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A Case Study

Role of biosensors in agro-food technology

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ABSTRACT : Sensor is a device which measures a physical quantity and transforms it into a signal which can be need by an observer or an instrument.Sensors are of various types (thermal, electric, mechanical, chemical, optical, acoustic and biological (*i.e.* biosensors). They are devices based on direct spatial competing between a biologically active compound and the signal transducer equipped with an electronic amplifier (Bose *et al.*, 2004).The biosensors have immense role in food technology. They are used as screening methods for detection of genetically modified food components (Tichoniuk *et al.*, 2008). Biosensors for the detection of food contaminants and toxin detection were studied by (Baeumner, 2003). Neethirajan *et al*. (2005) reported that biosensors are emerging as a highly promising tool for rapid diagnosis of pathogens and allergic components in food. Garcia and Mottram (2003) and Ivnitski *et al*. (2000) studied bacteriological food safety and discussed the role of biosensors in detecting pathogen like *Salmonella, E coli* in food likewise robust optical biosensors have been used to study beverage analysis (Luff *et al*., 1998). Heavy metals in food and water are also detected by biosensors (Nerayswamy, 2006). Detection of bacterial volatiles in food analysis using gas sensors were also deseribed in a patent by (Alocilja *et al*., 2002). Mascini and Palchetti (2000) studied that organophosphorus sand carbonate pesticides have gradually replaced organochlorines. Although, they have low environmental persistence but have higher toxicity. Biosensors are used to screen these compounds. Toxins in food have been detector using optical fluoro immunsensor capable of detecting multiple targets (mycotxin, bacterial toxins etc (Ligler *et al*., 2003).

KEY WORDS : Biosensors, GMF, Food contamination, Food safety, Toxicity

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Biosensor is described as a compact analytical device, incorporating a biological or biomimetic sensing element, either closely connected to or integrated within a transducer system (Garcia and Mottram, 2003). The principle of detection of biosensor is the specific binding of the analyte of interest to the complementary biorecognition element immobilized on a suitable support medium. The specific interaction results in a change in one or more physico-chemical properties (pH change, electron transfer, heat transfer) which are detected and may be measured by the transducer. The usual aim is to produce an electronic signal which is proportional in magnitude or frequency to the concentration of a specific analyte or group of analytes to which the biosensing element binds. (Turner *et al.*, 1987; Powner and Yalcinkaya, 1997). The application of biosensors to food analysis offers a rapid, cost-effective method for determining and measuring a wide range of target analytes, found in a variety of food stuffs within a diversity of situations. (Luong *et al.*, 1997) which include:

– The concentration of metabolites such as glucose, sucrose, alcohol, glutamate and ascorbic acid. (Mizutani *et al.*, 1998).
– The presence of contaminating agents such as micro-organisms, pesticides residues and antibiotics. (Baeumner, 2003).
– The residue from preservatives such as hydrogen peroxide and sulfites.

The advantages conferred by the use of biosensors include:

– A high degree of specificity for wide range of target analytes,
– Many sensor systems can be manufactured using mass

production techniques (e.g. screen printing), resulting in devices that are economic and disposable.

Biosensors can be designed for the use *in situ*; allowing real time monitoring of analytes. Biosensor system can be designed such as they can be operated at site, removing the need for centralized testing laboratory. This is one the major advantages of using biosensors. (Tapuhi *et al.*, 1996).

Components of biosensor :

– Biological element.
– Transducer.
– Signal processor.

Biological element :

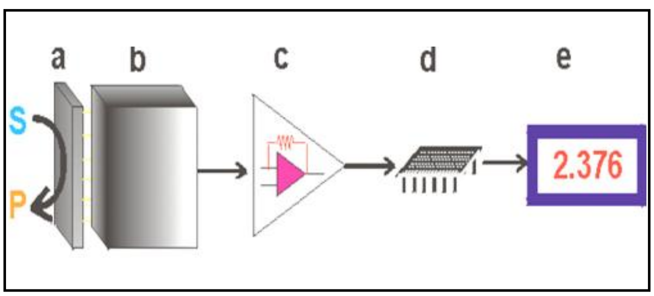
A sensitive biological element e.g., tissue, micro-organisms, enzymes, antibodies, nucleic acids and biologically derived materials like lipids, proteins etc. isolated from the cells are the main components of a biosensor. These sensitive elements can be created by biological engineering. These biological materials are ordinarily immobilized in close proximity to the transducer surface, thereby facilitating direct or mediated signal transfer to the transducer.

