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Biosensors for detection of food borne pathogens

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Abstract
A biosensor is an analytical device, which converts a biological response into an electrical signal. Food processing industry faces various challenges; one of the foremost challenges is the need for quick and cost effective methods to detect the presence of allergenic components and pathogens in the food. Biosensors pave way for the rapid detection of pathogens, allergens as well as pesticide residues in food. Detection of contaminants, verification of product contents, product freshness and monitoring of raw materials conversion are the areas of potential biosensor applications. Biosensors have the potential to produce an analytical revolution to resolve the challenges in the agricultural and the food industries. This review focuses on the application of biosensors for contaminants in food system.

Keywords: Biosensors, detection, pathogens, borne

1. Introduction
Bacteria are present everywhere, thus their existence in food is natural. The majority of bacterial strain are rendered either harmless or even beneficial to humans. However, several others, being pathogenic in nature cause infectious diseases. The World Health Organization (WHO) defines food borne illnesses as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food (WHO, 2007) [1]. The most probable carriers of food-borne pathogens like *E. coli*, *Salmonella*, *Listeria* and *Campylobacter jejuni*, are undercooked meat, poultry, sea foods, vegetables, milk and milk products. Many researchers identified the various food borne pathogens including *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 that have been generally found to be responsible for majority of food-borne illness (Alocilja and Radke, 2003; Chemburu *et al.*, 2005) [2, 3]. There are various methods towards pathogen detection, identification and prevention viz. conventional and rapid methods which largely rely on microbiological and biochemical analysis. Traditional conventional methods can be highly accurate, sensitive, and give both qualitative and quantitative information on the number and the nature of the micro-organisms tested, but these are labor intensive, cost-ineffective and non-amenable to integration for on-site diagnosis, which takes up to 5–7 days to get a result (Yang and Bashir 2008) [4]. While rapid methods are less time-consuming than the traditional conventional methods, which usually takes 30 mins or a few hours to achieve detection result (Lazcka *et al*., 2007; Sapsford *et al*., 2008) [5, 6].
Recently biosensors have been looked upon as attractive alternatives to the existing routine of conventional pathogen detection platforms. Biosensors are analytical devices composed of a biological recognition element (such as enzyme, antibody, receptor or microorganisms) linked to a chemical or physical transducer (electrochemical, mass, optical and thermal), which convert a specific bio-recognition results into a measurable signal. These devices have several advantages such as high degree of sensitivity and specificity of detection, minimal sample pre-enrichment and secondary enrichment steps, cost-effectiveness, miniaturization and portability for fast analysis, real time monitoring and reduced overall time required for detection in platforms of raw material reception, quality control laboratories or some other stages during the food processing (Luong *et al*., 1991) [7] as compared to conventional microbiological, immunological and molecular biological methods. The first "biosensor" was developed by Clark in 1956 [8] and further subsequently illustrated by Clark and Lyons in 1962[9] by sandwiching soluble glucose oxidase (GOx) between an outer dialysis membrane and the gas permeable membrane of an amperometric oxygen (O_2) electrode. Clark and Lyons found that the reduction in the concentration of dissolved oxygen is proportional to the concentration of glucose in the sample.

Generation of Biosensors

Biosensors can be divided into three generations depends on the level of integration i.e. the method of attachment of the biorecognition element or the bioreceptor molecule to the base of the transducer element (Arshak *et al*., 2009) [10].

1. **First generation biosensors:** are based on direct detection of substrate and product of the enzyme rection e.g. electrochemical biosensors for the detection of glucose concentration (Clark, 1956) [8]. Where the biocatalyst is either bound to or entrapped in a membrane, which in turn is immobilized on the surface of the transducer.
2. **Second generation biosensors:** in which co-reactants/ enzymes are co-immobilized the biologically active component using mediators on the surface of the transducer. It permits the elimination of semi-permeable membrane in order to improve the analytical quality and to simplify the performance.
3. **Third generation biosensors:** in which the biocatalyst are directly bind to an electronic device that transduces into measurable electrical and it further amplified the signal e.g. conducting polymer-based biosensors

