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Application of Biosensor in Food and Agriculture

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Abstract: *Biosensor technology is based on a specific biological recognition element in combination with a transducer for signal processing. Biosensors are detecting devices that rely on the specificity of cells and molecules to identify and measure substances at extremely low concentrations by producing an electronic signal which is proportional in magnitude or frequency to the concentration of a specific analyte or group of analytes, to which the biosensing element binds. Various studies conducted so far, proves that biosensor is more sensitive and helps in early detection of plant diseases like Karnal bunt, Late blight of potato, etc, than the conventional or traditional methods that are in use currently. Recent biological terrorism threats and outbreaks of microbial pathogens clearly emphasize the need for biosensors that can quickly and accurately identify infectious agents. The field of biosensors is constantly evolving to develop devices that have higher sensitivity and specificity, and are smaller, portable, and cost-effective. The potential growth in the world biosensor industry is remarkable, the emerging Biosensor market is expected to grow at over nine percent in the coming years thus becoming one of the fastest growing sectors in the World.*

Keywords: *Biosensor, analyte, food, agriculture.*

INTRODUCTION

Biosensor is a self contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transducer element (IUPAC). These instruments, that can detect and quantify specific plant pathogens and map these to defined positions within the field, would enable the farmers to perform a precise and target based application of pesticides and thereby reduce and optimise the use of agrochemicals. Conventional 'off-site' analysis requires the samples to be sent to a laboratory for testing. These methods are time consuming and require the use of highly trained and skilled personnels. In spite of the real need for obtaining analytical results in the shortest time possible, traditional and standard detection methods may take up to 7 or 8 days to yield an answer. This is clearly insufficient, and many researchers have recently geared their efforts towards the development of rapid methods.

The ideal scenario for precision agriculture is to have real-time, robust and low-cost biosensors, for both soil and air, which can be operated by

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personnel with limited or no training in various fields like disease diagnosis, pharmaceutical research, agriculture and homeland security.

Clark and Ann Lyons (1962) from the Cincinnati Children's Hospital developed the first glucose enzyme electrode. This biosensor was based on a thin layer of glucose oxidase (GOx) on an oxygen electrode. Thus, the read out was the amount of oxygen consumed by GOx during the enzymatic reaction with the substrate glucose. This publication became one of the most often cited papers in life sciences. Due to his incomparable contribution in the field of biosensor development, he is considered as the "Father of biosensors," especially with respect to the glucose sensing for diabetes patients.

COMPONENTS OF A BIOSENSOR

A biosensor is made by integrating three main components namely:

1. A bio-recognition element
2. Transducer
3. Display

Bio-recognition Element

Any biological element that works based on affinity can be used as a bio-recognition element. It can be an enzyme, an antibody, nucleic acid, hormone, cell, tissue etc. Its role is to interact specifically with the target analyte that results into a biochemical reaction which is consequently transformed through a transducer to a measurable signal.

Characteristics of an ideal bio- recognition element:

1. It should be highly specific
2. It must be stable under storage conditions
3. It must be amenable to immobilsation procedures.

Enzymes

Enzymes are often used as bio-receptors for the development of biosensors since they are highly specific for a desired molecule (substrate) and catalyze generation of the product, which is then directly, determined using one of the transducers. Some of the enzyme categories and their functions which are used for selective detection of their competent substrates as analytes by biosensor are given below;

1. Oxidoreductases-Oxidation/reduction reactions
2. Transferases- Transfer of molecular group from one molecule to another
3. Hydrolases- Hydrolytic cleavage
4. Lyases-Cleavage of C—C, C—O, C—N bonds by other means than oxidation or hydrolysis
5. Isomerases -Intramolecular rearrangement
6. Ligases- Joining of two molecules

The most successful commercially available biosensors are those for measuring glucose in blood samples representing about 90% of the global biosensor market utilizing glucose oxidase or glucose dehydrogenase (Monosík *et al.*, 2012). The performance of an enzyme based biosensor is very much dependent on the temperature, pH, the cofactors etc.

