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A COMPREHENSIVE REVIEW ON THE DESIGN AND OPERATION OF ENZYMATIC PART OF BIOSENSORS

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ABSTRACT

A biosensor is defined as a self-contained analytical device that combines a biological component with a physiochemical device for the detection of an analyte of biological importance. A biosensor is made up of two main components, the first one is a biological component and the second one is a transducer. When the interactions develop between the analyte of interest and the biological component, the transducer produces a digital electronic signal, which is proportional to the substance's concentration. Enzyme-based biosensors are developed on the base of biological recognition. The use of enzyme as biological recognition components in biosensors was very prominent due to their marketable accessibility or for the advantage of purification and isolation from various sources. Enzymatic biosensors have many advantages like they have a quick response, high specificity, and easy to be constructed. Biosensors have many uses in numerous fields of research. Biosensors are used to measure the freshness of food items, to examine the environmental pollutants, to detect the drug residues in veterinary items, to detect cancer cells and to check the food quality.

INTRODUCTION

Bio-sensing has been proven to be an advanced technique in several fields e.g. it has many unique applications in bio-medical, food and environmental fields. Modern biosensors are small in size and they can easily be transported. Biosensors can monitor rapid changes in biological fluids because they have the capability to measure an analyte in real time. Generally, biosensors are defined as "a self-contained analytical device that combines a biological component with a physiochemical device for the detection of an analyte of biological importance" (Hasan *et al*., 2014). A biosensor is an analytical device, that used

biological element (e.g. tissue, organelles, cells, nucleic acid, and micro-organisms) to interact with an analyte (Banica, 2012). A biosensor is made up of two chief components, a biological component which interacts with a target molecule and a transducer that produce an electronic signal as a result of this interaction. Detection of the analyte by biosensor happens due to the development of specific interaction between the analyte of interest and the recognition element. Changes will produce in one or several physical-chemical properties (pH, heat transference, electron transference, changes of potential or mass, and variation of optical properties, etc.) due to this specific interaction between the analyte of interest and the recognition element. These changes can be detected and measured by a transductor (Velasco-Garcia *et al*., 2003).

Biological sensors can be used to detect the existence of any of the substrates or by the appearance of a known product and they consisted of enzymes, cellular organelles, complete cells or animal or vegetal tissue (Davis *et al*., 1995). Biological sensors can be reused again because they are not consumed. The use of enzyme as biological recognition components in biosensors was very prominent due to their marketable accessibility or for the advantage of purification and isolation from various sources. Enzymatic biosensors have many advantages like they have a quick response, high specificity, and easy to be constructed.

Multi-enzymatic chains can be employed in various cases, in which the enzyme that mostly identifies the analyte does not directly act on it, instead of this, it interacts with a certain product that derived from it. Commercially existing enzymatic biosensors consist of mostly these enzymes; oxidoreductase, glucose oxidase, alkaline phosphatase, and horseradish peroxidase because these enzymes have very stable catalyzing reactions of oxide reduction (Rogers *et al*., 1998; Laschi *et al*., 2000). The biosensor is presently characterized as a powerful tool for the analysis in the food industry, medical, clinical, environmental and biotechnological application (Velasco-García *et al*., 2003). This device has important characteristics as compared to other technologies that used in the agro-food industry i.e. it is highly sensitive, specific and has short time response. This device has the ability to perform a function in real time and also has a low manufacture cost. They're used in food industries to ensure the quality of food. They can detect environmental pollutants and help in maintaining the environment clean (Cock *et al*., 2009).

The first biosensor was introduced in the 1960s and enzymatic biosensor's application and its bio-catalytic activity were also described. In 1962, a biosensor was made by Clark that

consisted of an oxygen electrode for the examination of electrochemical such as hydrogen or oxygen peroxide for the bio-analytical use. Afterward, the various uses of biosensors have been discovered in many fields of research, i.e. physics, biology, analytical chemistry, and bio-electronics. (Rodriguez and Raveendran, 2015). Many types of biosensors are made and being employed e.g. DNA based biosensor, enzyme-based, immune-sensors, piezoelectric, tissue-based and thermal biosensors (Mehrotra, 2016).

Components of biosensor

Commonly, three key elements are present in biosensor; a bio-transducer, bio-recognition, and an electronic system that comprises a signal amplifier, display, and processor (Hierlemann and Baltes, 2003). The bio-recognition part is also known as a bio-receptor and to interact with a specific analyte of interest, it exploits biomolecules as a receptor. The analyte should be highly selective and specific. The principle of detection of a biosensor is due to the specific interaction between the analyte of interest and the recognition element (Cock *et al*., 2009). Figure 1 represents the components of the biosensor.

