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INTERNATIONAL JOURNAL OF ADVANCED PRODUCTION AND INDUSTRIAL ENGINEERING

IJAPIE-2017-07-318, Vol 2 (3), 77-81

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Study of Biological Response into a Quantifiable and Processable Signal Using Earthworm as a Bio-receptor-An Overview

Ankita Rai[1], Dipankar Mishra[2]
[1](Amity Institute of Biotechnology, Amity University, Noida, India)
[2](Department of ITESM, Ambedkar Institute of Technology, New Delhi, India)
*Email: ankitarai378@gmail.com , dm.pusa@gmail.com

Abstract : *A biosensor is an analytical device that makes use of biological reaction of biochemical molecule for detecting the target analyte and converts such biological response into a form of signal that can be measured easily. It uses a specific biochemical reactions to detect chemical compounds present in given biological samples such as soil, water, blood, etc. This article deals with the principles and operation of biosensor, their existing and potential applications in the environment monitoring, food analysis and agricultural industries and to observe the effect of oxygen, moisture and soil pollutants on the behavior of an important decomposer i.e. earthworms which are used as a bio-receptor. By making use of earthworm as a bioreceptor, one can take preventive measures to control soil pollution at an early stage and can also maintain the ecological balance of the ecosystem. The bio-accumulative ability of earthworms can be utilized in a fruitful way and thus earthworm could be a useful living organism for the bio-monitoring of soil pollution.*

Keywords : *Bio-accumulative, Biochemical, Bio-monitoring, Bio-receptor, Decomposer, Ecosystem.*

1. Introduction

The most widely accepted definition of a biosensor is: "a self-sufficient analytical device that incorporates a biological molecule in close contact with an appropriate transduction element for the purpose of detecting the concentration of chemical or biological species present in any type of sample." [1] The first biosensor was developed by Clark and Lyons [2]. It was called the glucometer which makes use of the enzyme glucose oxidase that causes oxidation of blood glucose to glucono-lactone and hydrogen peroxide to generate a quantifiable signal. Glucose biosensor is an example of electrochemical sensor that involves oxidation and reduction of the sample and the enzyme. A biosensor consists of three main components: a bioreceptor, a transducer and an electronic System. The major advantages of a biosensor lies in its simplicity, selectivity, linearity, sensitivity, limit of detection and the speed of measurements.

Soil pollution has increased tremendously during the last decades due to the excessive use of pesticides and fertilizers in agriculture and industrial sectors. The adverse biological effects of various pollutants can be assessed using different living organisms or cells in form of 'analytical devices'[3]. The biological response of living organisms to environmental sample can indicate the toxicity of the whole mixture of chemical compounds present in that particular sample. Soils, which consist of useful minerals, oxygen, moisture, several organic compounds, etc. are the important sites of various biological phenomenon like decomposition, mineralization, nutrient incorporation etc. that supports the terrestrial ecosystem. A majority of toxic substances make their way into different layers of soil and cause soil pollution leading to soil contamination. These toxic substances pose threat to various living organisms and also the processes that takes place in the soil. Thereby the biological response of earthworm to soil contamination will be very helpful in monitoring the soil quality and the extent of toxicity.

It is well known that earthworms play an important role in enhacing the fertility of the soil by allowing the organic matter to diffuse through the soil and the nutrients retained in it to become accessible to bacteria, fungi and plants[4]. Also earthworms have a well developed nervous system that is responsive to even very low concentration of polluting agents. Since the culturing as well as handling of earthworms is quite easy as compared to other living organisms therefore, the earthworm is likely to be an excellent organism for this purpose.

2. Types of Biosensor

Biosensors can be classified on the basis of bio-recognition element or transduction element. The most common biological elements include enzymes, antibodies, micro-organisms, nucleic acids, and organelles. The most common transduction elements include electrochemical transducers, optical transducers, piezoelectric transducers and thermal transducers. When a chemical change takes place after analyte-receptor interaction and the concentration of one of the substrates or products is measured then the instrument is known as a metabolism sensor. When the analyte binds to the biorecognition element then the instrument is known as an affinity sensor. Finally, when the analyte binds to the

bioreceptor without chemically changing its properties but by altering an secondary substrate, then the biosensor is known as a catalytic sensor[5].The method of transduction relies upon the nature of physicochemical changes resulting from the sensing event.