Antibodies

The unique property of antigen- antibody specificity are crucial to their usefulness in a vast variety of immunosensors where only the specific analyte of interest, the antigen, fits into the antibody binding site (Vo-Dinh T and Cullum, 2000)

Nucleic acids

Biosensors based on DNA or RNA gain their high sensitivity and selectivity from the very strong base pairing affinity between complementary sections of lined up nucleotide strands (Borgmann *et al.*, 2011).

The electrochemical DNA biosensors, which rely on the conversion of the base-pair recognition event into a measurable electrical signal, are regarded to be suitable candidates for the rapid and inexpensive diagnosis of genetic diseases, the detection of pathogenic biological species of clinical interest, and for the compatibility with micro-fabrication technology (Lucarelli *et al.*, 2004 and Wang *et al.*, 2004).

Organelles

Each kind of organelle have different metabolic pathways and contain enzymes to fulfill its function. The most commonly used organelles include chloroplast, mitochondria and lysosome. Mitochondria actively participate in the metabolism of calcium ions to control the function and also modulate the calcium related signaling pathways. Experiments have proved that mitochondria have the ability to respond to high calcium concentration generated in the proximity by opening the calcium channel. In this way, mitochondria can be used to detect the calcium concentration in medium and the detection is very sensitive due to high spatial resolution. Another application of mitochondria is used for detection of water pollution. Detergent compound's toxicity will damage the cell and subcellular structure including mitochondria. The detergents will cause a swelling effect which could be measured by an absorbance change. Experiment data shows the rate of change is proportional to the detergent concentration, providing a high standard for detection accuracy.

Cells

Cells are often used in biosensors because they are highly sensitive to surrounding environment and they can respond to all kinds of stimulants (Prancazio *et al*., 1999). These bio-receptors are either based on bio-recognition by an entire micro-organism or a specific cellular component that is capable of specific binding to certain species. Cells can easily be immobilised to the surface and remain active for longer period compared to organelles. They are usually used to monitor the, treatment effect of drugs, pollution causing effects of herbicides (which are main aquatic contaminant), etc.

Tissues

Tissues are used as bio-recognition element for making biosensors. Advantages of tissues as biosensors include the following:

1. Easier to immobilize compared to cells and organelles.
2. The higher activity and stability in natural environment compared to other bio-elements.
3. The avoidance of tedious work of extraction, centrifuge and purification of enzymes .
4. Necessary cofactors for enzyme to function exist in tissues.

There also exist some disadvantages of tissues, like the lack of specificity due to the interference of other enzymes and longer response time due to transport barrier.

Transducer

A transducer is a device which converts one form of energy into another. The transducing systems can be electrochemical, optical, piezoelectric, thermometric, ion-sensitive, magnetic or acoustic one. There are different types of transducers, which are briefly described below:

1. ***Electrochemical:*** The basic principlefor this class of biosensors is that chemical reactions between immobilized bio-molecule and target analyte produce or consume ions or electrons, which affects measurable electrical properties of the solution, such as electric current or potential (Thevenot *et al*., 1999).
2. ***Amperometric:*** These are the most widespread class of biosensors. Most of biochemicals can now be detected and quantified amperometrically by their enzyme-catalyzed electro-oxidation or electro-reduction, or their enzyme-catalyzed hydrolysis/phosphorylation followed by electro-oxidation/electro-reduction, or their involvement in a bioaffinity reaction enabling electro-oxidation/electro-reduction (Heller, 1996).

   Amperometric biosensors are very sensitive and more suitable for mass production than the potentiometric ones (Ghindilis *et al*., 1998).These are low molecular weight redox couple, which shuttles electrons from the redox centre of the enzyme to the surface of the indicator electrode (Chaubey and Malhotra, 2002).
3. ***Potentiometric:*** This transducer measures difference in potential that is generated across an ion-selective membrane separating two solutions at virtually zero current flow.

