

## New Horizons of Biosensor Technology for Sustainable Agriculture

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### ABSTRACT

Biosensor is defined as 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. They are operated based on the principle of signal transduction. The measurement of biosensor responses to specific analyte in soil may allow real time monitoring of the elemental bioavailability by monitoring the induction of specific promoters, allowing a better assessment of bioaccumulation and biomagnification in food chains and therefore improving the risk assessment

for terrestrial ecosystems. Biosensors like ethanol biosensors are now developed for catering very sensitive issues related to raw and processed food and also as on-site quality testing tool. Tyrosinase, butyrylcholinesterase, alkaline-phosphatase, laccase based biosensors can be used to detect pesticides from soil, water, food samples. Since each biosensor is highly specific to a particular target molecule, it can be used for detect heavy metals or other toxic materials. Electrochemical based biosensors is mostly used to detect heavy metals like As, Pb, Hg from soil. It has the potential to quantify physiological, immunological and behavioral responses of livestock and multiple animal species. It is widely used for breath analysis of animals, monitoring animal stress, monitoring jaw movement of cattle to know the grazing efficiency, monitoring animal diseases. In brief, biosensors will emerge as most influential technology in modern precision agriculture to assist decision support systems for forecasting the calamities and assessing the losses rapidly, ensuring sustainable agriculture with enhanced crop productivity.

**Keywords** Biosensors, Sustainable agriculture, Portable biosensors, Soil management, Crop monitoring.

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### INTRODUCTION

Agricultural sector is one among the most susceptible sectors affected by climate inconsistency. Along with the increasing food emergencies, degradation of farmland, declining soil fertility, inefficient crop management practices, postharvest losses and the occurrence of extreme events such as drought and flood remains as major threats to sustainable agriculture.

Hence, climate resilient agriculture is necessary for climate change mitigation and to achieve agricultural as well as environmental sustainability. Biosensor technology has the potential to improve the agricultural productivity by adopting innovative tools and techniques by real time monitoring and managing the soil-crop system. This review highlights (1) various biosensors, (2) components, (3) working principles, (4) limitations and (5) their major applications in agricultural sector.

### Biosensors

Biosensor is defined as 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 (Thevenot *et al.* 2001). The first ‘true’ biosensor was developed by Leland C. Clark, Jr in 1956 for oxygen detection. He is known as the ‘Father of biosensors’ and his invention of the oxygen electrode bears his name, ‘Clark electrode’ (Koyun *et al.* 2012).

### Components of biosensor

There are four major parts involved in the working of biosensors namely analyte, bioreceptor, transducer and a signal amplifier (Fig.1). Analyte is the substance whose chemical composition is to be determined. Bioreceptor is a part which is sensitive to the substance which is to be detected. Enzymes have

been identified as the most widely used bio-receptor molecules. Recently, antibodies and proteins are also used as bio-receptor molecules in biosensors. The specificity of a biosensor comes from the specificity of the bioreceptor molecule used. Transducer is the part which transfigures the signals. It converts electrochemical signals into a measurable signal, mostly electrical signal that correlates with the quantity or presence of the chemical or biological target. Signal amplifier amplifies the electrical signal and it is processed by a signal processor (Srushtee and Sujata 2020).

### Working principle

Biosensors are operated based on the principle of signal transduction. Bioreceptor is allowed to interact with a specific analyte. The transducer measures this interaction and outputs a signal. The intensity of the signal output is proportional to the concentration of the analyte. The signal is then amplified and processed by the electronic system (Koyun *et al.* 2012).