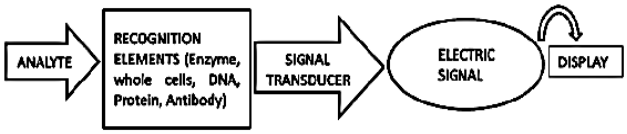

Fig. 1: Components of the biosensor.

Ways to use biosensors

The biosensor is an analytical device that has many useful applications in the field of research, medical, food industry and environment. Biomedical applications of bio-sensors are very helpful because they are less expensive, easy to handle and give a quick response. According to Castillo *et al.*, amperometric biosensors can be utilized in the following ways (Castillo *et al*., 2004). Biosensors can be used as "off line" device. By using biosensor based analytical equipment, collection and analysis of biological samples ensue. For example, blood glucose measuring devices.

Biosensors can be used as "in vivo" device. Extracellular variations (in the concentrations of the analyte of interest) can be continuously detected by the implantation of biosensors. Such implantable devices can only use animal models for preclinical research. Biosensors can also be used as "on-line" device. These devices are implanted within the biological material and also they are integrated with a sampling device.

Criteria for the classification of biosensors

According to the type of interactions between the biosensor and the analyte, biosensors are classified as i.e. enzymatic, antigen/antibody, nucleic acids interaction and surface attachment of biological elements as shown in Fig. 2.

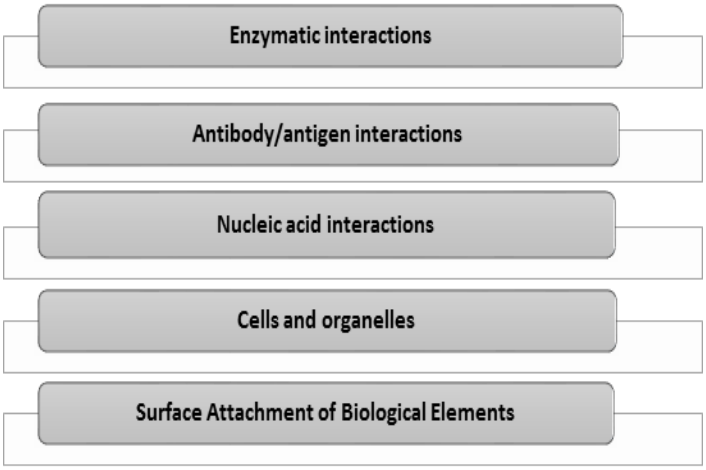

Fig. 2: Criteria for the classification of biosensors.

Enzymatic interactions

In 1967, the first time the enzyme-based sensor was introduced by Updike and Hicks. In this type of interactions, primarily the substrate specifically binds to an enzyme and converts to products (Updike and Hicks, 1967). The most commonly used this type of bio-receptor comprise polyphenol oxidase, urease, and glucose oxidase. Enzymatic interactions are more useful because of these advantages; highly specific, high selectivity, show more catalytic activity, which improves sensitivity and have less time of action. But there are also some limitations exist with this type of interaction i.e. these biosensors are expensive. Moreover, in these biosensors, the activity can be lost during immobilization of an enzyme on a transducer or deactivation can be occurred (due to inappropriate storage conditions. Thus, the enzymatic biosensors have a limited life span due to limited enzymes stability. Moreover, the enzymatic biosensors have more advantages e.g. they have the ability to catalyze many reactions and they can detect various analytes of interest particularly substrates, modulators, products, and inhibitors (Rajpoot, 2017).

Antibody/antigen interactions

Immunosensors have a specific property of antigen-antibody interactions. These interactions are similar to the lock and key model due to the specific binding of antigen and antibody.

Antibodies or immunoglobulins proteins can specifically bind to a specific molecule or antigen. There are some limitations regarding the use of immunosensors i.e. the antigen and antibody binding is irreversible, the interaction and the binding of the antibody with antigen are particularly dependent on conditions of the examination (e.g. pH and temperature). Also, the interaction of antigen and antibody can be interrupted by the organic solvents, and ultrasonic radiation (Marazuela *et al*., 2002).

Nucleic acid interactions

The nucleic acid biosensors consisted of a single strand of the nucleic acid molecule that can specifically identify and bind to its complementary strand. The nucleic acid is the backbone of genetics. It has a specific ability of base-pairing. If the known part of nucleic acid that called as probes is present, then the complementary sequence of bases can be made and by using an optically detectable component, (i.e. fluorescent label) can be labeled. Due to the formation of a hydrogen bond between the two strands of two nucleic acid, the nucleic acid interactions happen. These nucleic acid biosensors have shown a greater advantage in the recognition of viral infections, many types of cancer, and genetic disorders (Sohrabi *et al*., 2016).

