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Biosensors in Food Processing - A Review

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ABSTRACT

Biosensors are not commonly used for food microbial analysis; they have great potential for the detection of microbial pathogens and their toxins in food. They enable fast or real-time detection, portability, and multipathogen detection for both field and laboratory analysis. Several applications have been developed for microbial analysis of food pathogens, including E. coli O157:H7, Staphylococcus aureus, Salmonella, and Listeria monocytogenes, as well as various microbial toxins such as staphylococcal enterotoxins and mycotoxins. The technology is based on a specific biological recognition element in combination with a transducer for signal processing. Improving the preservation or increasing the yield of agricultural products is a common aim for the producers. However, the use of xenobiotics clearly represents a potential risk for consumers. Finally the concern on the safety issue is a key aspect for food industry, regulatory agencies and control laboratories. Along with instrumental analytical protocols there is the need for complementary techniques to detect quality parameters and safety threats which can be applied in delocalised analysis or as rapid screening methods, viz., Bioassays, immunoassays, chemical assay , lateral flow devices. In addition to these, biosensors represent an interesting alternative that may respond to a number of analytical problems.

Key words: *Biosensors, Food pathogens, Bio-reactors, Optical biosensors, Electro-chemical biosensors*

INTRODUCTION

Biosensor is a progressing interdisciplinary research between analytical chemistry, biology and microelectronics[16]. A biosensor can be defined as an integrated receptor transducer device, which is capable of providing selective quantitative or semiquantitative analytical information using a biological recognition element[20].The most widely accepted definition of biosensors is: "a self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction ele xxxx purpose of detecting (reversibly and selectively) the concentration or activity of chemical species in any type of sample." [9]. An enzyme is capable of recognizing a specific target molecule (Fig. 1). This biorecognition capability of the enzyme is used in biosensors.

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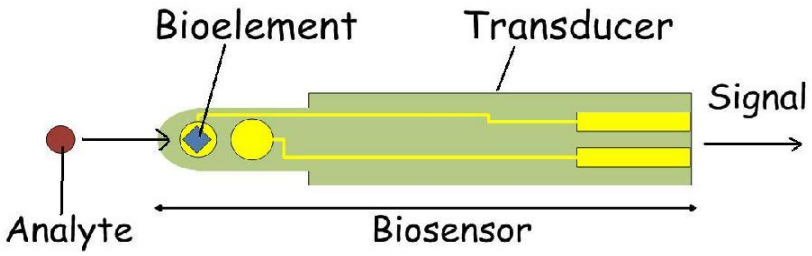
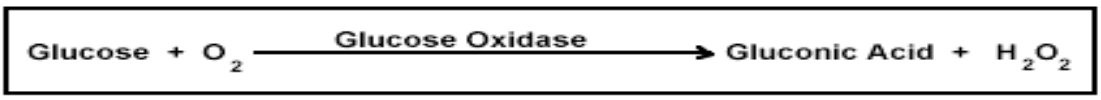
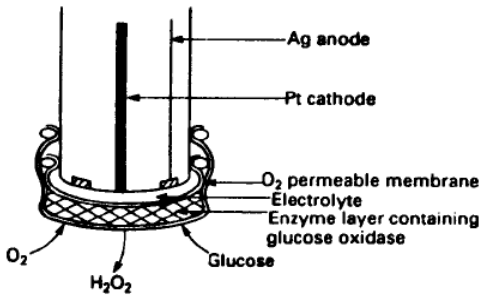


Fig. 1: Basic scheme of a biosensor

HISTORY OF BIOSENSORS

The biosensor was first described by Clark and Lyons in1962, when the term *enzyme-electrode* was adopted. The primary target substrate for this system was glucose so it led to the development of the first glucose analyzer[21].





