The history of the biosensor started in the year 1962 with the development of enzyme electrode by the scientist, Leland C. Clark. Biosensor is normally composed of transducers and biological components. The principle of detection of a biosensor is based on the specific interaction between the analyte of interest and the biological components. As a result of this specific interaction, different properties are changed, which can be detected and measured by transducers. The most important characteristics of biosensors are their specificity, high sensitivity, short response time; act as an integrated system, facility to automate them, versatility and low production cost. 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. The use of enzymatic biosensor technology in food processing, quality control and on-line processes is promising compared to conventional analytical techniques. Recently the biosensor market in the food industry has increased by fifteen fold amounting to $150 million. The use of biosensor in the food industry may include nutrient analysis, detection of natural toxins and antinutrients, food process monitoring by measuring enzyme activity and microbial contamination, and rapid detection of genetically modified organisms (GMOs), proteins, vitamin B complex, essential amino and fatty acids, and hazardous residual materials comprising pesticides and antibiotics. Biosensors are classified according to the transduction mechanisms and biological reception mechanism. The biosensor may be divided into basic four main groups based on the transduction mechanisms which include optical, mass, electrochemical and thermal biosensor. In addition, the biological component used in biosensors include enzymes, microbial, immune, and nucleic acids, biochips (protein, DNA, and cell chips), and biomimetic sensors, which utilize artificial bio recognition elements (electronic nose and electronic tongue).

**KEY WORDS:** Biosensors, Dairy, Food industry

their capacity to be incorporated into integrated systems, the facility to automate them, their capacity to work in real time, their versatility and low production Rassoly (2001), Mello and Kubota (2000) and Velasco-Garcia (2003).

The basic concept of biosensor can be illustrated by help of Fig. A. A specific biological component (say enzyme) recognizes a specific analyte and the transducer change in the biomolecule into electrical signal. The biological component is very specific to analyte to which it is sensitive. It does not recognize other analyte.

![Fig. A : Basic concept of biosensor](image)

The global biosensor market increased from $0.7 billion in 1997 to $8.9 billion in 2005. It is expected that this increasing trend will continue due to the increasing demand in the bionanotechnology sector. Currently, the biosensor market in the food industry has also increased by fifteen fold during the same period described above, amounting to $150 million. The need for biosensor to meet the increasing demand for new methods to evaluate food safety and functionally is expected to continually increase in the future.

The importance of biosensor in the food industry is to ensure the quality and safety of products and control process with effective, fast and economical methods (Liliana, 2009). Until now, the majority of marked food biosensors have been targeted for measurement of glucose, L-lactase, galactose, and alcohol (Warsinke, 1997). The use of biosensor in the food industry may include nutrient analysis, detection of natural toxins and antinutrients, food process monitoring by measuring enzyme activity and microbial contamination, and rapid detection of genetically modified organisms (GMOs). Pesticides and antibiotics can be measured using biosensors through enzymatic and immune reaction. Fungal toxins like aflatoxin; secondary metabolites including flavonoid compounds, which show functional activities; antinutrients such as trypsin inhibitor and lectin related with cytoplasmic membrane reactions are also measurable by complex formation between antigen and antibodies over biosensor transducers.

**Types of biosensors**:

Biosensors are classified according to the transduction mechanisms and biological reception mechanism. The biosensor may be divided into basic four main groups based on the transduction mechanisms which include optical, mass, electrochemical and thermal biosensor. In addition, the biological component used in biosensors include enzymes, microbial, immune, and nucleic acids, and biomimetic sensors (Jose, 2005).

**Biological components**:

**Indicator organisms**:

Indicator organisms are microorganisms or their metabolic products that may indicate the presence of a pathogen or harmful toxin (Escherichia coli fecal coliforms, enterococci, staphylococci, or Pseudomonas aeruginosa); or the possibility that faulty practices occurred during production, processing, storage, and distribution (coliform bacteria, Enterobacteriaceae, or total Gram-negative bacteria) (NRC and FPC, 1985). For some pathogens, Indicator systems are generally used to detect the potential contamination of pathogen because these are simple and economical than conventional method.

**Nucleic acid probes**:

The principle of selective detection is based on the detection of a unique sequence of nucleic acid bases through hybridization. Until now, culturing methods require 24 to 72 h for microbial examination. These methods are complex, time consuming, and strongly dependent on well-furnished laboratory facilities and expert personnel. The development of biosensors by using DNA and antibodies as sensing probes to shorten the time needed to measure and detect microorganisms of hygienic importance, such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*. The application of this biosensor to detect food poisoning bacteria and index microorganisms will be increasing in the near future (Edward, 1993).

**Bioluminescence**:

Chemiluminescence is the measurement of light emitted from a chemical reaction. When caused by biological enzymatically catalyzed reaction, this chemical reaction is often referred as bioluminescence. One example of a biosensor system utilizing chemiluminescence is the luciferase system. In this system, ATP from viable microorganism is detected and quantified by addition of the sample to the cofactor luciferin and the enzyme luciferase:

\[
\text{Luciferin} + \text{luciferase} \rightarrow \text{ATP} + \text{AMP} + \text{light} + \text{CO}_2
\]

This biosensor is capable of detecting bacteria in the range of 10^4 cells/ml in only a few minutes (Edward, 1993).

**Enzyme sensors**:

Enzyme-based biosensors primarily rely on two operational mechanisms. The first mechanism involves the catalytic transformation of analyte. The second mechanism involves the detection of analyte that inhibit or mediate the
enzyme’s activity. Basically, all enzyme sensors work by immobilization of the enzyme system onto a transducer.