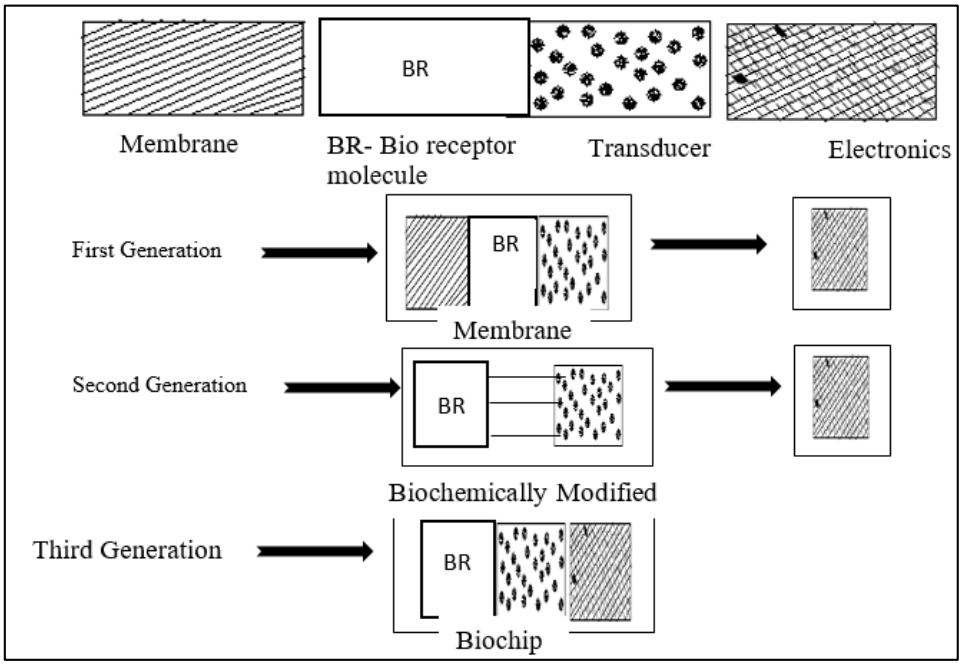


Fig 1: Three generations of biosensor

Working Principle of Biosensor

Biosensor is an analytical device that is a fusion of biology and sensing, which converts a biological response into an electrical signal proportional to the analyte concentration (Arora *et al*., 2011) [11]. This signal can obtained from a change in protons concentration, release or uptake of gases, light emission, absorption and by the metabolism of the target compound by the biological recognition element. It comprises two main components: a bio-receptor/bio-recognition component, which identifies the objective analyte and a transducer, for converting the recognition event into a measurable electrical signal. A bio-receptor can be a tissue, microorganism, organelle, cell, enzyme, antibody, nucleic acid, bacterial phages and bio-mimic etc. While transduction may be magnetic, electrochemical, piezoelectric, optical, thermometric and micromechanical or combinations of one or more of the above techniques. The transducer converts this biological signal into a measurable response such as current, potential or absorption of light through electrochemical or optical means. The amplifier responds to the small input signal from the transducer and delivers a large output signal that contains the essential waveform features of an input signal. Further, the amplified signal is then processed by the signal processor where it can later be stored, displayed and analysed. The detection principle is based on the ability of the transducer to transform a biochemical and/or physic-chemical change into a measurable signal (Thevenot *et al.*, 2001; Patel, 2002) [12, 13] as a result of a bio-recognition event between the biological recognition element and its target analyte.

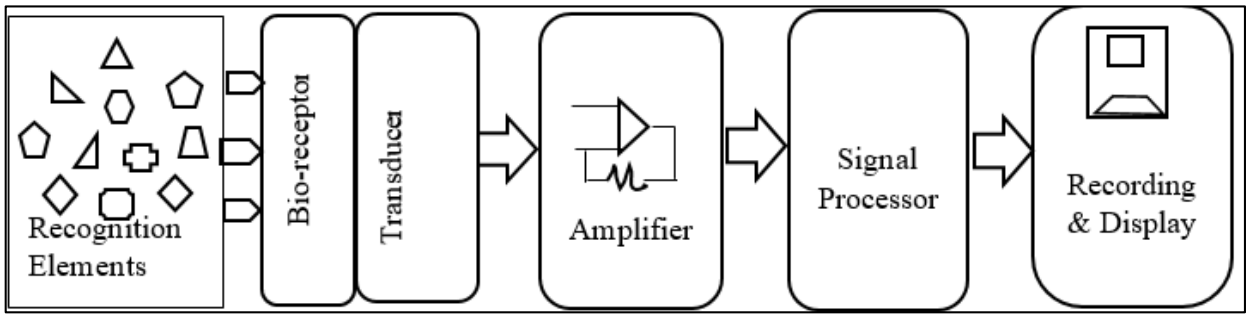


Fig 2: Schematic diagram of a biosensor (Adopted from Velusamy *et al*., 2010) [1]

Characteristics of biosensors

The fundamental characteristics of a biosensor (Stoytcheva *et al.*, 2009; Leonard *et al.*, 2004) [15, 16] comprise as

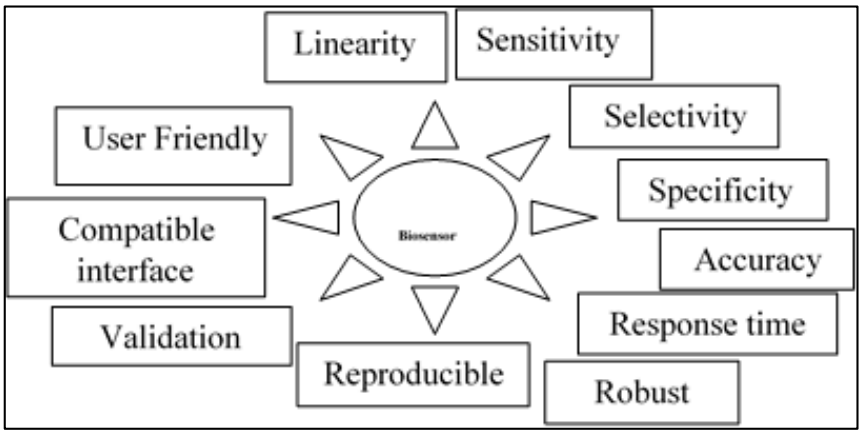


Fig 3: Characteristics of biosensors

Classification

Biosensors may be classified either according to the mechanism of biological selectivity (Bio receptor) or, on the mode of physio-chemical signal transduction (transducers).