Cells and organelles

Cells used as bio-receptors because of their high sensitivity to the surrounding environment. Cells have a greater capacity to attach to the surface. As compared to organelles, cells can do their activity for a large period of time because they are more stable at different pH and temperature. Cells based biosensors often used to control herbicides which cause water pollution. Organelles also have potential to perform several distinct functions independently. They carry out several important metabolic pathways and fulfill the body requirements by using specific enzymes. Some common organelles include chloroplast, mitochondria, and lysosome. Mitochondria have the potential to respond at a high level of calcium contents. That's why it can be utilized to monitor calcium concentrations with high sensitivity (Bragadin *et al*. 2001).

Tissues can also be used as biosensors because of the following benefits. They have low cost and easily available, tissues are a source for the plenty of enzymes. A natural environment is provided by the tissues where enzymes can perform the greater activity as compared to cells and organelles and essential cofactors are already present in tissues for the working of enzymes. But there are also some limitations of tissues as biosensors, for example, a large

variety of enzymes present in tissues that decreased the substrate specificity and the response time (Rajpoot, 2017).

Surface Attachment of Biological Elements

Biological elements link with the surface of the sensor. It is an important step in relating to the biosensor. Biological elements bind with the surface of biosensor and activate it. This method is accomplished through amino silane, epoxy silane, nitrocellulose or polylysine. Later, by using coatings of charged polymer, biological molecules can be immobile through the layer deposition method (Pickup *et al*., 2008).

Types of Biosensors

Biosensors have following types; electrochemical, impedimetric, amperometric, ion channel switch, pyroelectric, microarrays, gravimetric, surface plasmon resonance, Glucose, electronic, piezoelectric, and optical biosensors. Few important biosensors are following.

Electrochemical

The electrochemical biosensors have been made by immobilizing biological agents on metallic surfaces. Generally, these biosensors work by producing or using electrons as the enzyme catalysis. Usually, three electrodes include in this sensor substrate; i.e. a working electrode, a counter electrode, and a reference electrode. On the active surface of the electrode, the target analyte is usually found and the reaction outcome may be either the production of potential in the double layer or transfer of electron across the double layer. The concentration of an analyte of interest will direct the rate of flow of electrons (Lud *et al*., 2006).

Impedimetric biosensors

Impedimetric biosensors work on the principle of direct recognition of bio-molecular activities without using enzymes. These biosensors have many utilizing applications, for example, they can be used to recognize label-free antibiotic drug e.g. ciprofloxacin even a small quantity (3 pmol/L) (Giroud *et al*., 2009). Also, an Impedimetric aptasensor is made, which utilized for the recognition of cocaine in the plasma, saliva, and urine of human body.

Amperometric

Amperometric biosensors working based on the signal produced by the enzymes that are labeled and electro-active constituents produced as a result of an enzymatic reaction

(Dominguez *et al*., 2012). Amperometric biosensors have many advanced applications e.g. they utilized to investigate some molecules e.g. anabolic androgenic steroids, synthetic estrogens, penicillin, phenolic compounds and xenoestrogens. Depending upon the electron transfer method that utilizes to measure the degree of isolation of the biosensor constituents (cofactors, mediators, enzymes and transducer) and the biochemical reaction. The amperometric enzyme biosensor is divided into three main generations. Figure 3 shows the schematic representation of the amperometric biosensor.

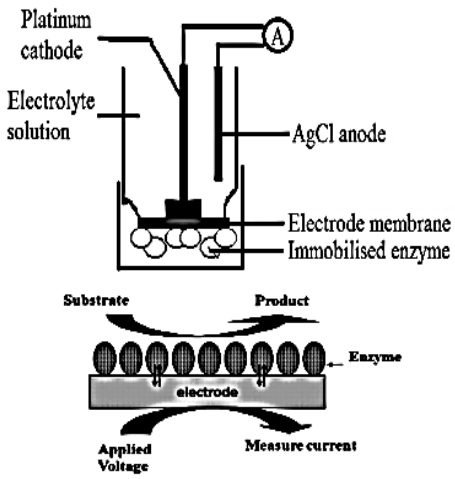

Fig. 3: Schematic representation of amperometric biosensors.