3. Elements of a Biosensor

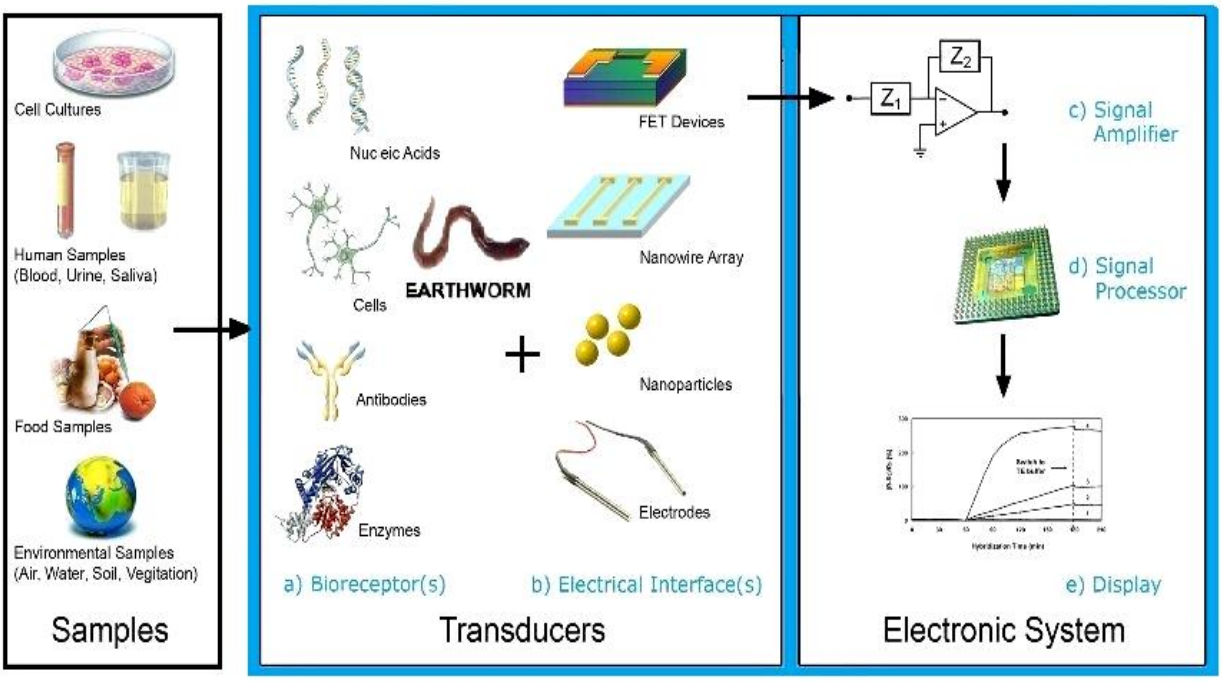

Figure:1-Elements of a Biosensor

Biosensor consist of three main elements- a bioreceptor, an electrical interface and an electronic system as shown in fig.1. Samples like water, soil, blood, urine, saliva, and food etc. can be immobilized on the biosensing membrane in order to assess its concentration. In the present manuscript, the soil containing moisture and oxygen is considered as a sample in order to determine its toxicity by using earthworm as a bioreceptor.

3.1 Bio-receptors: A bioreceptor is an immobilized extremely sensitive bio-recognition element (e.g. enzyme, DNA probe, antibody) that identifies the target analyte. There are various types of bioreceptors that can be used in a biosensor. Some of them are as follows:

a. Enzymes-These are polymers of amino acids which catalyze chemical reactions without being used up in the conversion of substrates to products. An enzyme is capable of identifying a specific target molecule. This specific nature of the enzyme is used in biosensors[6].

b. Antibodies- These are described as Y-shaped molecules consisting of four polypeptide chains - two light chains and two heavy chains that linked to each other through disulfide bonds. They are produced by B-cells in response to antigens (foreign body) that elicit an immune response inside the body. They are usually immobilized on the surface of the transducer through covalent attachment by conjugation of amino, carboxyl, or sulfhydryl groups. The antibody binds reversibly with a specific antigen.

c. Nucleic Acids- Biosensors based on nucleic acids like DNA and RNA gain their sensitivity and selectivity due to the strong base pair affinity between the complementary sections of strand. If the target nucleic acid sequence is known, complementary sequences can be synthesized, labeled, and then immobilized on the surface of biosensor.