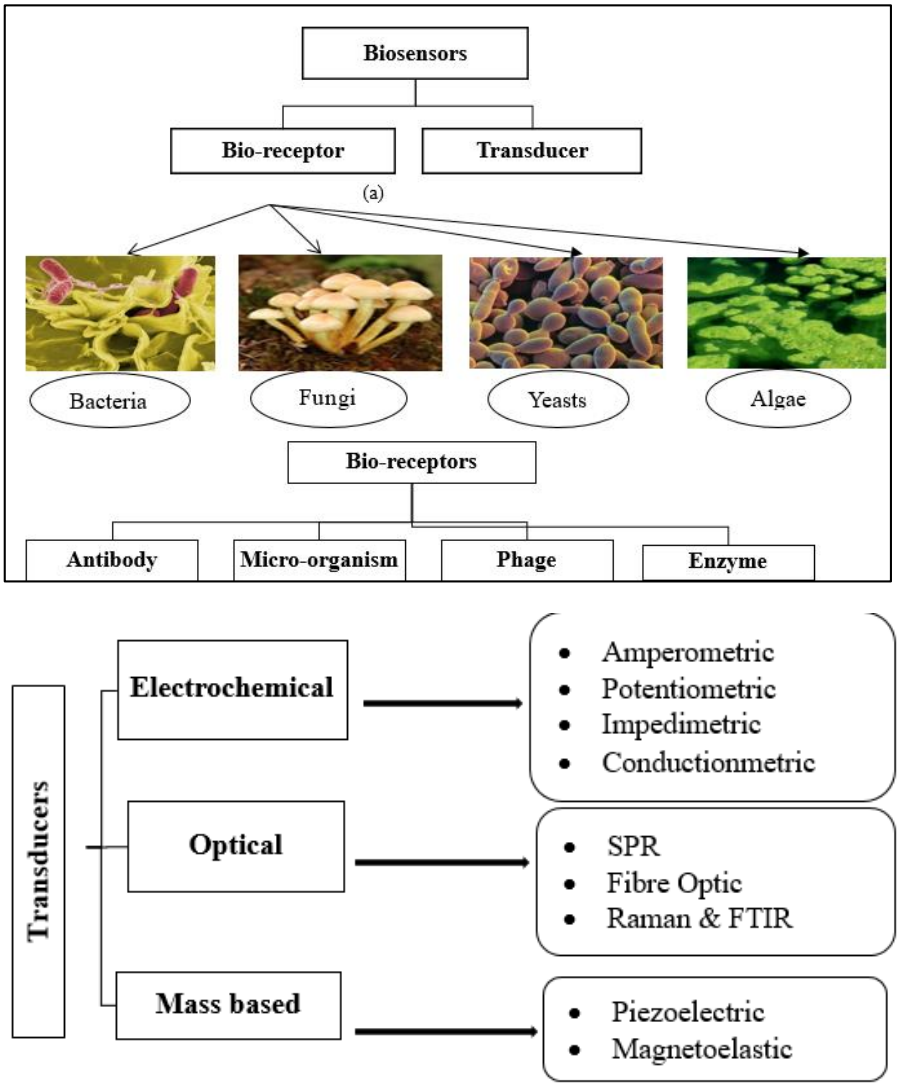


Fig 4: Classification of biosensors (a): Basic components of biosensors, (b): Bio-receptors & (c): Transducers)

Bio-receptors

A bio-receptor is a biomolecule that exploits a biochemical mechanism for recognition, which are responsible for binding the target analyte to the sensor for measurement (Velusamy *et al.*, 2010) [14]. Bio-receptors can broadly be classified into five distinct major categories. These categories includes antibody-antigen bio-receptor, enzymatic bio-receptor, nucleic acids (DNA) bio-receptor, cellular structures or cellular bio-

receptor, biomimetic bio-receptor and bacteriophage bio-receptor.

Antibody bio-receptor

Antibodies are common bio-receptors used in biosensors, which may be polyclonal, monoclonal or recombinant based on their selective properties and synthesis. Willis *et al*. (2013) [17] found that it has a three-dimensional structures of antigen and antibody molecules where an antigen and its antigen-specific antibody interact in a way alike to a lock and key fit.