Source [21]

PRINCIPLE OF BIOSENSORS:

The basic principle of biosensor technology is to convert a biologically induced recognition event (e.g., enzyme, antibody) into a detectable signal, via a transducer and processor. The end result is a display depicting both the presence and the concentration of the target analyte. The bioreceptor is a biomolecule that recognizes the target analyte[21]. A bioreceptor can be a tissue, microorganism, organelle, cell, enzyme, antibody, nucleic acid and biomimic[20]. The transducer converts the recognition event into a measurable signal[21]Transduction may be optical, electrochemical, thermometric, piezoelectric, magnetic and micromechanical or combinations of one or more of the above techniques[20].

Biosensor = Bioreceptor + Transducer

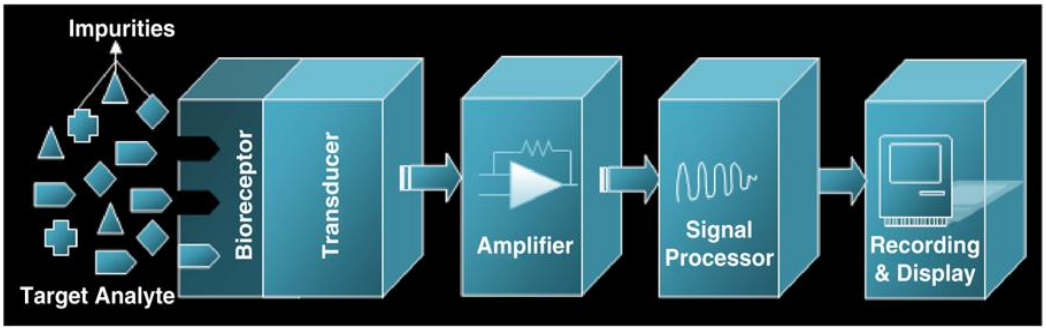


Fig. 2: Schematic diagram of a biosensor[20]

Analytical chemistry plays an important role in food quality parameters because almost every sector of industry and public service relies on quality control. A food quality biosensor is a device, which can respond to some property or properties of food and transform the response(s) into a detectable signal, often an electric signal. This signal may provide direct information about the quality factor(s) to be measured or may have a known relation to the quality factor[17].

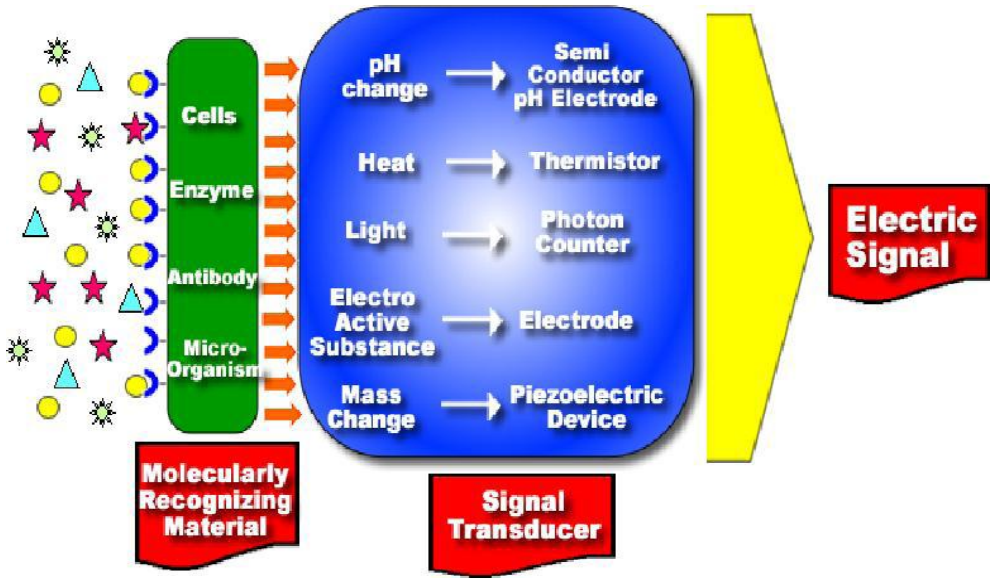


Fig. 3: Principle of biosensor[17]

The main methods of immobilization, particularly for enzymes, include physical adsorption, entrapment in a matrix (using gels, polymers, or printing inks), covalent binding, or electrochemical polymerization and photo polymerization. Physical adsorption is generally based on interactions such as van der Waals forces between the biological element and the transducer.