First generation biosensors

These biosensors generate an electrical response by measuring the analyte's concentration and the products of enzymes reaction diffuse through the surface of the transducer. They are also known as mediator-less amperometric biosensors (Dzyadevych *et al*., 2008). In this generation of biosensors, the enzymes are fixed on a surface of the transducer and it has the ability to covert the substrate into an electroactive substance and the byproduct is measured. Two main categories of enzymes belong to these biosensors; oxidase and dehydrogenases. Both of these enzymes require coenzymes (e.g. NADH. NADPH, NAD^+, $NADP^+$, ATP, FAD, FADH) during catalysis and these coenzymes need to be regenerated for the catalysis of subsequent reactions by an enzyme. Figure 4 shows the diagram of the first-generation biosensor.

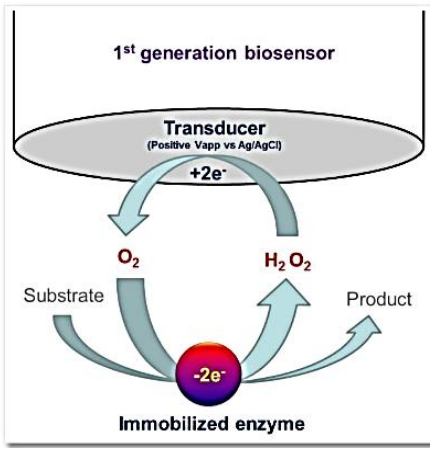

Fig. 4: Schematic representation of a first generation biosensor,

The most common cofactor is flavin adenine dinucleotide (FAD) in oxidase enzymes. This cofactor is bonded covalently to the oxidase enzyme. The production of hydrogen peroxidase or oxygen consumption can be observed by the biosensors based on oxidase. These biosensors are dependent on oxygen because they require molecular oxygen as an additional substrate. During the catalysis by oxidase enzyme, the following reactions occur;

$$S + E - FAD \rightarrow E - FAD - S \rightarrow E - FADH_2 + P$$

$$E - FADH_2 + O_2 \rightarrow E - FAD + H_2O_2$$

There are some limitations exists while using first generation biosensors i.e. these biosensors utilize oxygen as an electron acceptor so, any changing in oxygen concentration can reduce the linearity of biosensors. This dependence of first-generation amperometric biosensors on oxygen can limit their use in living systems, e.g., they are not applicable in ischemic conditions. Dehydrogenase-based first generation biosensors, give the following reactions (Dzyadevych *et al*., 2002);

$$S + E + NAD^+ \rightarrow S - E - NAD^+ \rightarrow E + P + NADH + H^+$$

$$NADH \rightarrow NAD^+ + H^+ + 2e^-$$

In these biosensors, the NADH concentration is directly dependent on the concentration of the analyte of interest. For the perfect detection of a monitored analyte, sufficient cofactor's concentrations should be present. First generation biosensors have many advantages e.g. they are highly sensitive and require low response time almost around one second (Rocchitta *et al*., 2012). However, there is also some limitation of these biosensors for example to produce a reproducible surface and response of the sensor, electrode pretreatment of this biosensor required. Moreover, the prolonged use of amperometric biosensors outcomes in the

deterioration of transducer's surface and change the response of biosensor, especially when using them in complex natural media (Prodromidis and Karayannis, 2002).

Second generation biosensors

Second generation biosensors also are known as mediator amperometric biosensors. A schematic diagram of the second-generation biosensors is shown in figure 5. In these biosensors, mediators are used as an oxidizing agent and they carry electrons. This methodology made these biosensors to work at a low potential and avoid oxygen dependence. Most common mediators that used in these biosensors are following; ferricyanide and ferrocene but alizarin yellow, azure A and C, inorganic redox ions, methyl violet, methylene blue, Prussian blue, phenazines and toluidine blue are also using extensively (Chaubey and Malhotra, 2002). Mediators can be either directly fixed on the surface of the electrode or added to the sample. As compared to other electroactive compounds in the sample, the mediators should have a low redox potential. Some additional developments are made by substituting oxygen with an electron acceptor that has the ability to transfer electrons from the enzyme (E) redox center to the electrode. The following reactions occur;

$$S + E(ox) \rightarrow P + E(red)$$

$$E(red) + 2M_{OX} \rightarrow E(ox) + 2M_{RED} + 2H^{+}$$

$$2M_{RED} \rightarrow 2M_{OX} + 2e^{-}$$

M_{OX} and M_{RED} are the oxidized and reduced forms of the mediator. M_{RED} is oxidized at the electrode's surface and give a current signal related to the detected analyte concentration. Due to the immobilized ions, second-generation biosensors generally have low stability that's why they are using less commonly as compared to first generation biosensors (Rocchitta *et al*., 2016).