3.2 Electrical Interface: It is a machine specific module that allows the operator to use standard Remote Control systems across a fleet of different machines. It includes FET devices (Field effect transistors), nanoparticles, nanowire array and electrodes.

3.3 Electronic System: It includes a signal amplifier, a signal processor and a display. Signal can be amplified by two different ways. The first type of signal amplification technique include nano signal amplification in which by taking the advantages of good conductivity and unique optical properties of the nanomaterials, the transfer of electron is accelerated in order to obtain sensitized optical signal. The second type of signal amplification technique is based on molecular biological amplification. PCR amplification is the most primitive applied strategy that falls under this category. It can simply amplify the DNA samples of interest and the signal is detected by

using the PCR product as target. Also, by incorporating functional nanoparticles, several new strategies like rolling circle amplification (RCA), hybridization chain reaction, loop-mediated amplification and target DNA recycling amplification have been evolved and implemented so as to intensify the electrochemical, optical and visual signals. Signal processing is one of the most crucial part of the electronic system where signal will be processed in two distinct phases. First phase is called analog signal processing that occurs when signal is coming from the transduction element of the biosensors while the second phase is digital signal processing and data acquisition system. The signal will be filtered first and then amplified so as to increase the intensity of the signal. Then it is required to eliminate the noise from the signal so as to get an appropriate signal without any disturbances. Data acquisition system helps in converting the resulting signal in form of energy into digital numeric values that can be easily interpreted by a computer. This data can be illustrated via graphical representation of an electric signal generated by the transducer in a user friendly way. The process flow diagram of working of a biosensor is shown in fig:2.