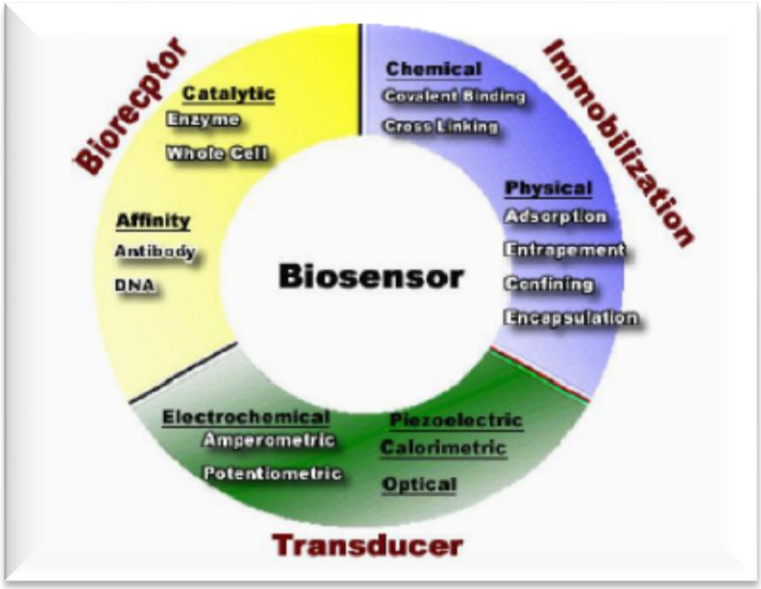


Fig. 4 Components of biosensor[17]

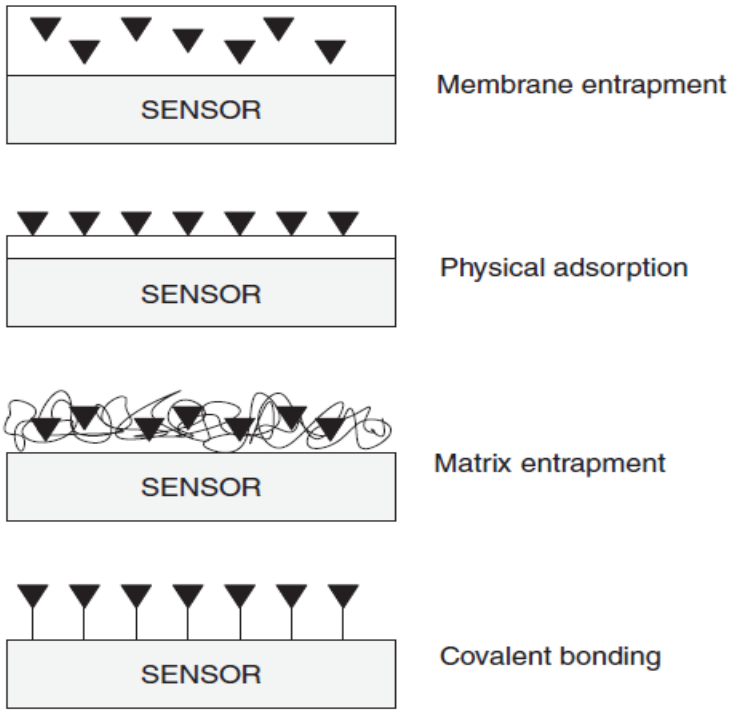


Fig. 5: coupling of bio-recognition element with the transducer on a substrate

CLASSIFICATION OF BIOSENSORS:

Various types of biosensors have been developed, which can be divided into 2 general classes: direct- and indirect-detection biosensors. Direct-detection sensors (Figure: 6A), in which the biological interaction is directly measured in real time, are typically non-catalytic elements such as cell receptors or antibodies. Indirect-detection sensors as shown in Figure: 6B. These rely on a primary recognition reaction that binds the analyte to a substrate followed by a secondary recognition reaction that binds antibodies as the recognition element are called immunosensors. Direct-detection biosensors are simpler and faster but typically yield a higher limit of detection than indirect-detection systems.