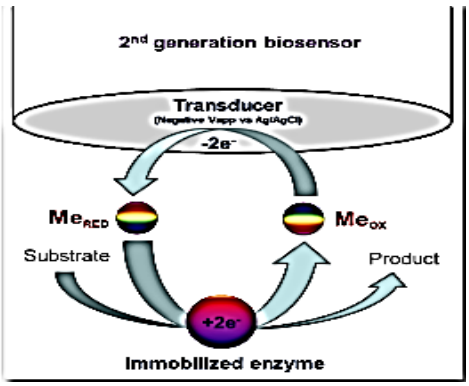

Fig. 5: Schematic diagram of a second generation biosensor.

Third generation biosensors

In third generation biosensors, there is a direct transmission of an electron between enzyme and electrode because they rely on bioelectrocatalysis (Dzyadevych *et al.*, 2008). There are three elements present in these biosensors; the electrode as the entrapping surface, the redox polymer to make sure the signal transmission and the enzyme as a bio-recognition element (Prodromidis and Karayannis, 2002). Third generation biosensors are not usually utilized for analysis but nanotechnology and polymer science advancement make them promising, due to the possibility of very short response time and comparatively independent of the concentrations of oxygen or cofactor (Rocchitta *et al.*, 2016). Figure 6 shows the diagrammatic representation of third generation biosensor.

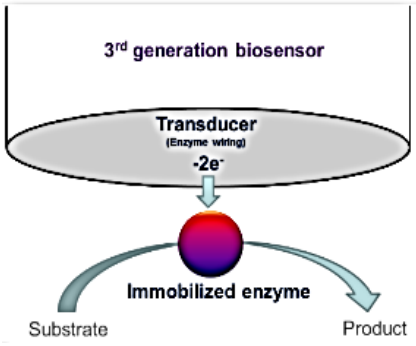

Fig. 6: Schematic diagram of a third generation biosensor.

Ion channel switch

A biosensor that utilizes an ion-channel switch is highly sensitive for the detection of biological molecules. These biosensors utilized to sense an extensive range of the target molecules in their quantitative amounts for example bacteria, drugs, proteins, and toxins. Ion channels linked on the gold electrode by embedding in a tethered bilayer membrane and makes an electric circuit. In ion channels, the target molecules control the flow of ions through a channel by bounding to the ion channel in a specific way. Due to this, an important change produces in electrical conduction. By using gramicidin (a chain of the dimer peptide) inside the tethered bilayer membrane, biosensors based on ion channel can be designed. If the dimmer is braked, then the flow of ionic current stopped through the membrane. However, afterward isolating the membrane from the surface of the metal by a hydrophilic spacer, the extent of variations in the electrical signal is significantly amplified (Krishnamurthy *et al.*, 2010).

Optical biosensors

Optical biosensors first presented in the late 1980s for commercial purpose, then a huge number of optical biosensors has been designed. These biosensors used in research fields such as bacteriology, cell biology, cell adhesion, epitope making, ligand fishing, molecular engineering, nucleotide-nucleotide binding, nucleotide-protein, and virology. Figure 7 represents the diagram of the optical biosensor. They are highly sensitive, specific, cost-effective and small in size (Damborsky *et al*., 2016). An inexpensive, multiplexed and easily portable system can be developed from these biosensors. Various pollutants in the environment can be detected by using optical biosensors. These biosensors measured the generated electrical noise. Optical biosensors used the bioluminescence mechanism for real-time processing monitoring. Bioluminescence exploits the release of illumination by certain microbes. Lux gene (the gene responsible for light emission) of the bacterial system was used as a reporter gene for the examination of the sample (Nigam and Shukla, 2015).

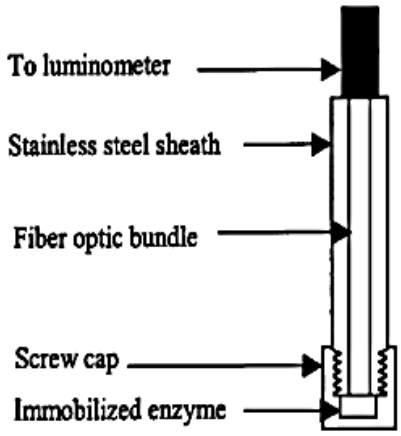

Fig. 6: Schematic diagram of an optical biosensor.