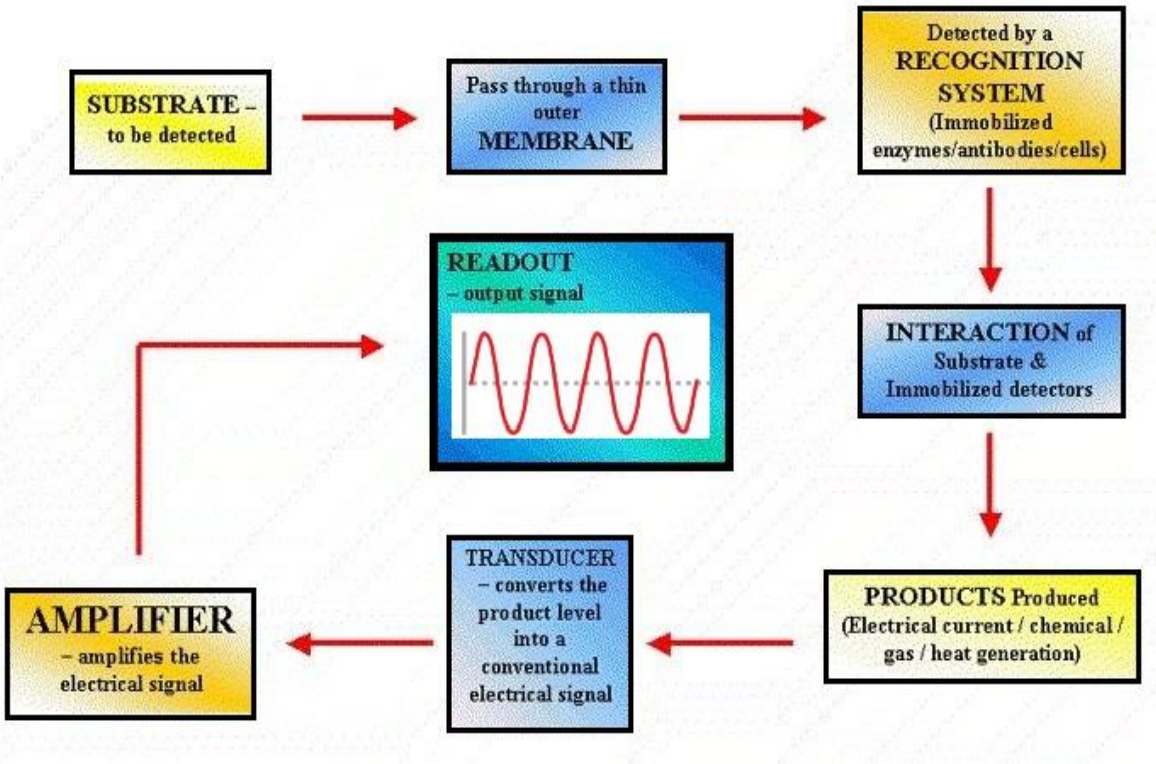

Figure:2-Process Flow diagram of a biosensor

4. Signal detection methods:

a. Amperometry: It is based on the principles of electrochemistry in which the potential is set across the two electrodes and the current is measured that is generated by the oxidation or reduction of electroactive species and interrelated to the concentration of the analyte of interest.

b. Potentiometry: It involves the measurement of the potential difference between the working electrode and the reference electrode which depends on the concentration of the target analyte.

c. Voltammetry: This is the most flexible technique in the electrochemical analysis in which current is measured in d.c. or a.c. mode. It may include sensors based on chemically inert electrodes, chemically active electrodes and modified electrodes.

d. Optical detection: It is usually based on the interaction of microorganisms with the analytes resulting into a luminescent, fluorescent or other optical signals and then the observed optical signals are compared with the concentration of target analyte.

e. Piezoelectric: Piezo-electric transducers utilize gold in order to detect the precise angle at which electron waves are emitted when the substance is exposed to crystals, such as quartz, which vibrate under the effect of an applied electric field. The change in frequency is directly proportional to the mass of absorbed material.

5. Choice of Bio-receptor

In this article effort has been made to incorporate earthworm as a bioreceptor. Earthworm play an important role in enhancing the soil fertility and are exceptionally important in decomposition process by consuming dead and decaying organic matter, breakdown it, and intermixing it with soil

mineral particles to form water-stable aggregates. The bio-accumulative ability of earthworms makes them a useful living organism for the bio-monitoring of soil pollution [7]. The species *Eisenia fetida* is most commonly used in ecotoxicological studies, and is identified as a useful bioreceptor for assessing the soil toxicity[8]. Earthworms have a well-developed nervous system that is responsive to even low concentration of toxic agents. Also, it is practically feasible to measure different parameters on earthworm like reproduction, growth and excretion of substances and biochemical responses. Moreover, the culturing and handling of earthworms is comparatively much easier. Therefore, earthworm is considered to be a suitable organism for this purpose.

5.1. Effect of oxygen and moisture content on the behavior of Earthworms

Earthworms absorb and lose moisture through their skin simply by the process of diffusion. If soils are dry, earthworms may migrate to deeper layers of soil, die, or go back to a hibernation condition called diapause. Earthworms can survive under submerged conditions if the water has high oxygen content. In most cases, however, earthworms will not survive under excessive water logging conditions.

5.2. Effect of soil pollutants on the behavior of earthworm

Earthworms have a well-developed nervous system that is extremely sensitive to even minute levels of polluting agents. Thus, the nerves of the earthworm get affected when exposed to soil pollutants and recover quickly once they are removed from the pollutants. Hence this test can be used as an early warning indicator when potential toxicants are beginning to rise in the soil. Appropriate measures can therefore be taken much earlier than at present. Also, while burrowing, earthworms consume large amounts of soil and thus they are rendered to the toxicant through their intestine as well as through their skin as a result of which the toxicants are concentrated from the soil into their body[9]. This makes them suitable for assessing the bioavailabilty of many harmful substances in the soil.

