin B-6 | *Suvarum* | Oxygen electrode (adsorption on cellulose nitrate membrane) | 0.5-2.5 ng/ml |
| Vitamin B-12 | *E. coli* | Oxygen electrode (trapped in porous acetyl cellulose membrane) | $5-25x10^{-9}$ m |
| Pyruvate | *Streptococcus faecium* | $CO_2$ gas sensing electrode (direct immobilization on sensor membrane | 0.22-32mM |
| Tyrosine | *A. phenoogenes* | Ammonia gas sensing electrode | $8.2x10^{-2}$ -1mM |
| Phosphate | *Chlorella vulgaris* | Oxygen electrode (polycarbonate membrane) | 8-70 mM |

*Microbial sensors:*

Microbial cells attached to the physical transducer are used to measure the concentration of a substrate that is utilized. The metabolic activity of the cells is measured by an electrochemical change which is proportional to the substrate concentration. These sensors are described in Table 3 (D'Souza, 2001).

*Nucleic acid biosensor:*

In recent year DNA and RNA have been employed for the detection of food borne pathogens. These sensors involve detection of a unique sequence of nucleic acid bases through hybridization. DNA biosensors are constructed by immobilization of the oligonucleotide sequence/single standard DNA (probe) on to a different electrode to measure the hybridization between DNA probe and their complementary DNA strands.

**Applications of biosensors in food:**

*Determination of food component:*

They may be used for the analysis of carbohydrates and determination of organic acid, vitamins, alcohols, phenols, and amino acids in food.

*Sensory analysis:*

Two types of biosensors namely Electronic nose (E-Nose) and Electronic tongue (E-tongue) are being used in organoleptic evaluation of different foods.

E-Nose is an instrument which is designed to mimic the human olfactory system. It is based on the principle of change in the sensor resistance when the sensor is exposed to odors or vapors (Lawrence and Masih, 2011). E-Nose is used in identification of hop varieties and aroma of orange juice, coffee, and whiskey samples; aging process to know cheese maturity, fish freshness, and degradation of cooking oil; detection of contaminant like diacetyl in orange juice and trichlonoanisole in wines; quality control to recognize acceptable and rejectable samples in raw material and finished product; wine and brewing industries for on-line measurement of ethanol; dairy industry to classify the various types of cheese and strains of bacteria (Thakur and Kingsly, 2007).

E-tongue is a multisensory system for liquid analysis based on chemical sensor arrays and a suitable recognition method. It can be used for the detection of all types of dissolved compounds, including volatile compounds which give odors after evaporation. E-tongue is used in process monitoring (batch fermentation process of starter cultures for cheese production), foodstuff recognition (to distinguish regular and diet cola drink), freshness evaluation, quality control (to assess bitterness in beer) and quantitative analysis (to assess sourness in red and white wines) (Peris, 2011).

**Table 4.** Detection of pathogens in food

| Pathogen | Sources of contamination | Biosensors |
|---|---|---|
| *Salmonella* | Contaminated food products | Piezoelectric antigen-antibody |
| *Listeria monocytogene* | Fresh and processed food as meat, shell fish, unpasteurized milk | Fibre-optic and piezoelectric biosensor |
| *Campylobactor* | Poor cooked poultry, sea food, dairy products | SPR optical biosensor |
| *E. coli* | Fresh fruit and vegetables, contaminated water, poorly cooked animal foods | Ameperometric and nucleic acid biosensor |

**Table 5.** Biosensors to determine pesticides and fertilizer residues in food

| Pesticide | | | Fertilizer residue | | |
|---|---|---|---|---|---|
| Pesticide | Bioreceptor | Transduction system | Fertilizer | Bioreceptor | Transduction system |
| Methyle parathion | *Sphingomonas*, *Flavobacterium* | Fibre optics | Nitrate | Nitrate reductase | Potentiometric |
| Methyle parathion | Methyle parathion hydrolase | Amperometric | Nitrite | Nitrite reductase | Amperometric |
| Organophosphorus | Organophosphorus hydrolase | Amperometric | Phosphate | Pyruvate oxidase | Amperometric |
| Triazophos | Acetyle Cholinesterase | Amperometric | Urea | Urease | Amperometric, Potentiometric |
| Monocrothopos, Malathion, Lannate | Acetyle Cholinesterase | Amperometric | | | |

**Table 6.** Biosensors to determine heavy metals in food

| Heavy metals | Bioreceptor | Transduction system |
|---|---|---|
| Hg, Ag, Pb and Cd | Invertase and glucose oxidase | Ultra microelectrode |
| Cd, Cu, Ni and Zn | Urease | Optical |
| Lead | Bovine serum albumin | Piezoelectric |
| Cu and Hg | Glucose oxidase | Amperometric |
| Hg | DNA based biosensor | Optical fiber |

*Contaminant analysis:*

Cock and Verdugo (2009) compiled the different biosensors used to detect pathogens (Table 4), and to determine pesticides and fertilizers residues (Table 5), and heavy metals (Table 6) in different foods.

*Biosensorsi in food packaging:*

A biosensor/barcode called as food sentinel system is being developed to detect pathogen in food packages. A specific-pathogen antibody is attached to the barcode's membrane forming part; the presence of contaminating bacteria shall produce the formation of localized dark bar, that shall render the barcode unreadable upon scanning. Toxin guard is also being developed to detect pathogens. In this antibodies are incorporated into the plastic packaging films. When the antibodies encounter a target pathogen, the packaging material display a clear visual signal (Otles and Yalcin, 2008).

*Monitoring of fish quality:*

Biogenic amines are produced in meat, fish, cheese, wine, milk due to microbial decarboxylation of amino acids. Biosensors based on direct coupling of amine oxidase and horseradish peroxidase maybe used to detect quality of fish.

**Merits and limitation of biosensors:**

There are several advantages of using biosensor in food industry. Use of biosensors is analyte specific, rapid and does not require extensive sample preparation. Analyte is measured in its natural micro-environment as it is not modified during sample preparation. The instrument is small, compact, portable, versatile, simple to use and gives reproducible results. Biosensors can be employed in monitoring the process on line due to their rapid response and continuous signals. The system can be used for simultaneous multiple assays. Possibly, they would not be very expensive when produced on large scale.

Besides several merits of using biosensors in food industry, there are also many constraints. Development of biosensors involves a team of experts from diverse field such as food science and technology, electro-chemistry, biochemistry, optics, electronic engineering, and microbiologist etc. Linear response may not be maintained over a range of concentrations of the analyte. Membrane carrying biosensors may pose problems of fouling and biocatalyst which is the least stable part of biosensor would limit the life of biosensor. Also, sometimes the lack of applicability of a single-analyte targeting by the biosensors for food *e.g.* in taste or smell analysis may limit their use.

**Conclusion:**

Biosensors are emerging as a revolutionary analytical technique having diverse applications in food industries. These compact and portable devices are based on biological interaction coupled with physical transducer and electronic. A large variety of biosensors have been invented for food analysis. For example, Immunosensor that uses antigen or antibody has a great potential for rapid detection of pesticide residue in food and environment and various enzymes are also applied on transducer for analysis of food composition. Similarly a microbial sensor uses microbes as bioreceptor for analysis of different component in food. In recent years, DNA biosensors have been reported for the detection of food borne pathogens. Biosensor technology can offer the food industry a new, rapid monitoring and measuring devices whose speed, sensitivity, stability and ease of operation exceed the current methodologies. However, before the potential of biosensors as a tool of quality and safety assurance is realized problems associated with their use *e.g.* short life of biomaterial, limited commercial availability etc. would have to be resolved and further research is needed for improved performance of biosensors.

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