4. ***Electrical:***

   (i) **Conductometric (Impedimetric) :** When ions or electrons are produced during the course of biochemical reaction, the overall conductivity or resistivity of the solution is changing. The measured parameter when using this transducer is the electrical conductance/resistance of the solution. Conductance measurements have relatively low sensitivity. When using a sinusoidal voltage (A. C) the electric field is generated which finally minimize undesirable effects such as Faradaic processes, double layer charging and concentration polarization (Mohanty and Kougianos, 2006). The inverse value of resistance is called conductance and thus the name conductometric has been used.

   (ii) **Optical:** The output transduced signal that is measured is light. This biosensor can be based on fluorescence or optical diffraction. A fluorescence-based device detects the change in frequency of electromagnetic radiation emission which is caused by previous absorption of radiation and also by generation of an excited state lasting for a very short time.

5. ***Calorimetric (thermometric):*** These biosensors are constructed by immobilization of biomolecules onto temperature sensors. Once the analyte comes in contact with the biocomponent, the reaction heat which is proportional to the analyte concentration is measured. The total heat produced or absorbed is proportional to the molar enthalpy and the total number of molecules in the reaction. The measurement of the temperature is via a thermistor, and such devices are called as enzyme thermistors. Thermal biosensors do not require frequent recalibration and are insensitive to the optical and electrochemical properties of the sample (Mohanty and Kougianos, 2006). Calorimetric biosensors were used for food, cosmetics, pharmaceutical and other component analysis (Antonelli *et al*., 2008, Bhand *et al*., 2010, Ramanathan *et al*., 2001)

6. ***Piezoelectric:*** The piezoelectric biosensor is based on measuring frequency changes directly related to mass change on the sensor surface, these sensors utilise crystals which undergo an elastic deformation when an electrical potential is applied to them. An