Microarrays

Microarrays help in the simultaneous identification of several substances as they are bi-dimensional receptor arrays. Microarrays also have many advantages in the biomedical field, because they have the potential to detect several small sized molecules. A microarray was prepared by Peng and Bang that was utilized to examine three veterinary active molecules (i.e. chloramphenicol, clenbuterol, and tylosin). By using an indirect format, this microarray was designed, in which small molecules are fixed on the glass slide surface. By using a secondary antibody labeled Cy5, the antigen-antibody linking outcomes were measured. In terms of IC50, the detectability obtained for drugs; chloramphenicol, clenbuterol, and tylosin was 0.14mg/L, 0.53mg/L and 10.53mg/L (Peng and Bang, 2006).

Surface plasmon resonance

Surface plasmon resonance (SRP) is used for the analysis of interactions made by the nucleic acid. By linking the free electrons and electrical field existing in the metal, surface plasmons or surface plasmon polariations are produced. SRP can be described as "the oscillation of conduction electrons over the interaction between positive and negative permittivity material stimulated via incident light". By using the biosensor-SPR methodology, great benefits can be obtained in the field of protein-DNA interactions. Surface plasmons are extensively used in several different fields e.g. pharmaceuticals (Kantiani *et al*., 2009). Fernandez et.al synthesized an SPR biosensor that is utilized to detect antibodies in milk, by multiplexed analysis. This multiplexed portable biosensor consists of six channels and used to detect three different antibiotics e.g. chloramphenicol, fluoroquinolones, and sulfonamides (Fernandez *et al*., 2010). This biosensor has the ability to detect antibiotics with an extensive range of the spectrum. SPR is also utilizing to increase the surface sensitivity for various spectroscopic analysis that commonly includes Raman scattering, second harmonic generation, and fluorescence.

Glucose biosensors

Blood glucose biosensors is a characteristic device for testing in household situations as of it's comfortable to use and reliable qualitative results. For the revealing of many targets e.g. recreational drugs (Xiang and Lu, 2011) and protein biomarkers, a unique DNA sensor which attached to a general glucose meter was established. Recently a glucose biosensor was developed by using gold microelectrodes coated via single-walled Carbon Nanotubes (SWCNTs), by the Electrophoresis Deposition Process. For layer a poly (pyrrole)/glucose oxidase film, this nanostructured was successfully utilizing.

Applications of biosensors

Biosensors have much-advanced application in many fields such as medical, pharmaceutical, clinical and food industries. There are some important applications of biosensors such as determination of freshness of food items, detection of drug residues, and examination of cancer cells, etc. Some advanced applications of biosensors are shown in figure 8. Following are some major applications of biosensors.

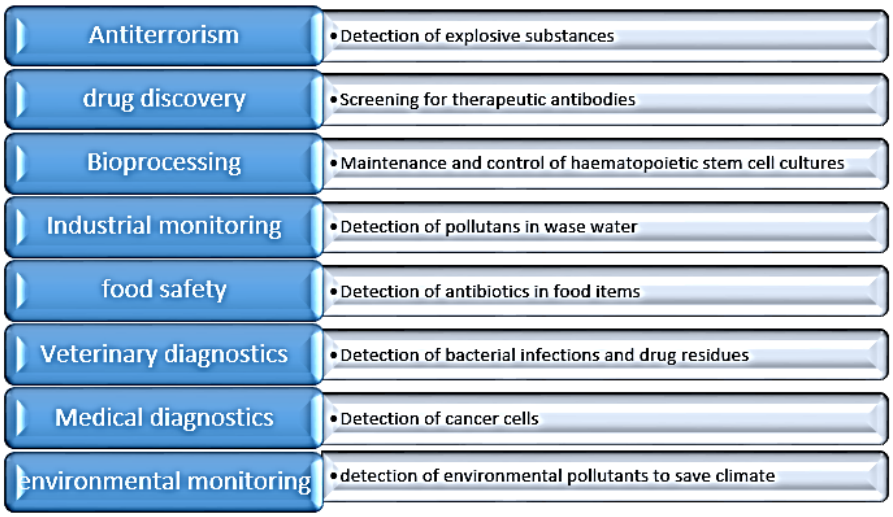

Fig. 7: Applications of the biosensors.