Enzyme-based biosensor using penicillinase immobilized to detect penicillin in milk as it flows from truck to dairy (Caras and Tamata, 1980). The inactivation of index enzymes such as polyphenol oxidase (PPO), lipoygenase, and alkaline phosphatase (ALP) occurs by various heat treatment procedures in food processing. Biosensor is reported to measure the activities of the alkaline phosphatase, polyphenol oxidase for evaluating pasteurization of milk and blanching of vegetables, respectively.

**Immunosensor:***

Antibody based biosensor is also known as immunosensor. The high degree of specificity in antigen-antibody reactions is attractive in the development of biosensors. Although the use of antigen-antibody complex specificity may be utilized in all types of sensors, recent technology has been developed that utilizes mass changes on a piezoelectric crystal to measure this specific biological activity (Aizawa, 1991 and Luong and Guilboult, 1991). The change in mass that is due to the binding of biological material, such as antigen to antibody, will change the frequency of vibration of the crystal. This change of vibration may then be converted to an electrical signal by the crystal. A rapid *Salmonella* detection system utilizing this technology may soon be available.

**Microbial sensors:**

Microbial sensors are defined as microorganisms that are associated with a transducer. Given a sample with an unknown concentration of substrate, this type of biosensor measures the activity of the microorganism within the sensor. An example of a microbial biosensor is the ammonia gas sensor (Karube, 1991). This sensor uses nitrifying bacteria as the detector immobilized on an oxygen electrode. As the bacteria oxidize the ammonia, oxygen is consumed in direct proportion to the amount of ammonia substrate.

**Transducer element:***

**Optical biosensor:**

Optical transducers are particularly attractive as they can allow direct “label-free” and “real time” detection of bacteria. The phenomena of surface plasmon resonance (SPR), has shown good biosensing potential and many commercial SPR systems are now available (e.g. BIAcoreTM) (Homola, 1999). Haines and Patel (Haines, 1995) employed such an assay for detection of *Salmonella* and *Listeria*. Perkins and Squirrell (Perkins and Squirrell, 2000) have described the use of light scattering in combination with conventional SPR-based platforms with potential for the enhanced detection of bacterial cells. The example given was for the detection of *Bacillus subtilis* var. *niger* spores down to a concentration of $10^7$ spores/ml.

**Acoustic wave-based biosensor:**

Acoustic wave biosensors are based on the detection of mechanical acoustic waves and incorporate a biological component. These are mass sensitive detectors, which are operated on the basis of an oscillating crystal that resonates at a fundamental frequency (Babacam, 2000). The vast majority of acoustic wave biosensors utilise piezoelectric materials as the signal transducers. The most commonly used piezoelectric materials include quartz (SiO2) and lithium niobate (LiTaO3). Acoustic wave biosensors offer label-free, on-line analysis for antigen–antibody interactions, and also provide the option of several immunoassay formats, which allow increased detection sensitivity and specificity.

**Thermistor:**

This type of biosensors is constructed combining enzymes with temperature sensors. When the analyte comes in contact with the enzyme, the heat reaction of the enzyme is measured and is calibrated against the analyte concentration (Gregory, 1998).

**Electrochemical biosensor:**

Electrochemical biosensors are mainly used for detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc. The electrochemical biosensors can be classified based on the measuring electrical parameters as: (i) Impedimetric, (ii) Amperometric and (iii) Potentiometric (Sethi and Love, 1998).

**Impedimetric biosensor:**

The measured parameter is the electrical conductance/resistance of the solution. Impedimetric biosensors are less frequent compared to potentiometric and amperometric biosensors; nevertheless, there have been some promising approaches. Hybridization of DNA fragments previously amplified by a polymerase chain reaction has been monitored by an impedance assay (Davis, 2007).

**Amperometric biosensors:**

Amperometric biosensors are quite sensitive and more suited for mass production than the potentiometric ones (Ghindilis, 1998). Amperometric detection of micro-organisms involves the measurement of the current generated through electro-oxidation/reduction catalysed by their enzymes. They are often used on a large scale for analytes such as glucose, lactate, and sialic acid. Amperometric microbial biosensors have been widely developed for the determination of biochemical oxygen demand (BOD) for the measurement of biodegradable organic pollutants in aqueous samples.
Another application of amperometric microbial biosensors is the detection of heavy metal ions for environmental control as well as detection of microorganism for safety aspects. Amperometric sensors measure current such as the common glucose oxidase system (Babacan, 2000; Haines and Patel, 1995), which is based on the enzymatic reaction:

\[
glucose + oxygen + glucose oxidase \rightarrow H_2O_2 + gluconate
\]

Potentiometric biosensors:

Potentiometric biosensors are based on ion-selective electrodes (ISE) and ion-sensitive field effect transistors (ISFET). A potentiometric biosensor consists of a perm-selective outer layer and a bioactive material, usually an enzyme. The enzyme-catalysed reaction generates or consumes a species, which is detected by an ion selective electrode. The developed light addressable potentiometric sensor (LAPS), based on the FET has proved to be suitable for detection of microbial contamination in dairy-food product (Aizawa, 1991). Application of this biosensor is the detection of pestis, Bacillus globigii spores and to detect S. typhimurium to levels as low as 119 CFUs.

Conclusion:

Although conventional methods for the detection and identification of microbial contaminants can be very sensitive, inexpensive and present both qualitative and quantitative information, they can require several days to yield results. Biosensors have demonstrated potential for food microbial analysis; they provide miniaturized systems that can be integrated with online process monitoring systems to analyze samples. Because of their small size, the sensors can be incorporated into various food industries.

LITERATURE CITED