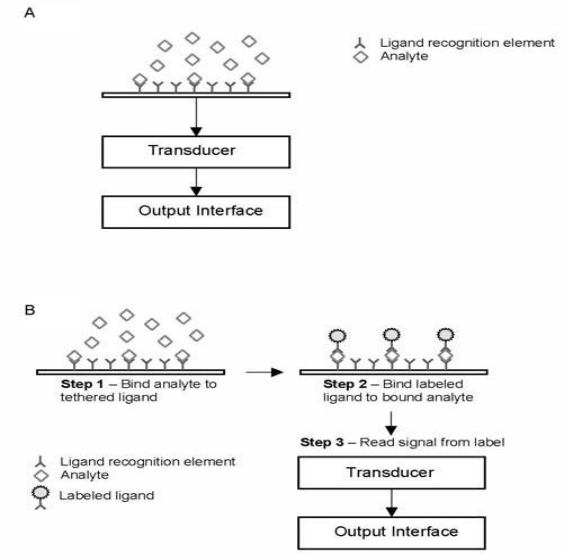


Fig. 6: Generic schematic of biosensors:

A. Direct detection biosensors where the recognition element is label-free;

B. Indirect-detection biosensors where the analyte is first bound to a surface with subsequent labeling[2].

SPR- Surface Plasma Resonance, QCM- Quartz Crystal microbalance, SAW-Surface Acoustic Wave[20] .

BIORECEPTORS

a) Antibody Bioreceptors:

Antibodies are common bioreceptors used in biosensors. Antibodies may be polyclonal, monoclonal or recombinant, depending on their selective properties and the way they are synthesized. In any case, they are generally immobilized on a substrate, which can be the detector surface, its vicinity, or a carrier.An antigen-specific antibody fits its unique antigen in a highly specific manner, so that the three dimensional structures of antigen and antibody molecules are matching. Due to this three-dimensional shape fitting, and the diversity inherent in individual antibody make-up, it is possible to find an antibody that can recognize and bind to any one of a large variety of molecular shapes. This unique property of antibodies is the key that makes the immunosensors a powerful analytical tool and their ability to recognize molecular structures allows one to develop antibodies that bind specifically to chemicals, biomolecules, and microorganisms[20].

b) Enzyme bioreceptors

Biosensors that make use of enzymes as the biorecognition elements are a well developed and widely studied area. Enzymes are chosen based on their specific binding capability and their catalytic activity and the chosen enzyme with a suitable substrate should provide sufficient electron transfer to the working electrode[20]. 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[11]**.**

c) Nucleic acid bioreceptors

Biosensors based on nucleic acid as biorecognition element are simple, rapid, and inexpensive and hence it is widely used in pathogen detection. In contrast to enzyme or antibodies bioreceptors, nucleic acid recognition layers can be readily synthesized and regenerated. DNA damage is one of the most important factors to be considered when nucleic acid bioreceptor are used.