### Types of transducers

There are different types of transducer elements used for signal transduction. Ion-selective electrode (ISE), glass electrode, gas electrode and metal electrode are used in potentiometric analysis to detect  $K^+$ ,  $Cl^-$ ,  $Ca^{+2}$ ,  $F^-$ ,  $H^+$ ,  $Na^+$ ,  $CO_2$  and  $NH_3$ . Metal or carbon electrode and chemically modified electrodes (CME) are used in amperometric analysis to detect  $O_2$ , sugars, alco-

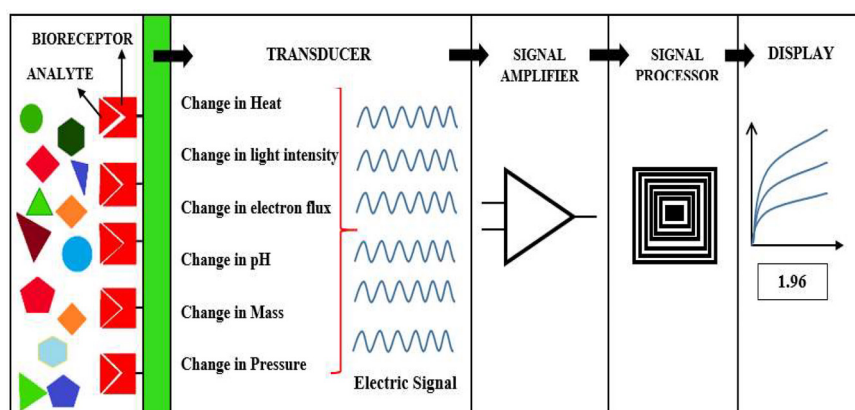


Fig. 1. Components of a biosensor unit.

hols, phenols and oligonucleotides. Metal electrodes are used in conductometric analysis to detect urea, charged species and oligonucleotides. To detect nutrient availability in soil, Ion-sensitive Field-effect transistor (ISFET) and Enzyme FET (ENFET) commonly used (Chauhan and Urooj 2019).

### Classification of biosensors

Based on transduction principle, biosensors are classified to electrochemical biosensors, optical biosensors, calorimetric biosensors and piezo-electric biosensors. Based on bio element used, biosensors are classified to enzyme biosensors, nucleic acid biosensors, ligands biosensors, saccharides biosensors, oligonucleotides biosensors and proteins biosensors. Based on the type of analyte to be detected, biosensors are again classified to DNA biosensors, glucose biosensors, toxins biosensors, mycotoxins biosensors and drugs or enzyme based biosensors (Srushtee and Sujata 2020). Major biosensors and their features are mentioned below:

*Electrochemical biosensor:* The principle of electrochemical biosensors is that the electro active analyte is oxidized or reduced on the working electrode surface, which is subjected to some predefined pattern of fixed or varying potential and the variation on electron fluxes leads to the generation of an electrochemical signal, which is measured by electrochemical detector (Viswanathan *et al.* 2009).

*Calorimetric biosensor:* Many enzyme catalyzed reactions are exothermic, generating heat which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration. Change in heat before and after entry of analyte is measured using thermistors.

*Piezoelectric biosensors or acoustic wave biosensors:* Piezoelectric materials, typically quartz crystals are used to generate acoustic waves. Their surface is usually coated with antibodies which bind to the complementary antigen present in the sample solution. This leads to increased mass which reduces their vibrational frequency; this change is used to determine the amount of analyte present in the sample solution.

*Optical biosensors:* These involve determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process (Mudgal *et al.* 2020).

In surface plasmon resonance (SPR) sensor, the sensor chip is composed of a glass surface coated with a thin layer of metal that provide the physical conditions necessary for the SPR reaction. Polarized light is incident on the reverse side of this chip, propagating an electron charge density wave phenomenon that arises on the surface of the metallic film. The extent of binding between the analyte and the immobilized bioelement is easily observed and quantified by monitoring this reflectivity change (Mudgal *et al.* 2020).

In whole cell biosensor, lux or gfp-inserted reporter genes are under control of an inducible promoter that can be used to detect the bioavailability of specific analyte. Typically, the activation of the promoter is induced when the analyte binds, acting as the selective bioreceptor for the analyte, or through complex cellular events that lead to the expression of the reporter gene (Saini *et al.* 2019). Microbial fuel cells (MFCs) are devices that use bacteria as catalysts to oxidize organic and inorganic matter and generate electrical current. Microorganisms in the anode chamber function as a biological recognition element, whereas proton exchange membranes and electrodes act as a transducer. The bacteria can sense the sudden presence or a change in the level of target analytes and then give the response on its output electric current (Do *et al.* 2020).