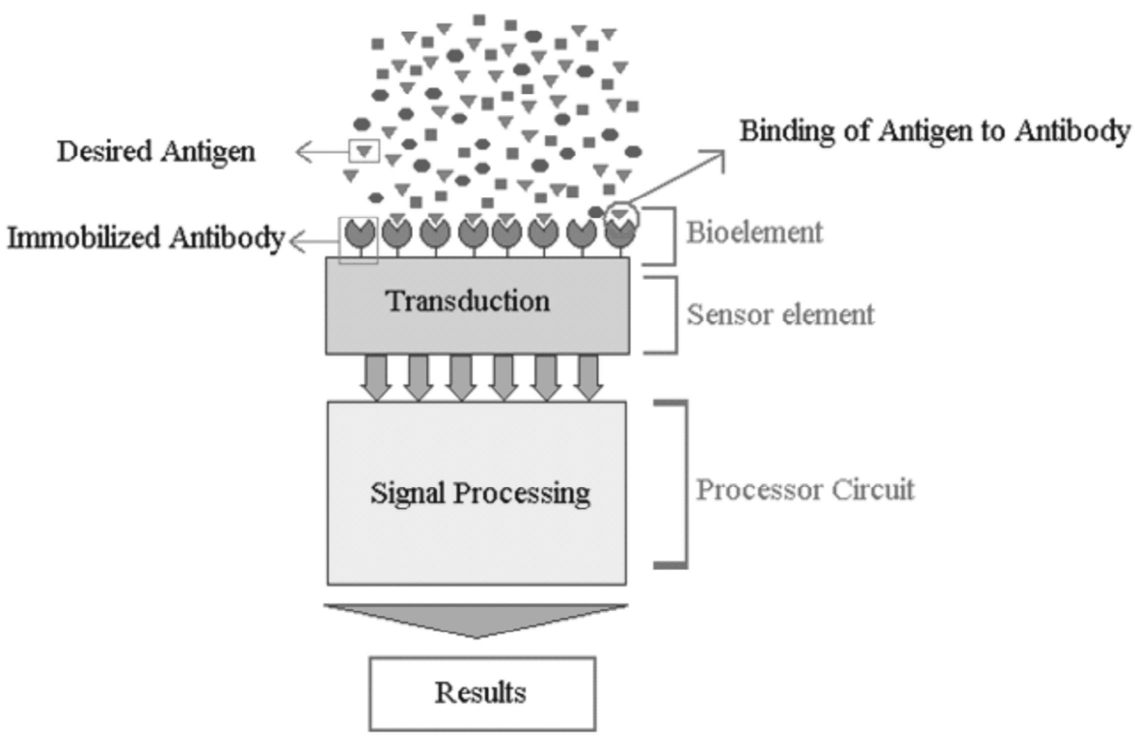

Figure 1: Basic principle of a biosensor

alternating potential (A.C.) produces a standing wave in the crystal at a characteristic frequency. This frequency is highly dependent on the elastic properties of the crystal, such that if a crystal is coated with a biological recognition element the binding of a (large) target analyte to a receptor will produce a change in the resonance frequency, which gives a binding signal. In a mode that uses surface acoustic waves (SAW), the sensitivity is greatly increased. This is a specialised application of the Quartz crystal microbalance as a biosensor.

Immobilization of the Biomolecules

Very important part of a biosensor fabrication is the immobilization of bio-component. It is the procedure by which biomolecules are cemented or fixed on the transducer. Performance of biosensors with immobilized molecules depends also on factors such as pH, temperature, contaminants, thickness and stability of the materials (Kissinger, 2005). Some of the chemicals used for the immobilization procedure are listed below.

1. Cellulose
2. Silica gel
3. Succinimide
4. Polyacrylamide,
5. Polyvinyl alcohol
6. Polycarbonate
7. Glutaraldehyde,
8. Hexamethylene
9. di-isocyanate, 1, 5-difluoro 2, 4-dinitro-benzen, and
10. bisdiazobenzidine-2,2- disulphonic acid

Display

The signals from the trasnducer are passed to a microprocessor where they are amplified, analysed and converted to concentration units and trasnfered to a display output, or a monitor from where it can be read by an observer in measurable unit.

Basic Principle of a Biosensor

The analyte of interest when binds to the immobilized bio-recognition element, reacts with it producing a signal. This signal is transduced by various transducers mentioned above and amplified by various microprocessors. The transducer can be connected to a display output to get the result in a readable form (Figure 1).

Figure 2: Application of biosensors

Advantages of biosensor

1. Require very low sample volume.
2. Provide more accurate readings than conventional methods.
3. Economical
4. Miniaturization is possible: Amperometric biosensors modified by silver nanoparticles showed improved biocompatibility utilized in pesticide detection (Huang *et al*., 2008)
5. The performance of multi-analyte analysis is enabled in the same device, which also shortens analysis time.

Disadvantages

In spite of the great many advantages the biosensor offers, two important factors still need to be addressed.

1. Reproducibility.
2. Reusability.

Figure 3: Glucose meter

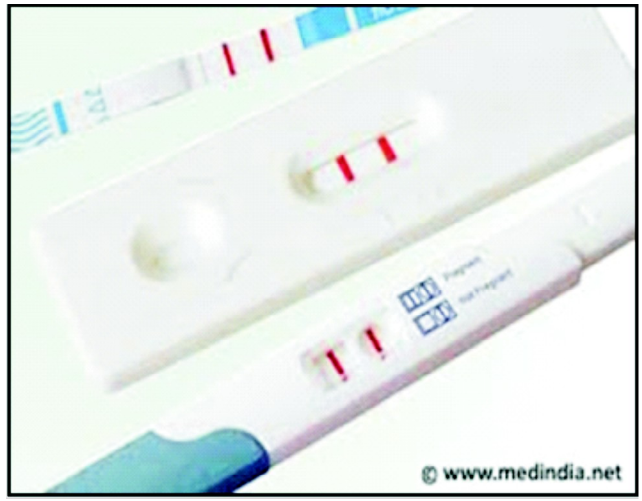

Figure 4: Pregnancy meter

Applications of biosensors

Biosensors can be used in versatile sectors like Health and medical sector, Industrial sector, Environment, Agriculture, Defence sector etc.