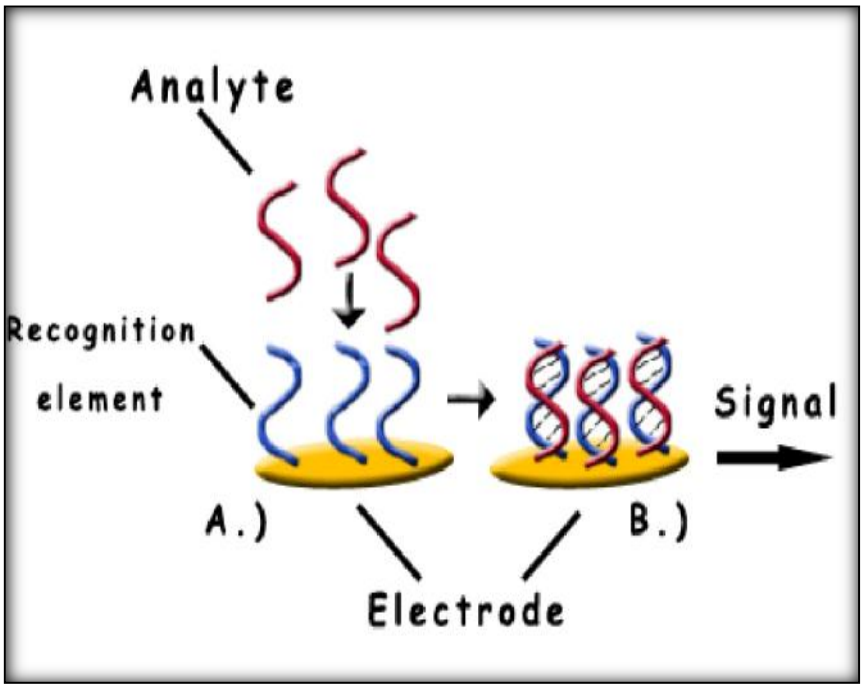


Fig. 8: General DNA biosensor scheme

Target DNA is captured at the recognition layer (A), and the resulting hybridization is transduced into a measurable electronic signal (B)[8].

d) Cellular bioreceptors

In cellular structures/cells based bioreceptors biorecognition is either based on whole cell/microorganism or a specific cellular component that is capable of specific binding to certain species. The cellular systems, enzymes and non-enzymatic proteins are the three major sub-classes of this category. Since the biosensors based on enzyme bioreceptors.