### Applications of biosensor technology

Biosensors have wide applications in food analysis, to study biomolecules and their interactions, drug development, crime detection, medical diagnosis, environmental monitoring, industrial process control, manufacturing pharmaceuticals, wastewater treatment and agriculture. Biosensor immobilizes the activity of enzymes and also the microbial load in the food which keeps the food fresh for long duration and also improves its quality (Kundu *et al.* 2019). Applications of biosensors in agriculture (Fig. 2) are depicted below:



Fig. 2. Applications of biosensor technology in agriculture.

#### *Applications in crop monitoring and soil management*

In crop monitoring, electrochemical biosensors is more efficient since enzyme-substrate interaction is very specific. Applications include the monitoring the crop growth as well as crop quality. Biosensors can be used in soil science to study the bioavailability of various nutrients, nutrient transformation, enzymatic activity, microbial activity and moisture stress. The measurement of biosensor responses to specific analyte in soil may allow real time monitoring of the elemental bioavailability by monitoring the induction of specific promoters, allowing a better assessment of bioaccumulation and biomagnification in food chains, and therefore improving the risk assessment for terrestrial ecosystems (Renella and Giagnoni 2016). DeAngelis *et al.* (2005) reported that green fluorescent proteins (GFP) biosensors are useful for estimating nitrate transformation in the rhizosphere region of *Avena fatua* grown in rhizotrons amended with different concentration of sodium nitrate. GFP reporter gene placed under the regulatory control of the promoter of *Escherichia coli* nitrate reductase gene (*narG*) confers the reduction of nitrate to nitrite. Amine and Palleschi (2004) reported that the biosensors based on various enzymes can be used as phosphate and nitrate biosensor. A nitrate biosensor contains a permeable bio catalytic membrane that allows nitrate ions to enter a chamber where active

enzymes such as nitrate reductase reduce nitrate to  $\text{N}_2\text{O}$  and can be detected by a  $\text{N}_2\text{O}$  transducer (Sinfield *et al.* 2010). The chemistry of the enzymatic reduction of nitrate involves a flow of electrons from an electron donor, such as NADH, or a mediator through the enzyme NaR to achieve reduction into nitrite Sohail and Adeloju (2016). Saputra *et al.* (2017) reported that Foster Resonance Energy Transfer (FRET) bi-sensors can be used as monitoring tools to assess the performance of biochar amended contaminated soil. Tapia *et al.* (2018) reported that MFCs can be used for sensing soil water content. An increase in the current has observed at the time of irrigation supplied via sprinkler in test crop *Sedum* sp (Chiranjeevi *et al.* 2012). Vakilian (2019) demonstrated an optical biosensor based on gold nanoparticles aggregation which was capable of detecting mild, moderate and severe drought stress in tomato plants by ultrasensitive determination of sly-miRNA-1886. Wang *et al.* (2019) demonstrated a biosensor using anti- Vitronectin-like proteins to monitor invisible damage of plant cells induced by cadmium [Cd(II)] and lead [Pb(II)].

#### *Applications in pest management*

Infectious plant diseases are caused by pathogenic microorganisms such as fungi, bacteria, viruses, viroids, phytoplasma and nematodes. Current immunological techniques like enzyme-linked immunosorbent assays (ELISA), direct tissue blot immunoassays (DTBIA), DNA-based techniques such as polymerase chain reaction (PCR) and real time PCR (RT-PCR) are proposed for pathogen identification and detection. Lateral flow biosensor for *Phytophthora* sp., *Erwinia amylovora*, Potato virus Y, ELISA kit for the detection of *Citrus tristeza virus*, *Acidovorax avenae* ssp. *citrulli* and *Botrytis cinerea* are commercially available in market (Khater *et al.* 2017). Zhan *et al.* (2018) demonstrated a novel method for visual detection of *Phytophthora infestans* by integrating universal primer mediated asymmetric PCR with gold nanoparticle (AuNP)-based lateral flow biosensor.