Transducer :

It is the instrument that transforms the signal resulting from the interaction of analyte with the biological element, into electrical signal that can be more easily measured and quantified.

Associated electronics or signal processors :

These instruments are primarily responsible for the display of results in a user friendly way.



– The platform for bioreaction where conversion of substrates to products take place
– Transducer
– Amplifier
– Processor
– Electronic displayer

Types of bio elements used in a biosensor :

– Enzymes
– Antibodies
– DNA
– Organelles, microbial cells
– Micro-organisms

Types of biosensors :

– Electrochemical biosensor
  Potentiometric biosensor
  Amperometric biosensor
– Optical biosensor
– Piezoelectric biosensor
– Calorimetric biosensor
– Fluorometric boisensor

Electrochemical biosensor :

These are based on monitoring the electro active species that are either produced or consumed by the action of the biological components e.g. enzymes and cells. Transduction can be performed using one of the several methods under 2 broad categories.

Potentiometric biosensor :

These are based on monitoring the potential of a system at a working electrode, with respect to an accurate reference electrode under conditions of essentially zero current flow. In operation, potentiometric measurement s are related to the analyte activity of a target species, described by the Nernst equation:

$$E = \frac{E_0 + RT}{n F \ln a}$$

(where ; E: Electromotive force, E_{0}: Standard potential for a, R: Gas constant, T: Absolute temperature at Kelvin, F: Faraday constant, n: Number of exchanged electrons, a: Activity of the species to be detected.)

These sensors operate by measuring the potential difference between sensing electrode resulting from accumulation of charge density. The electrical potential developed is usually proportional to the logarithm of the target analyte in the solution. However its use in food analysis was described by Dutta *et al.* (2001). Potentiometric biosensor system was assessed for the estimation of monophenolase activity in apple juice.

Amperometric biosensor :

Generally the use of amperometry as the method of transduction has proved to be the most widely reported. In contrast to potentiometric devices, the principle operation of amperometric biosensors is defined by a constant potential applied between the working and reference electrode. The imposed potential encourages redox reactions to take place causing a net current to flow. The magnitude of current is proportional to the concentration of electro active species present in the solution. Both cathodic (reducing) and anodic (oxidizing) reactions can be monitored amperometrically. Many of the amperometric biosensors described to date have been based on the use of enzymes. Typically oxidase enzymes have been the most frequently exploited catalysts used for these biosensor formats. Broadly the oxidase enzyme reaction can be described as follows:

$$\textbf{Substrate} + \textbf{oxygen} \rightarrow \textbf{product} + \textbf{hydrogenperoxide}$$

These biosensors tend to monitor the oxygen consumed using Clark Oxygen Electrode or hydrogen peroxide generated. Both are electrochemically active: oxygen can be electrochemically reduced and hydrogen peroxide can be oxidized. The current generated will be proportional to the concentration of enzyme (*0* the target analyte) present. Amperometric enzyme based biosensors are designed to monitor rancidification process in olive oils (Campanella *et al.*, 1999).

Optical biosensors :

These biosensors are based on measuring responses to illumination or light emission. Optical biosensors can employ a number of techniques to detect the presence of target analytes and are based on well found methods (Tothill, 2001) including Chemiluminescence *i.e.* emission of light and heat in a chemical reaction, fluorescence in which molecular adsorption of photons with longer wavelength but lower energy and phosphorescence which is a kind of photoluminescence *i.e.* emission of light by a bioluminescent plankton. Energy is absorbed a substance and is released actively and slowly in the form of light.

Surface plasmon resonance (SPR) is the most common method of transduction in optical biosensors. SPR occurs when surface plasmon waves are excited at a metal (e.g. gold liquid interface). Incident light is reflected from the surface that is not in contact with the sample solution. As a result of SPR, there is a reduction in intensity of the reflected light, at a specific angle and wave length. Since the angle is dependent on the reflective index of material present on the metal surface, SPR has been exploited to measure the binding of antibodies to antigens, immobilized at the sensor surface. In effect, biomolecular binding events will cause changes in the refractive index at the surface layers. These are used to determine the food quality, including the biotin and folate in infant formula milk and detection of food allergens. (Indyk *et al.*, 2000 and Mohammed *et al.*, 2001).