Enzyme bio-receptor

Another type of commonly used bio-receptors involves enzymes, which are generally taken as bio-receptors based on their specific binding capabilities as well as their catalytic activity which depends upon the integrity of their native protein conformation. Sassolas *et al*. (2012) [18] found that the detection of pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli* and *Campylobacter jejuni* can be done by labeling the antibody with most frequently used enzymes such as Horseradish Peroxidase (HRP) and beta-galactoxidase.

Bacteriophage bio-receptors

Recently, bacteriophages are used as bio recognition elements for the identification of various pathogenic microorganisms. These powerful bacteriophages (phages) are viruses of 20-200 nm in size (Singh *et al*., 2009) [19] that bind to specific receptors on the bacterial surface in order to inject their genetic material inside the bacteria. Phages recognize the bacterial receptors via its tail spike proteins. Many researchers have studied the application of phage as a bio recognition element for the detection of various pathogens such as *E. coli* (Singh *et al.,* 2009) [19], *S. aureus* (Balasubramanian *et al.,* 2007) [20] and *B. anthracis* spores by using different sensing platforms (Xie *et al.,* 2009) [21].

Nucleic acid bio-receptors

In the case of nucleic acid bio-receptors for pathogen detection, the identification of a target analyte's nucleic acid is achieved by matching the complementary base pairs (adenine: thymine (A:T) and cytosine: guanosine (C:G) pairing in DNA) that are generally the genetic components of an organism. Since each organism has unique DNA sequences, any self-replicating microorganism can be easily identified. Wide range of physical, chemical and biological activities of nucleic acid based biosensors, many researchers studied on it for the detection of food pathogen like *E. coli* O157:H7 (Chen *et al.,* 2008) [22], *Cumpylohacter jejuni* (Uyttendaele *et al.,* 1997) [23], *Salmonella* spp. (Lermo *et al.,* 2007) [24] etc.

Biomimetic receptors

An artificial receptor that is fabricated and designed to mimic a bio-receptor (antibody, enzyme, cell or nucleic acids) is generally termed a biomimetic receptor. Silbert *et al*. (2006) [25] demonstrated a new platform for visual and spectroscopic detection of bacteria. The detection principle is based on the interaction of membrane-active compounds secreted by bacteria with agar embedded nanoparticles comprising phospholipids and the chromatic polymer polydiacetylene (PDA). Where PDA undergoes dramatic visible blue-to-red transformations together with an intense fluorescence emission that are induced by molecules released by multiplying bacteria. This can be used for detection of both Gram-positive and Gram-negative bacteria. In addition, Biochromic conjugated polymer (BCP) sensors are another type of biomimetic based biosensor for pathogen detection, demonstrated by (Song *et al.,* 2002) [26] where biologically active cell membrane components is incorporated into conjugated polymers with desirable optical properties.

Transducers

The transducer plays an important role in the detection process of a biosensor by monitoring the activity of the biological component for a substrate by the oxygen consumption, hydrogen peroxide formation, and changes in NADH concentration, fluorescence, absorption, pH change, conductivity, temperature or mass. Thus, the biosensor can be classified in several types based upon the transduction methods they employ such as : potentiometric [ion-selective electrodes (ISEs), ion-sensitive field effect transistors (ISFETs)], amperometric, impedimetry, calorimetric, optical, piezoelectric and mass based transducers and they contain many different subclasses and they can be further divided into label and label-free (non-labeled) methods. Where, the label and label-free detection methods depend on the detection of a specific label and the direct measurement of a phenomenon occurring during the biochemical reactions on a transducer surface respectively.

Optical-based biosensors

Optical-based biosensors can use a large number of spectroscopy like absorption, reflection, refraction, dispersion, infrared, Raman, chemi-luminescence, fluorescence, and phosphorescence. Optical biosensors are most commonly used for the detection of bacterial pathogen due to their sensitivity and selectivity (Dey and Goswami, 2011) [27]. Surface plasmon resonance and fluorescence are most commonly employed for the bacterial detection due to their sensitivity. Optical techniques using fiber optics, laser, prism and waveguides are also employed for the detection of bacterial pathogen.

Raman and Fourier transform infrared spectroscopy

Whole organism fingerprinting techniques involves vibrational spectroscopies such as Raman scattering and Fourier transform infrared (FT-IR). Many researchers investigated that Raman spectroscopy is an optical technique based on light scattering, used for rapid detection of bacterial pathogens. Schmilovitch and co-workers operated a dispersive system spectrophotometer at 785 nm diode laser for the detection of Gram-positive and Gram-negative bacteria (Schmilovitch *et al.,* 2005) [28] and obtained a clear distinction between clean samples and samples containing bacteria. Recently, Raman chemical imaging spectroscopy (RCIS) is used to detect the trace levels of pathogen without the use of amplification or enhancement techniques (Kalasinsky *et al*., 2007) [29], which combines Raman spectroscopy, fluorescence spectroscopy, and digital imaging.