Saha (2016) developed a nano biosensor for detection of bract mosaic virus in banana. Antigen of banana bract mosaic virus (BBrMV) was added to nanoprobe solution of red color containing antibody of BBrMV conjugated to Au nanorods. Brezolin

*et al.* (2018) reported that olfactory electrochemical biosensors based on Odorant-binding proteins (OBPs) are highly sensitive and selective, which are prerequisites for practical applications. Farkhanda (2013) proposed that biosensor devices could be a combination of several factors, such as temperature, moisture, movement, feeding and behavior of the termites, in the sensing technology.

#### *Applications in post-harvest technology*

Post-harvest agriculture is a complex aggregate of operations consisting of harvesting, sorting, storage, processing and packaging. Smart packaging methods have been used not only to facilitate product handling, but also to preserve nutrition value, extend their shelf life and reduce spoilage (Park *et al.* 2015). Commercial ethanol biosensors are composed of chromagen and immobilized enzymes like alcohol oxidase and peroxidase. Oxidation of the chromagen causing a color change from red to yellow according to the ripeness degree (Kundu *et al.* 2019). Food additives and flavor enhancers such as glutamate is very often used in processed food and beverage industry. Biosensors have been developed for detecting excessive usage of artificial preservatives, food additives and artificially ripening agents which is conventionally being done by HPLC and gas chromatography techniques (Kulkarni *et al.* 2014). Acetylcholinesterase (AChE) based electrochemical biosensors can be used to detect  $\text{CaC}_2$  in mango fruits. Amperometric current was found to be decreasing linearly with an increase in  $\text{CaC}_2$  concentration from 1 nM to 100 nM, which suggested that the inhibition of AChE activity on addition of  $\text{CaC}_2$  (Ramachandra *et al.* 2016).

Various biosensors are available for the detection of quality indicators such as flavonoid content in citrus fruits, glucose content in fruits. Huang *et al.* (2016) demonstrated that Ag nanoflower-based electrochemical immunosensor is used for the detection of *Escherichia coli* present in food. Bacterial antibody was immobilized on the surface of Ag nanoflowers through covalent conjugation. The performance of amperometric glucose biosensor to measure the glucose content of fruits was compared to that of a commercial glucose assay kit. There was no significance different between two methods, indicating the

introduced biosensor is reliable Ang *et al.* (2015).

#### *Applications in pesticide residue analysis*

Chromatography is one of the most important techniques in pesticide residue analysis. The detectors most commonly used are flame ionization detector, electron capture detector, and mass spectrometer detector. The excellent sensitivity of chromatographic methods is evidenced by the low detection limits. However, these methods have some limitations, such as complex and lengthy analysis, highly skilled operators and high costs of reagents and materials (Wang *et al.* 2014). In this sense, the use of biosensors is becoming more popular to complement the analysis, and as an alternative to replace the existing classical methods.

Biosensors based on acetylcholinesterase and organophosphorus hydrolase (OPH) can be used to determine organophosphates residues. If organophosphate is not present in sample, the acetylthiocholine substrate is converted to acetic acid and thiocholine by the action of the enzyme present on the biosensor. The thiocholine, in turn, is oxidized by applying voltage. Since, in the presence of an inhibitor, the conversion of acetylthiocholine decreases or is zero. Similarly, Tyrosinase, Butyrylcholinesterase, Alkaline-phosphatase, Laccase based biosensors can be used to detect pesticides like Chlortoluron, Diazinon, Malathion, Pirimicarb respectively from soil, water, food samples (Muenchen *et al.* 2016).