1. **Medical sector:** The most commercial biosensor which holds 90% of the biosensor market is the blood glucose biosensor (Figure 3). This was the initial impetus in the development of biosensors. Another important medical biosensor used commercially is the pregnancy meter (Figure 4).
2. **Environmental applications**
   - The detection of river water contaminants such as heavy metal ions
   - Determining levels of toxic substances before and after bioremediation.
3. **Industrial sector**
   - Determination of drug residues in food, such as antibiotics and growth promoters particularly in meat and honey
   - Detection of toxic metabolites such as mycotoxins in food
4. **Agricultural sector**
   - Determination of various qualitative aspects in agricultural produce, for eg. the gluten strength in wheat, starch content in rice, sugar content in fruits, etc.
   - For determination for various plant pathogenic microorganisms like fungi, bacteria etc (Tothil *et al*., 2001)
   - For determination of pesticide residue in agricultural products.
5. **Defence sector:** For the easy, and rapid detection of various biowar weapons, that can cause direct loss to humans by causing human epidemics, or indirect loss by causing epiphytotics and epizootics.

Application of biosensors in plant pathology

Label free biosensors that can detect and quantify specific plant pathogens on-field might enable farmers to target application of pesticides precisely, reducing their use.

Currently antibodies are the preferred detection systems since they offer all the advantages mentioned above and many pathogen-specific antibodies are available. The main application of biosensor in plant pathology are its usefulness in the detection of;

1. Various plant pathogens (bacteria, fungi, virus, etc)
2. Fungal spores
3. Toxins- mycotoxins, and
4. Nutrient abnormalities

Biosensors can be used for easy detection of harmful fungal spores. The spores of, Ug- 99 race of *Puccinia graminis tritici* causing wheat rust, brown spot causing pathogen of rice *Drecslera oryzae* etc. which are used for bioterrorism, causing large scale human destruction and famine can easiy be detected by using biosensors.

Table 1
Biosensor-based studies of plant pathogen detection

Sl. No.	Analyte	Sensor type	BRE used	Sensitivity	Reference
1.	Cowpea mosaic virus	SPR*	mAb	12.5 ìg/ml	Torrance *et al.* (2006)
3.	Fusarium culmorum	SPR	Nucloride hybrisisation	0.06 pg	Zezza *et al.* (2006)
4.	Lettuce mosaic virus	SPR	pAb	Not reported	Candresse *et al.* (2007)
5.	Puccinia striiformis	SPR	mAb	3.1 × 105 sp/ml	Skottrup *et al.* (2007b)
6.	Phyththora infestans	SPR	mAb	2.2 × 106 sp/ml	Skottrup *et al.* (2007c)
7.	Orchid viruses	QCM **	pAb	1 ng/ml	Eun *et al.* (2002)
9.	Aspergillus niger	SPR	pAb	103 cfu/ml	Nugaeva *et al.* (2007)

*Surface Plasmon resonance **Quatz crystal microbalance.

Nanoparticles have been utilized with other biological materials such as antibody for detecting *Xanthomonas axonopodis* that causes bacterial spot disease (Yao *et al.*, 2009). Gold nanoparticle-based optical immunosensors have been developed for detection of karnal bunt disease in wheat using surface plasmon resonance (SPR) (Singh et al., 2009). In addition to single probe sensors, nano-chips made of microarrays which contain fluorescent oligo probes were also reported for detecting single nucleotide change in the bacteria and viruses with high sensitivity and specificity based on DNA hybridization (López *et al.*, 2009).

Fluorescent silica nanoparticles (FSNPs) combined with antibody as a biomarker have been studied as the probe, which successfully detected plant pathogens such as *Xanthomonas axonopodis* pv. *Vesicatoria* that cause bacterial spot diseases in *Solanaceae* plant (Yao *et al.*, 2009).

CONCLUSION

The diagnostics market is expanding rapidly and covering a wide range of disciplines. Application of biosensors in the medical sector has been highly successful but it still faces various enigmas for its accurate usage in agriculture. Even though various studies have proved biosensor as a successful analytical device for detection of plant diseases, it still has to be modified and sharpened for it's on-field application. A vast number of researches are being undertaken in diagnostics companies and research institutions to develop biosensors, however, moving the technology to the market faces many challenges, for which efforts and funds need to be mobilized to manufacture biosensors on a large scale so as to benefit and be of use to the general public.

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