Fourier transform infrared (FT-IR) spectroscopy: is a non-destructive computational technique which involves the collection of spectra based on calculation and evaluation of the coherence of a radiative source with the aid of space-domain or time domain measurements of the electromagnetic radiation or any other type of radiation. It has considerable potential for application in the foodborne pathogen detection. The FT-IR spectrometry is demonstrated to differentiate *E.*

coli O157: H7 from other bacteria inoculated into apple juice (10^9 CFU/mL) (Al-Holy *et al*., 2006) [30]. Another report demonstrated the use of FT-IR spectrometry to differentiate between intact and injured *Listeria* and to distinguish this strain from other selected *Listeria* strains (Lin *et al*., 2004) [31]. It has been reported that FT-IR technique can be used directly on the surface of food to produce biochemically interpretable "fingerprints" (Ellis *et al*., 2002) [32]. FT-IR has also been utilized for the compilation or recognition of various food borne pathogens such as *Yersinia, Staphylococcus, Listeria, Klebsiella, Enterobacter, Citrobacter, Salmonella*. Devis and co-workers reported that FT-IR spectrometry can be implemented to detect *E. coli* O157: H7 from ground beef (Davis *et al*., 2010) [33].

Surface plasmon resonance

Surface plasmon resonance (SPR) is a robust tool that can measure the binding kinetics of two molecules without the help of any fluorescent tag, uses reflectance spectroscopy for the pathogen detection. Whereas 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 change of angle of reflected light as a function of change of density of medium against time. SPR is also used for direct label-free detection of pathogens. Many researchers reported the detection monitoring of foodborne pathogens on basis of SPR based biosensors such as *L. monocytogenes* (Bhunia *et al.,* 2004; Koubova *et al*., 2001; Taylor *et al*., 2006)[34, 35, 36], *Salmonella*, (Bhunia *et al*., 2004; Koubova *et al*., 2001; Oh *et al*., 2004; Taylor *et al*., 2006) [34, 35, 36, 37], *E. coli* O157:H7 (Meeusen *et al*., 2005; Subramanian *et al*., 2006; Taylor *et al*., 2005; Taylor *et al*., 2006; Waswa *et al*., 2007) [36, 38, 39, 40, 41], and *C. jejuni* (Taylor *et al*., 2006) [36].

Electrochemical biosensors

According to the IUPAC definition, an electrochemical biosensor is a self-contained integrated device, which is able to provide specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct and spatial contact with the transduction element. Velusamy and co-workers reported that these techniques are an addendum of conventional antibody based enzyme immunoassays (ELISA), comprises the catalysis of substrates by an enzyme conjugated to an antibody and products' production. It can be detected in the form of ion or oxygen consumption and pH change due to generation of electrical signals on a transducer (Velusamy *et al.,* 2010) [14].

Amperometric method

Amperometric transduction is the most common electrochemical detection method in which the sensor potential is set at a value where the analyte produces current. So, the applied potential serves as the driving force for the electron transfer reaction, and the current produced is a directly proportional to the rate of electron transfer. These sensitive biosensors can be used in order to detection of various foodborne pathogens such as *E. coli* O157:H7 (Abdel-Hamid *et al*., 1999; Chemburu *et al.,* 2005; Ruan *et al*., 2002; Varshney *et al*., 2005) [3, 42, 43, 44], *Salmonella* (Abdel- Hamid *et al.,* 1999; Brooks *et al*., 1992; Yang *et al*., 2001) [42, 45, 46], *C. jejuni* (Chemburu *et al.,* 2005) [3] and *L. monocytogenes* (Crowley *et al.,* 1999; Chemburu *et al*., 2005) [3, 47]. This approach is used for determining the presence, amount, and/or concentration of an analyte in a microfluidic sensor, where the sensor is a part of an integrated sample acquisition system and/or analyte measurement device (Reymond *et al.,* 2007) [48].

Potentiometric detection

Potentiometric biosensors involve the utilization of high impedance voltmeter in order to transduce the biological reaction into a potential signal, which measures the electrical potential difference or electromotive force (EMF) between two electrodes at near zero current. The potentiometric devices simply comprised of an immobilized enzyme membrane which surrounds the probe from a pH-meter and the hydrogen ions are generated or absorbed here via catalyzed reaction, which generates a proportional potential to the logarithms of the concentration of the active species, measured in relation to a reference electrode. Since potentiometry generates a logarithmic concentration response, the technique allows the detection of extremely small concentration changes. Ercole and co-workers demonstrated a method for the detection of *E. coli* cells in vegetable food using the potentiometric alternating bio-sensing (PAB) system based on Light-addressable potentiometric sensor (LAPS) (Ercole *et al*., 2003) [49]. Where LAPS is used as a transducing component for sensing pH variations due to ammonia production by a urease- *E. coli* antibody conjugate.