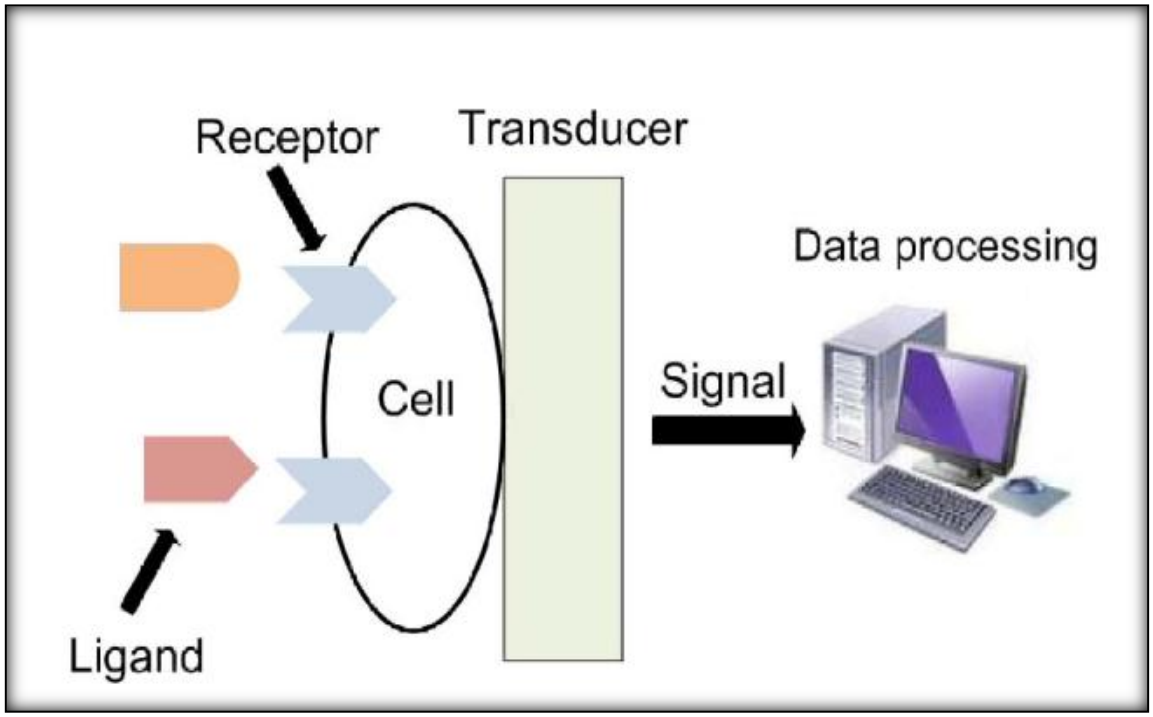


Fig. 9: scheme of cell-based biosensors. (Gordon and Bobby 2005)

e) Biomimetic receptors

A receptor that is fabricated and designed to mimic a bioreceptor (antibody, enzyme, cell or nucleic acids) is often termed a biomimetic receptor. Using biomimetic polymer sensor, presented a new platform for visual and spectroscopic detection of bacteria

f) Bacteriophage Bioreceptors

Recently, bacteriophages are employed as biorecognition elements for the identification of various pathogenic micro organisms. These powerful bacteriophages (phages) are viruses that bind to specific receptors on the bacterial surface in order to inject their genetic material inside the bacteria. These entities are typically of 20–200 nm in size.Phages recognize the bacterial receptors through its tail spike proteins. Since the recognition is highly specific it can be used for the typing of bacteria and hence opened the path for the development of specific pathogen detection technologies.

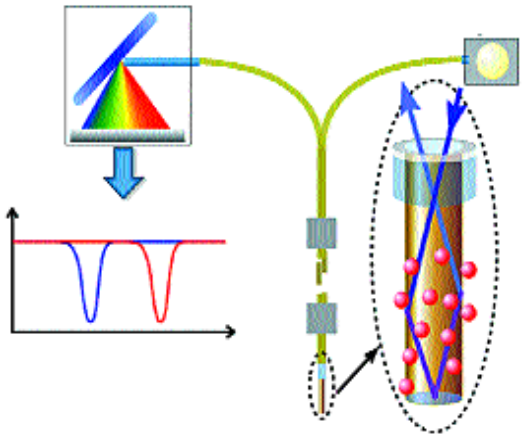
2. TRANSDUCERES

The transducer plays an important role in the detection process of a biosensor

A. OPTICAL BASED BIOSENSORS

Optical biosensors have received considerable interest for bacterial pathogen detection due to their sensitivity and selectivity

i. **Fiber optic biosensor:** White light passes through the optical fiber into the gold-tipped sensor probe, where bio-molecular binding events cause changes in the **refractive index** that affect the spectrum of the **reflected light.**
ii. **Surface Plasmon Resonance:** It is able to detect minor changes in the refractive index, which occur when cells binds to receptors immobilized on the transducer surface.It measures the change of the **angle of the reflected light** as a function of change of **density of medium** against time[8].