Biosensor is initially treated with a control and the base signal ( $I_0$ ) is measured. Then the biosensor is incubated in a solution containing the substrate containing inhibitor and the base signal ( $I_i$ ) is measured. Thus, the amount of inhibitor can be related to the inhibition percentage (%IR) obtained from following equation (Skladal and Mascini 1992).

$$\% \text{ IR} = [(I_0 - I_i) / I_0] \times 100$$

Portable biosensor based on the inhibition of the acetylcholinesterase enzyme using screen-printed electrodes and designed potentiostat for determining toxic pesticides in water and food samples has been assessed and validated for in-field use purposes

(Mostafa 2010). Isolated thylakoids can be immobilized on an electrochemical detector, thus enabling the direct detection of their photochemical activity (Bettazzi *et al.* 2007). A study was conducted by Masojidek *et al.* (2011) to compare the standard algal growth inhibition test using *Desmodesmus subspicatus* with a novel PSII-biosensor assay in the detection of photosynthetic herbicides in liquid samples. When the herbicide was added to the medium, a decrease in signal peaks during the illumination period was observed in a concentration-dependant manner due to the blocked electron transport between the PSII complex. The response time of the PSII- biosensor is immediate and the whole test procedure can be completed in 1 h while the standard procedure of the algal growth inhibition test takes 72 h.

Zhao *et al.* (2020) developed a smart plant-wearable biosensor for the *in-situ* detection of methyl parathion on the surface of crops. Organophosphorus hydrolase (OPH) can hydrolyze methyl parathion to release the electroactive *p*-nitrophenol (PNP). This smart plant-wearable biosensor modified with OPH and equipped with gelatin electrolyte can be attached on the surface of agricultural products, such as leaf of spinach and fruits of apple, which allows for the real-time and *in-situ* analysis of methyl parathion by the electrochemical determination of the corresponding hydrolysis product.

#### *Applications in environmental monitoring*

Biosensors have a vital role in monitoring pollutants present in soil and water. Since each biosensor is highly specific to a particular target molecule, it can be used for detect heavy metals or other toxic materials. Electrochemical based biosensors is mostly used to detect heavy metals like As, Pb, Hg from soil (Hernandez-Vargas *et al.* 2018). Whole cell biosensors detects heavy metals and other aromatic hydrocarbons present in soil (Cui *et al.* 2018). Microbial fuel cell based biosensors also can be to detect toxic pollutants in soil and water samples (Cui *et al.* 2019). Coelho *et al.* (2015) studied the response of two bacterial bioreporters, pCHRGFP1 *Escherichia coli* and pCHRGFP2 *Ochrobactrum tritici* to increasing concentrations of chromate in two different soils. Data obtained showed that the biosensors tested

are sensitive to chromate presence in soil and may constitute a rapid and efficient method to measure chromate availability in soils.

#### *Applications in animal husbandry*

Biosensors has the potential to quantify physiological, immunological and behavioral responses of livestock and multiple animal species. They are used for disease detection and isolation, health monitoring and detection of reproductive cycles, as well as monitoring physiological wellbeing of the animal (Sadeghi *et al.* 2015). Biosensors can be used for breath analysis of animals, monitoring animal stress, monitoring jaw movement of cattle to know the grazing efficiency, monitoring animal disease (Pereira *et al.* 2015).

#### **Limitations of biosensors**

Although many biosensor related patents are filed each year, very few play a prominent role in food industry, environmental monitoring, agricultural or veterinary applications. Application and commercialization of biosensor technology is lagging behind the output of research laboratories. There have been many reasons for the slow technology transfer from the research laboratories to the market place. Cost considerations, stability and sensitivity issues, quality assurance, instrumentation design are some of the reasons. Methods of sensor calibration, methods of producing inexpensive and reliable sensors, stabilization and storage of biosensors remains as other barriers for this technology transfer.

#### **CONCLUSION**

Biosensors are nowadays ubiquitous in different areas of agriculture. A range of molecules with bio recognition properties can be used as the sensing element in biosensors. A wide range of transducers are also available to engineer new biosensing devices. There are different ways to combine biology, chemistry, physics, mathematics and engineering in order to develop new biosensors with applications in agriculture. The promise shown by biosensor technology is very real, however there are some technological obstacles that need to be overcome. Constraints and difficulties in the way of technological adoption and

dissemination need to be addressed through research and developmental activities. In future, there will not be any sector untouched by biosensors. Biosensors will emerge as most influential technology in modern precision agriculture to assist decision support systems for forecasting the calamities and assessing the losses rapidly, ensuring sustainable agriculture with enhanced crop productivity.

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