Impedimetric detection

Impedimetric detection principle is based on the changes in the conductance of the medium due to microbial metabolism of the inert substrates into electrically charged ionic compounds and acidic-by-products (e.g. amino acids, lactic acid and acetic acid). This causes a change in conductance of medium and electrical impedance. Recently, the integration of impedance with biological recognition technology for detection of pathogens has found wide-spread use (Yang and Bashir, 2008) [4]. Where bacterial growth in a medium which is related to the function of time at a given temperature can be monitored by carefully monitoring and measuring electrical impedance and conductance. Impedimetric transduction technique has been applied to detect and/or quantify variety of foodborne pathogens. Yang *et al*. (2004) [50] is reported that the impedimetric transduction technique can be applied to detect and/or quantify variety of foodborne pathogens e.g. viable *Salmonella*.

Conductometric detection

Conductimetric biosensors are based on the principle of change of conductivity of the medium when microorganisms metabolize uncharged substrates, such as carbohydrates, to intermediates, such as lactic acid, which leads to a change in electrical conductivity or current flow. Thus, it is simply consists of two metal electrodes separated by a certain distance and an AC voltage applied across the electrodes causes a current flow and the amount of charged metabolites is directly proportional to the growth rate of the organism and is easily quantifiable. According to the Association of Official Analytical Chemists, Intl. (AOAC) (1996) [51], the impedance principle was accepted as a first action method (Gibson *et al*., 1992) [52] and is most indicated to monitor quality and detect specific food pathogens, detection of bacteria and sanitation microbiology (Feng, 1992) [53]. Conductometric biosensor exploited for detecting foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* spp. (Muhammad-Tahir and

Alocilja, 2003) [54]. Recently, a direct-charge transfer conductometric biosensor is developed for the detection of *B. cereus* in various food samples (Pal *et al.*, 2008) [55].

Mass-sensitive biosensors

The principal mode of mass analysis relies on the account of piezoelectric crystals, which can be made to vibrate at a specific frequency with the application of an electrical signal of a specific frequency (Pramanik *et al.*, 2013) [56]. Thus, the frequency of oscillation is dependent on the applied electrical frequency to the crystal as well as the mass of the crystal (Velusamy *et al*., 2010) [14]. The detection of *L. monocytogenes* has been conceivable with the development of a quartz crystal microbalance biosensor (Singh *et al*., 2013) [57] where quartz is used as a piezoelectric material. There are mainly two types of mass based sensors used as:

(1) Surface acoustic wave (SAW) and

(2) Bulk wave (BW) or quartz crystal microbalance (QCM).

When antibody coated piezoelectric sensor's surface is placed in a solution containing pathogens, the attachment of the agent to the antibody coated surface results in an increase in the crystal mass, and this provides increase to a corresponding frequency shift. Su and Li (2005) [58] demonstrated a quartz crystal microbalance (QCM) immunosensor to detect the *S. typhimurium* with simultaneous measurements of the resonant frequency and motional resistance. They also reported with a surface acoustic wave (SAW) sensor for the rapid detection of *S. typhimurium* (Chen and Barbaree, 2002) [59], *E. coli* O157:H7 (Berkenpas *et al.*, 2006) [60]. Recently, Huang *et al*. (2009) [61] and Xie *et al.* (2009) [21] demonstrated a ME biosensor for the real-time *in-vitro* detection of *B. anthracis* spores by immobilizing bacteriophage as bio recognition element.

Nanosensors

Nanosensors can be used for the detection of food spoilages, for instance, an array of thousands of nanoparticles designed to fluoresce in different colors on contact with food pathogens, reduces the time from days to hours or even minutes for pathogen detection (Bhattacharya *et al.*, 2007) [62] which can be placed directly into the packaging material, where they would serve as 'electronic tongue' or 'noses' by detecting chemicals released during food spoilage (Lange *et al*., 2002; Garcia *et al*., 2006) [63, 64]. Nanosensors based on microfluidics devices can also be used for detection of pathogens efficiently in real time and with high sensitivity (Baeummer, 2004) [65] such as nanocantilevers are used for detection of pathogens which depends on their ability to detect biological-binding interactions, such as between antigen and antibody, enzyme and substrate or cofactor and receptor and ligand, through physical and/or electromechanical signaling (Hall, 2002) [66], where they consist of tiny pieces of silicon-based materials that have the capability of recognizing proteins and detecting pathogenic bacteria and viruses (Kumar, 2006) [67]. Balasubramanian *et al.* (2005)[68] reported the use of a commercially available Cyranose-320™ electronic nose system to identify *S. typhimurium* in inoculated beef samples which containing an array of 32 conducting polymer sensors and used to obtain the odour patterns of the headspace of the meat samples. It also used to analyse the volatile organic compounds from vacuum packaged beef strip. Many researchers reported the use of a conducting organic polymer based e-nose sensors for foodborne pathogen detection where it exhibit a change in resistance due to adsorbing gas by the sensor, which is sensed and delivered as the output (Arshak *et al*., 2009)[10]. Magan and co-workers also reported the use of a commercially available Blood- hound™ BH114 electronic nose unit which includes 14 conducting polymer sensors for the detection of the volatile profiles which is produced by uninoculated skimmed milk media or that inoculated with bacteria (*Pseudomonas aureofaciens, P. fluorescens, and B. cereus*) or yeasts (Magan *et al*., 2001) [69].