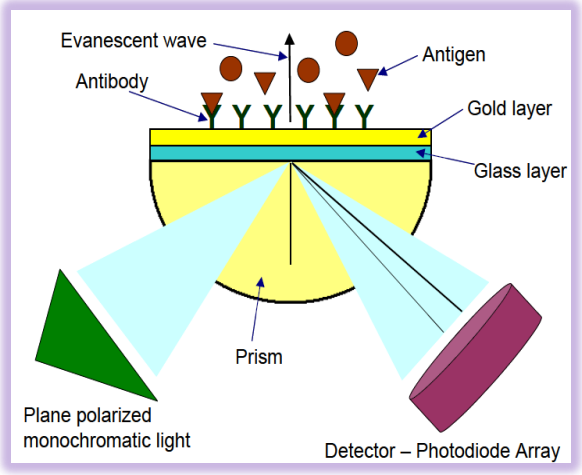


Fig. 10 i) Fiber optic biosensor **ii) Surface Plasmon Resonance**

A common method which uses reflectance spectroscopy for the pathogen detection is surface plasmon resonance (SPR). SPR is able to detect minor changes in the refractive index, which occur when cells binds to receptors immobilized on the transducer surface and it measures the change of the angle of the reflected light as a function of change of density of medium against time. Direct label-free detection of pathogens is also possible using this method. SPR based biosensors have been reported by many researchers for the detection of foodborne pathogens such as L.monocytogenes [3, 10, 18] .

B.ELECTROCHEMICAL BIOSENSORS

Electrochemical biosensors are based on monitoring electroactive species that are either produced or consumed by the action of the biological components (e.g., enzymes and cells). Transduction of the produced signal can be performed using one of several methods under two broad headings: Potentiometric Biosensors and Amperometric Biosensors

Potentiometric biosensors : 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 process, potentiometric measurements are related to the analyte activity (of a target species) in the test sample.

Amperometric Biosensor: The use of amperometric biosensors in signal transduction has proved to be the most widely reported using an electrochemical approach. Both "one-shot" (disposable) sensors and on-line (multimeasurement) devices are commercially available, monitoring a wide range of target analytes. In contrast to potentiometric devices, the principle operation of amperometric biosensors is defined by a constant potential applied between a working and a reference electrode. The applied potential results in redox reactions, causing a net current to flow. The magnitude of this current is proportional to the concentration of electro active species present in test solution and both cathodic (reducing) and anodic (oxidizing) reactions can be monitored amperometrically. Most of the amperometric biosensors described use enzymes as the biorecognition element. Typically, oxidase and dehydrogenase enzymes have been the most frequently exploited catalysts used for these biosensor formats [17].

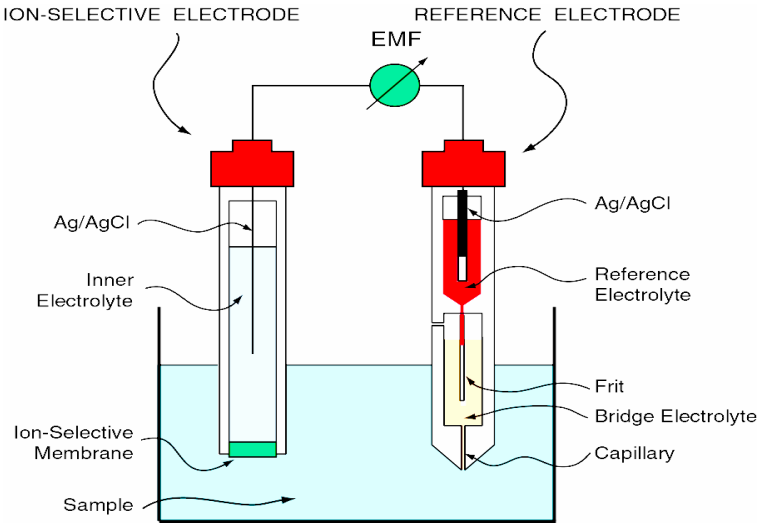


Fig. 11: Potentiometric biosensors[8]

Impedimetric biosensor: The integration of impedance with biological recognition technology for detection of pathogens has led to the development of impedance biosensors that are finding wide-spread use in the recent years.

C. MASS BASED BIOSENSORS

Piezoelectric biosensors: Piezoelectric quartz crystals can be affected by a change of mass at the crystal surface; this phenomenon has been successfully exploited and used to develop **acoustic biosensors.**

QCM (quartz crystal microbalance) biosensors: The surface of the crystal can be modified with recognition elements (e.g., antibodies) that can bind specifically to a target analyte.

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