Role of biosensors in detecting environmental pollutants

Pollution has been increasing in the environment day by day i.e. CO_2 and other greenhouse gases increase the air pollution, a variety of fertilizers, herbicides, and pesticides increase the water pollution and the dumping of dangerous wastes and non-biodegradable materials, herbicides and pesticides increase the soil pollution. Anthropogenic activities are adding various chemicals to an environment like cosmetics, pesticides, and medicines, these chemicals are using all over the world and essential for current societies. Furthermore, anthropogenic activities also cause the contamination of water resources with biological micro-pollutants for example bacteria and virus. For the monitoring of environmental pollutants, various procedures have been adopted but still many undetected contaminants (endocrine disruptions, hormones, toxins, and pharmaceuticals) are present in the environment that need to be recognized and measured. On the base of chemical structure and mode of action, the pollutants in the environment are classified. Biosensors have been designed for the estimation of pollutants (organic and inorganic) in the environment (Rodriguez-Monaz *et al*., 2004). Biosensors detecting environmental pollutants have many key advantages e.g. small in size, portable, and have the ability to examine pollutants in complex media with the minimum sample preparations. Several biosensors are restricted to be used for a specific toxicant or a limited number of pollutants. However, biosensors have been proved an effective analytical tool for detecting environmental pollutants.

Heavy metal ions

Mostly bacterial biosensors utilized for the analysis of heavy metals in the environment because they have heavy metal resistant genes in them. Several bacteria have been studied for the potential to act as possible bio-receptors molecules for the recognition of cobalt, copper, mercury, silver, tin, and zinc, etc. These biological receptors show resistance properties toward these metals. For metals detection, many biosensors developed that are based upon the bioluminescent phenomena. These biosensors have both the genes of metal resistant and luciferin proteins (Nigam and Shukla, 2015).

Table 1: detection of heavy metals by biosensors.

Analyte	Type of interaction	Recognition bio-catalyzer	Transduction system
Copper and mercury	Bio-catalytic	Glucose oxidase	Amperometric
Arsenic, cadmium, and bismuth	Bio-catalytic	Cholinesterase	Electrochemical
Cadmium and lead	Bio-catalytic	*Staphylococcus aureus* or Recombinant *Bacillus subtilis*	Optical
Mercury(II) and lead(II) ions	Bio-catalytic	DNA	Optical
Copper	Bio-catalytic	Recombinant *Saccharomyces cerevisiae*	Amperometric
Copper and mercury	Bio-catalytic	*Spirulina subsalsa*	Amperometric
Organophosphates, urea, and ethanol	Bio-catalytic	*Flavobacterium sp., Bacillus sp.,* and *S. ellipsoideus*	Potentiometric
Mercury, cadmium, and arsenic	Bio-catalytic	Urease enzyme	Electrochemical

Phenols and cyanide

Phenols and their derivatives are listed as hazardous materials. They are known as toxic compounds as they are present in several industrial discharges which are linked with the production of detergents, disinfectants, plastics, pesticides, and medicines, etc. organophosphorus pesticides and chlorinated phenoxyacids produce chlorophenols and nitrophenols as main degradative compounds. These compounds cause severe toxicity in living organisms e.g. genotoxicity and mutagenicity. These compounds also decrease another life process like enzyme-catalyzed reactions, photosynthesis, and respiration. That's why phenol and its derivatives are considered as hazardous pollutants. Cyanide is also a very toxic compound and it blocks respiration by binding with cytochrome oxidase. For monitoring the presence of cyanide, an oxygen electrode has been designed. It exploits an immobilized bacteria (*P. fluorescens* NCIMB 11764) (Lanyon *et al*., 2005).

Organophosphorus (OP) compounds

Organophosphorous (OP) compounds are being utilized as insecticides for controlling insects, pests, and weeds in agriculture. These pesticides are very toxic and present in large amount in environmental samples. That's why certain concentration limits have been set by the European Commission for their application. For the detection of OP compounds in a various environmental sample, certain enzyme biosensors have been designed on the base of specific enzyme inhibition by these OP compounds. For example, biosensors have been construed for the assessment of OP compounds that have an inhibitory action on acetyl cholinesterase and colin oxidase (Choi *et al*., 2001).

Herbicides, triazines and nitrate compounds

Biosensors having amperometric and optical transducers are used to detect the presence of herbicides and triazines in the environmental samples. These compounds triazines obstruct the mechanism of photosynthesis. Nitrate compounds are being utilized in large amounts in the environment e.g. for maintaining the fertility of soils and preserving food items, these nitrate compounds are being utilized. Consumption of these compounds by a human can cause severe problems in them. These compounds can react with hemoglobin irreversibly and inhibit oxygen transport that can lead to diseases i.e. methemoglobinemia. These compounds can also harm aquatic organisms when they are present in large quantities in water bodies. Amperometric and conductimetric enzymatic biosensors are mostly utilized for the assessment of nitrate compounds. By utilizing immobilized nitrate reductase from *Aspergillus niger*, the electrodes of conductimetric biosensors can be modified (Wanekaya *et al*., 2008). Figure 9 represents the of action of nitrate reductase.