In case of earthworms, electrochemical (amperometric and potentiometric) signal detection method can be utilized for detecting the biological responses generated by the earthworm. In this technique, two electrodes are placed on the earthworm in order to measure the potential difference between the working electrode and the reference electrode generated from the interaction of the analyte with the biological element. This signal is detected with the help of a device called transducer.

6. Applications of Biosensor

a. Indication of soil pollution

Earthworm as a bioreceptor represent useful tool in soil monitoring and assessment as an early warning of adverse ecological effects. Soil characteristics such as presence of oxygen, moisture content also play an important role in determining the bioavailability of pollutants. The biological response of an earthworm to pollutant exposure varies because of the variety of pollutants present in the environment. Thus, by detecting these biological responses and behavioural changes with the help of a biosensor, the toxicity of soil can be estimated. The biological and behavioural response of earthworm to oxygen, moisture and soil pollutants at sub-lethal range can also be used as bioassays, giving rise to early indication of soil pollution leading to its contamination. Such responses can be measured in the form of signals with the help of an electrochemical biosensor. In case of earthworm, Electrochemical (amperometric and potentiometric) signal detection method can be utilized for detecting the biological responses generated by the earthworm. In this technique, two electrodes can be placed on the earthworm in order to measure the potential difference between the working electrode and the reference electrode generated from the interaction of the analyte with the biological element. Continuing research will reveal the best procedures and full applicability of using earthworm as a bioreceptor. They can provide a real-time measurement of the bioavailability of soil pollutants, as well as other heavy metals they can be designed to detect.

b. Health Care: Diagnosis of cancer disease

Existing cancer diagnostic techniques rely greatly on the study of cell and tissue morphology using several staining and microscopic methods that are very invasive and time consuming procedures. Furthermore, the removal of tissue may have a probability of missing cancer cells at the early onset of the disease. Therefore, diagnosis of cancer using biosensor becomes more convenient and beneficial in clinical testing for cancer due to its unique properties like quick response time, high reliability, low cost, high sensitivity, and less technically demanding as compared to microarray based analyses. However, major technical improvement is still required in order to utilize its potential to the fullest. For cancer diagnosis, multi-array sensors would be advantageous for multi-marker study. Both monoclonal and polyclonal antibodies have been utilized in cancer diagnostic tests for detecting cancer cells. A wide range of recognition elements such as antibodies have been used for detection of biomarker. More recently, artificial elements like nanomaterials, aptamers, binding proteins and synthetic peptides have been made-up as affinity molecules for detecting the target analyte.[10,11].

c. Industrial Process Control: Detection of heavy metals

Whole-cell bacterial biosensors have a great potential to be utilized as a useful tool in detection of heavy metals in soil or water sample. Devices have been designed to assess the concentration of heavy metals.They utilize genetically modified microorganisms and enzymes such as urease, cholinesterase, glucose oxidase, alkaline phosphatase, and peroxidase introduced into electrochemical and optical transduction elements[12].

d. Food Industry

Biosensors are also utilized for the measurement of carbohydrates, alcohols, and acids in various food products. Such devices are frequently used in quality testing and assurance laboratories. Potential use of enzyme based biosensors in assessing the food quality control include

measurement of amino acids, amines, amides, carbohydrates, carboxylic acids, gases, cofactors, inorganic ions, alcohols, and phenols[13].

7. Conclusion

In this article, the basic concepts and different elements of biosensors has been discussed briefly. In addition, this article highlights the possible utility of using earthworms as a bioreceptor, by observing the biological responses and behavior of earthworm under the effect of oxygen, moisture and soil pollutants present inside the soil. Earthworm as a bioreceptor represent useful tool in soil monitoring and assessment as an early indication of adverse ecological effects. These responses can be measured in the form of signals with the help of an electrochemical biosensor. Presently, the above study is at the research level but continuous research in this area will further disclose the best procedures and complete applicability of using earthworm as a bioreceptor. They can provide a real-time measurement of the bioavailability of soil pollutants, as well as other heavy metals they can be designed to detect. Also, wide range of applications of biosensors in different fields were discussed briefly in this article.

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