Piezoelectric biosensor :

The term piezoelectric means the ability to produce electric potential. Some substances like piezoelectric quartz crystals can be affected by the change of mass at the crystal surface. For practical applications, the surface of the crystal can be modified with recognition elements (e.g. antibodies that can bind specifically to a target analyte. If the crystal is placed in alternating electric field, the crystals are subjected to mechanical deformations. At a particular frequency, a mechanical or acoustic resonance is induced. The frequency of this response will be dependent on the size and mass of the crystal (Tapuhi *et al.*, 1996).

Calorimetric sensor :

These sensors based on the transduction method are designed to detect heat generated or consumed during a biological reaction. Many biochemical reactions are accompanied by heat absorption or production by using sensitive heat detection devices; biosensors for specific target analytes have been constructed. A thermometric biosensor system is to determine sucrose in sugar cane (Thavarungkul *et al.*, 1999).

Fluorometric biosensor :

Carlson *et al.* (2000) developed a fluorometric biosensor to detect and quantify aflatoxins, that are commonly found in a variety of agricultural products. They are produced by *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius*. They are carcinogenic in nature and may also cause death of a human if ingested at a higher rate.

Applications of biosensors in the field of agro- food industries:

There are numerous applications of biosensors in the field of agro- food industries. In addition to detection of micro-organisms it's also helpful in detecting food allergens and heavy metals.

Biosensors for heavy metal detection in soil, food and water:

Biosensor technology is a powerful alternative to conventional analytical technique, harnessing the specificity and sensitivity of biological systems in small, low cost devices. They have a lot of applications in agriculture. Biosensors detect the concentration of herbicides, pesticides and heavy metals in soil. They can also be used to forecast the possible occurrence of soil borne disease which has not been feasible with existing technology hence, reliable preventions and decontamination of soil borne diseases can be done at an earlier stage (Bachmann *et al.*, 2000 and Neethirajan *et al.*, 2005).

Heavy metal ions (Al^{3+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+} etc.) in the food have been determined using optodes that utilise immobilised chelating ligands that nearly specifically interact with metal ions. These optodes require fairly rigorous control of pH of the medium and can result in devices with very low detection limits and several optodes have been developed for the monitoring the chemical species. (Nerayswamy, 2006) .

Biosensors for food allergen detection :

The emerging health issue related to food induced allergic reactions present an important challenge to the food industry. The National Institute of Health estimates that six to seven million people in United States suffer from food allergies. Some of these people may develop serious or life threatening allergic reactions when exposed to causative protein. The major food allergens that account for more than 90 per cent of the all food allergies are found in peanuts, soybeans, milk, egg, fish, shell-fish, tree nuts, wheat.

Seafood is widely eaten around the world in coastal areas. It is rich in proteins and vitamins but can be potentially harmful if contained with histamine. Histamine is a biogenic amine involved in local immune response to allergy, which can be easily detected by electrochemical biosensor (Saaid *et al.*, 2008). It can accumulate in seafood when the bacterial infection is commenced in fishes the amino acid histidine converts to histamine and if that fish is consumed by humans it causes histamine poisoning, showing the symptoms of peppery mouth sensation, skin rashes, headache and dizziness. Histamine contamination does not alter the fishes normal appearance and odour. Therefore, a histamine biosensor using immobilised enzyme diamine oxidase (DAO) has been developed for rapid monitoring of histamine levels in tiger prawn (*Penacus monodon*) (Michaela *et al.*, 2000). The histamine biosensor has a response time of less than one minute and optimum pH operation 7.4.

Biosensors for microbial detection in food and water :

One of the main application of biosensors is envisioned in the area of water safety to detect water borne pathogens which pose major disease challenge in both developed and developing countries. WHO estimated 1.6 million deaths in 2005 which occurred from consequences of water related diseases traditionally the predominant techniques used to identify the water borne pathogens rely upon culture based approaches which is time consuming. Current practices for preventing microbial diseases reply upon careful control of various food and water borne pathogens. The main disadvantage of conventional detection methods are multi-step procedure and requires long time. Contamination of food and water by bacterial pathogens (such as *Escherchia coli, Salmonella typhimurium, Staphylococcus aureus, Streptococci* sp. etc.) results in numerous food and water borne diseases (Bernabei *et al.*, 1993). Infectious diseases account for nearly 40 per cent of the total 50 million annual estimated deaths worldwide.