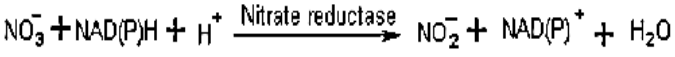

Fig. 8: mechanism of action of nitrate reductase.

Miscellaneous

For the detection of urea in milk, a disposable microbial biosensor was constructed by the assembly of urease enzyme generating bacteria and ammonium ion electrode. A bacterial biosensor has been designed that was modified by immobilized Pseudomonas putida ML2, used for the detection of benzene found in the air on the base of analysis by flow injection. This biosensor can sense benzene in the range of 0.025 to 0.15µmol in the air sample (Nigam and Shukla, 2015). For the recognition of volatile compounds, a biochip based algal

biosensor was developed, in which immobilized algal cells of genus Chlorella and Klebsormidium were used. Some biosensors are being utilized for the identification of vapors of methanol (200-1000 ppm) and formaldehyde (0.051-1ppm) (Pearson *et al*., 2000).

Role of biosensors in detecting pathogens

The contamination of food items from microbes led to the development of rapid, low-cost methods of examination to ensure the safety of life of the consumer. Different biosensors are being utilized to detect food contamination. A unique aptamer-based electrochemiluminescent biosensor used for ochratoxin A detection in wheat (Rhouati *et al*., 2013). Mycotoxin detection by biosensors is a new approach. The biosensors used for detection of mycotoxin can analyze multiple samples in one run and detect families of mycotoxin, simultaneously. Marine toxins mostly formed by micro-algal species and dinoflagellates. These toxins can assemble in filter-feeding bivalves e.g. shellfish. Mostly mouse bioassay test used for detection of marine toxins but it has some ethical issues. Fonfria *et al* developed an optical (SPR) biosensor method for the recognition of paralytic shellfish killing toxins in mussels clams, oyster, cockles, and scallops.

Veterinary drug residues determination by biosensors

Drug residues presence in veterinary food items can increase the risk of drug sensitization and can cause allergic reactions and disturbance in the normal microbial flora of the human gut. A dual biosensor was developed by Marchesini *et al.* that allows the detection of six fluoroquinolones including flumequine. It was a multichannel optical biosensor that detects fluoroquinolones in poultry muscle through a competition based assay (Marchesini *et al*., 2007).

Determining the freshness of food items by biosensors

Determining freshness in food items is an important effort by various food labeling regulations. Biosensors have been developed to detect the compounds which synthesized during the period of storage of food items such as fish, fruits, meats, and vegetables. These compounds generate abnormal odors, flavors and are harmful to the life of the consumer. Fresh food items experience following problems which distort their quality e.g. inadequate environment for storage, incorrect way of food packaging, inadequate provision of temperature and the oxygen level (Cock *et al*., 2009). Therefore profitable biosensors made that use immobilized enzymes like alcohol peroxidase and alcohol oxidase and a chromogen. These biosensors measure ethanol accumulation in broccoli, cauliflower, cabbage, and

lettuce. These biosensors can also be used to examine the development of putrefaction in tubercles like potatoes and for many other applications where ethanol accumulation can deteriorate the food quality (Smyth *et al*., 1999).

Examine of cancer cells by using biosensors

Oncological biosensors have developed by biological researchers for the treatment of breast cancer (Atay *et al*., 2016). As compared to radiology imaging tests, biosensors have the ability to directly detect the malignant influence of the tumor. A small quantity of sample is required by both detector and biological elements for testing. These biosensors can determine the stage of cancer and check the efficiency of treatment. These biosensors are more capable, productive, and less costly as compared to radiology imaging. Many biosensors developed for the finding of the various type of cancers (Rajpoot, 2017).

CONCLUSION

A biosensor is a rapidly developing technology. Biosensors are sensitive, selective, rapid, and also have low costs. Numerous powerful, precise and sensitive biosensors of huge significance have been developed e.g. electrochemical biosensors, impedimetric biosensors, amperometric, ion channel switch, optical biosensors, microarrays, surface plasmon resonance, glucose biosensors, electronic biosensors, piezoelectric biosensors, gravimetric biosensors, and pyroelectric biosensors. Biosensors are the ideal analytical devices for the monitoring of environmental pollutants and food contaminants. There are many important applications of biosensors such as determination of freshness of food items, detection of drug residues, and examination of cancer cells, etc. Further enhancements can be made in biosensor technologies to make them more valuable and also to increase their applications.

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