Microbial Biosensors

Microbial biosensors are based on the use of microorganisms or microbial products as a biological sensing element with a transducer to produce a signal proportional to the analyte concentration. The choice of the biological material will depend on a number of factors such as the specificity, storage, operational and environmental stability. Selection also depends on the analyte to be detected such as chemical compounds, antigens, microbes, hormones, nucleic acids or any subjective parameters like smell and taste. Enzymes, antibodies, DNA, receptors, organelles and microorganisms as well as animal and plant cells or tissues have been used as biological sensing elements. Viable microbes metabolize various organic compounds either anaerobically or aerobically resulting in various end products like ammonia, carbon dioxide, acids etc that can be monitored using a variety of transducers and converted into quantifiable electrical signal which are based on direct measurements of a physical phenomena occurring during the biochemical reactions on a transducer surface, where signal parameters such as pH change, oxygen consumption, ion concentrations, potential difference, current, or resistance, can be measured by electrochemical transducers.

Riedel reported that the viable cells are mainly used when the overall substrate assimilation capacity of microorganisms is taken as an index of respiratory metabolic activity for estimation of biological oxygen demand (BOD) or utilization of other growth or metabolically related nutrients like vitamins, sugars, organic acids and nitrogenous compounds (Riedel, 1998) [70]. Arikawa and co-workers found that the viable microbial biosensor involves the inhibition of microbial respiration by the analyte of interest, like environmental pollutants (Arikawa *et al*., 1998) [71].

Immobilisation of biomaterials

Immobilization is a natural phenomenon existing in the universe. Immobilization technology has played a major role for formation of close proximity between the biomaterial and the transducer; and stabilizing it for reuse (D'Souza, 2001) [72]. The biological material directly immobilized on the transducer or membranes, which can subsequently be mounted on the transducer. Biomolecules can be immobilized either through adsorption, entrapment, covalent binding, cross-linking or a combination of all these techniques (D'Souza, 1989, 1999; Bickerstaff, 1997) [73, 74, 75]. Where the selection of a technique and/or support depends on the nature of the biomaterial and the substrate and configuration of the transducer used. Covalent binding is most commonly used for the immobilization of enzymes and antibodies rather than the immobilization of cells. Besides this cross-linking successfully used for the immobilization of cells in various supports and entrapment and adsorption techniques are more useful when viable cells are used. A common approach is to

retain the cells in close proximity to the transducer surface using membranes like the dialysis membrane. Many researchers reported that microbial cells immobilized by entrapment in a variety of synthetic or natural polymeric gels for use in industrial processing (Bickerstaff, 1997; D'Souza, 1999; Ramakrishna and Prakasham, 1999) [74, 75, 76].

Applications of Biosensors

Applications of Biosensors for food security are described in mainly two areas: detection of foodborne pathogens like *Salmonella*, *Listeria monocytogenes*, *Campylobacter*, and *Escherichia coli*, and detection of chemical contaminants such as pesticides, fertilizers, heavy metals, food additives and antibiotics. Pathogens detection is important in the prevention of microbiological hazards acquired from production to consumption of food products.

Biosensors used for the detection of bacteria

Salmonella

Salmonella are Gram-negative bacteria naturally found in the gastrointestinal tract of warm blooded animals and humans and becomes the principal biological contamination hazard in food processing plants (White *et al.*, 2002) [77], can cause enteric fever and salmonellosis associated to the consumption of contaminated food products (Nowak *et al*., 2007; Lu *et al*., 2009)[78,79]. Piezoelectric antigen-antibody biosensors can be used for the rapid detection of *Salmonella* based on the immobilization of amphiphilic antibodies, by applying the Langmuir-Blodgett (LB) monolayer method, where the antibodies are captured and distributed uniformly in a liquid having affinity to the antibody and a little interaction between the antibody and the polar surface (antibody film) is occurred due to submerging a support probe into a polar and a non-polar surface and the resonance frequency will be affected due to increased mass in the crystal support due to affinity takes places with antigen (Guntupalli *et al*., 2007) [80].

Listeria monocytogenes

Listeria monocytogenes is a Gram positive, flagellate microaerophilic *cocco bacillus*, can cause *listeriosis* due to the consumption of fresh and processed foods such as meats, shellfish, unpasteurized milk and vegetables (Sánchez *et al*., 2009) [81]. The fiber-optic biosensors can be applied for the detection and identification of *Listeria monocytogenes* at low concentrations and allow the reaction between receptor and analyte, where excitation of atoms is related to the receptor-analyte reaction, where the fluorescent light generated by a wave, results in changes in resonance that are captured by the biosensor (Geng *et al*., 2004) [82].