Application of electrochemical biosensors is for detection of these food and water pathogenic bacteria (Ivnitski *et al.*, 2000). Apart from sensing pathogens, biosensors significantly detect the vapours evolved on the spoilage of product. New Zealand Meat Industry Research Institute is using biosensors to pinpoint the food spoilage. Apparently bacteria release gases which cause the packages to explode in transit. Optical biosensor detects diacetyl vapours evolved on the spoilage of meat (Shiers and Honeybourne, 1993). A patent describes the detection of bacterial volatiles in food analysis by using gas sensors and spectral food prints with advantages of indentification of particular microorganism (Alocilja *et al.*, 2002).

Listeria monocytogenes was also found in domestic animals causing encephalitis in humans showing symptoms like inflammation of brain, fever, headache etc. This bacterium was detected by an optical biosensor (Woifbeis, 1995).

Biosensors for beverage industries :

The beverage industries need rapid and affordable method to assure the quality of products where high performance liquid chromatograph (HPLC) or specific enzyme methods for toxin detection in beverage industries can be costly and laborious. Application of biosensors reduces the labour and cost (Luff *et al.*, 1998). The detection of lactose in milk is also determined by lactose biosensor based on Langmuir Blodgett film (Sharma *et al.*, 2004).

Biosensors for meat industry :

Examples of successful commercialised sensing instruments are meat check and bio check sensors. Meat sensor is a four electrode array attached to a knife which can be inserted into meat to measure glucose gradient immediately below the surface. The size of gradient is related to microbial activity on the surface of meat and is regarded as sound indicator of meat quality. The device provides in seconds what microbiology laboratory tests take. The biocheck method transforms glucose sensor into a device capable of detecting and quantifying microorganisms in aqueous solutions. The system transforms electrons from the respiratory pathway of microorganisms and is capable of detecting bacteria in two minutes (Maines *et al.*, 1996).

Biosensors for detecting Avian flu :

A team of Arkanas University has developed a biosensor for field rapid screening of avian influenza virus. This inexpensive device detects the avian influenza strain H5N1 from poultry in just less than 30 minutes.

Tiny biosensor :

A tiny portable biosensor is a big gun to fight against food contamination. Atrazine, a toxic herbicide in food and underground water, measures are taken to prevent their entering into food chain. A sensor allows the detection of this herbicide at a very low level (0.006 micrograms per litre) which is much lower to toxify. Hence, earlier determination can be done and steps can be taken to detoxify the food and water (Turner *et al.*, 1987).

Biosensors as loc concept :

LOC (Lab on a chip) is a generic term to describe the devices which integrate different laboratory functions on a single chip, capable of handling extremely small fluid volumes down to less than a pica litre. LOC devices represent a subset of Micro Total Analysis System dedicated to the integration of total sequence of lab processes to perform chemical analysis. LOC technologies allow chemical and biological process to be performed on a small glass plate with fluid channels known as microfluidic capillaries (Caramen *et al.*, 2007).

Biological threat agents :

The RAPTOR is a portable automated fibre optics biosensor for detection of biological threat agents. It performs rapidly (3-10 minutes). Fluorescent sandwich immunoassays on the surface of short polystyrene optical biosensor obtained a positive result is obtained reducing the logistical burden for field operations (George *et al.*, 2000). Biosensors for the detection of cancers are also developed carbon nanotube based biosensor is coated by antibodies to detect the cancer cells in tiny drops of water. This approach is used towards developing a biosensor for cancer detection.

Conclusion :

Apart from so many applications of biosensors their use is still underexploited because of an inherent conversation and inflexible regulatory system within this industry. The overview of biosensors on detection of food and environmental contamination demonstrates that previous challenges such as detection limit sensitivity and specificity have been overcome by many biosensors. Potential markets include the medical, military, food, and environmental industries. Those industries combined have a market size of $563 million for pathogen detecting biosensors and are expected to grow at a compounded annual growth rate of 4.5 per cent. The food market is further segmented into different food product industries.Often a biosensor is a portable inexpensive and suitable analytical tool but a reason for availability of bioelements make it difficult to adapt easily. But the increasing thrust on food quality and safety requires such analytical tools to specify the best quality, freshness and safety.

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