Campylobacter

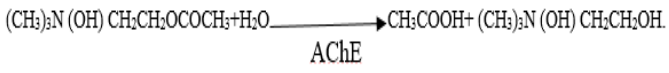
The genus *Campylobacter* are Gram negative bacteria, comma-shaped or "S" flagellates microaerophiles with motility (Ryan and Ray, 2010) [83], can cause gastroenteritis infections and Campylobacteriosis illness due to consumption of contaminated food that is a major problem in poultry production. These disease has side effects including reactive arthritis and muscle pain due to alterations in the immune system (WHO, 2000) [84]. Wei and co-workers found that a SPR optical biosensor has great sensitivity and the specific antibody for *Campylobacter* populations of at least 10^3 CFU/ml that is capable of rapidly detecting concentrations of *Listeria monocytogenes* (Wei *et al*., 2007) [85].

Escherichia coli

Escherichia coli is a Gram negative *bacilli* normally found in the intestine of humans and warm blooded animals (Darnton *et al*., 2007) [86], can cause the hemolytic uremic syndrome (Waswa *et al*., 2007) [41] and infection due to the consumption poorly cooked animal foods or foods that were washed with contaminated water (WHO, 2005) [87]. There are different biosensors for a rapid detection of *E. coli*. Amperometric biosensors can be applied for a rapid detection of *E.coli.* The detection principle is based on detection of hydroxyl radicals which is produced by *E. coli* oxygen reduction during aerobic metabolism (Tang *et al*., 2006) [88]. Many researchers developed a techniques in which bienzyme electrochemical coupled with immunomagnetic separation technique, in order to detect *E. coli* O157:H7 (Ruan *et al*., 2002) [43] and *S. typhimurium* (Yang *et al*., 2001) [46].

Biosensors for environmental monitoring

Many researchers developed a large number of biosensors based on enzyme inhibition for the detection of a variety of compounds in context of food and environmental analysis. For the determination of pesticides, antibiotic residues and heavy metal, a large number of enzymes has been used. Heavy metal ions (Cu, Hg, Cd and Pb) can be detected with the help of enzymatic method with high precision (Krawczynski vel Krawczyk *et al*., 2000) [89] and enzymes used are urease (Lee and Russel, 2003; Rodriguez *et al*., 2004) [90, 91] and butyrilcholinesterase (BChE) (Mourzina *et al*., 2004) [92]. Most common enzymes are used for pesticides detection are cholinesterase (AChE, BChE), organophosphorus-hydrolase (OPH), and urease where acetylcholinesterase (AChE) catalyses the hydrolysis of acetylcholine to acetic acid and choline (Velasco-Garcia and Mottram, 2003) [93] as



Antibiotics are a group of antimicrobials that are extensively used in dairy cattle management as therapeutics and to prevent outbreak of diseases e.g. mastitis (Suhren *et al*., 1996) [94], and the most commonly used antibiotics in dairy animals are β-lactam, tetracycline, amino glycoside, sulfonamide and macrolides. Presence of these residues in milk may cause consumers various problems such as allergic reactions and cancers in human beings besides decreased antimicrobial susceptibility among pathogenic bacteria (Katz and Brady 2000; Khabir *et al*., 2004) [95, 96]. In addition, antibiotic residues can also have adverse effects during manufacturing of fermented milk products in terms of starter failure (Mayra-Makinen, 1995) [97]. There are different methods have been developed for the detection of antibiotic residues, which include screening methods and chromatographic techniques and bacterial spore based assay (SBA).

Other antimicrobial agents like aflatoxins and β-lactam are detected in milk using a spore inhibition based-enzyme substrate assay (SIB-ESA) (Kumar *et al*., 2013) [98]. Where the detection principle of aflatoxin M_1 comprises of spores of Bacillus spp. and in addition, detection of β-lactam antibiotics is based on the principle of resistance mechanism of some β-lactamase generating *Bacillus* spp., while some other spore forming bacteria such as *B. cereus* and *B. licheniformis* produce β-lactamase enzyme due to induction by β-lactam antibiotics and the enzyme production is proportional to the

concentration of inducer present in milk.

Conclusion

Conventional analytical applications can be replaced by biosensors. Biosensors show greater potential for the detection of pathogens, pesticide and drug residues. Also used in hygiene monitoring, heavy metals and other toxic substances in the food. Rapid, sensitive, specific method have to be developed for the detection of foodborne pathogenic bacteria to ensure food safety. The use of micro-organisms is making a great impact for environmental monitoring purposes. In addition, use of nano-materials/nanoparticles as a composite with conducting polymer would lead to the innovation of biosensors and many other nano-electronic devices and the basic requirements of biosensor like enhanced sensitivity and very low detection limit can also achieve. In fact, a variety of enzyme, antibody, nano-sensors and microbial-based biosensors using optical, electrochemical, and acoustic-signal transducers have been reported to measure a significant number of contaminants from a variety of compound classes.

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