Octa Journal of Environmental Research **Apr - Jun, 2015**
International Peer-Reviewed Journal ISSN 2321 3655
Oct. Jour. Env. Res. Vol. 3(2): 212-218
Available online http://www.sciencebeingjournal.com

Review Article

Octa Journal of Environmental Research
(Oct. Jour. Env. Res.) ISSN: 2321-3655
Journal Homepage: http://www.sciencebeingjournal.com
Scientific Planet

BIOSENSORS FOR ENVIRONMENTAL MONITORING: AN UPDATE

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Received: 2nd April 2015 **Revised**: 15th April 2015 **Accepted:** 20th April 2015

Abstract: Environmental safety is the key requirements of our well-being. Rapid urbanization and industrialization have greatly contributed several pollutants such as heavy metals, inorganic and organic compounds, pesticides, toxins, endocrine disrupting hormones, *etc.* to the environment. The presence of such pollutants is becoming a major global challenge for environmental security and human health. Although regulatory bodies are trying to control such pollutants at the source of entry, but still requires an effective and integrated environmental management program. Therefore, beside to minimizing/reducing or eliminating the amounts of hazardous effluents into the environment, there is an emergent need to methods that can detect and monitor these environmental pollutants in sensitive and selective manner to enable effective remediation. Application of modern nanotechnology based biosensors has a great potential for environmental surveillance and detection of pollutants. Such biodevices are portable and give rapid responses in real time. In this paper, the author has discussed the key concepts behind the development of biosensor and explored the most relevant applications in the area of environmental monitoring. In addition, the author has also reviewed new developments, challenges in the development of biosensor along with future research direction in biosensing technology for sustainable environment monitoring.
Keywords: Biochemical oxygen demand (BOD), bioreceptor, biosensor, environmental monitoring, real-time, transducer.
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INTRODUCTION

At the present time, one of the major problems we are facing is that of environmental pollution, which is increasing day by day and causing severe damage to the earth. Environmental pollution comprises of five basic types of pollution, *i.e.* water, soil, air, noise and light. Out of them, soil and water are the most adversely affected due to increasing urbanization and industrialization in the recent past. The water and soil are significantly polluted by untreated industrial and agricultural wastes, including heavy metals, pesticides, herbicides, dioxins and other toxic substances. Such compounds may adversely affect the ecosystem. Most of the pollutants can accumulate and persist for a long time in the environment due to their non-degradable nature. In addition, they can also enter into the food chain. Therefore, detection and monitoring of such environmental pollutants in soil, water and air are very important for the overall environmental safety and security of biological system. While highly sensitive and selective, conventional analytical techniques, *i.e.* chromatography and spectroscopy are time tracking, expensive and require a lot of skill. Hence, there is an emergent need of easy, rapid, specific, sensitive and portable methods for analyzing environmental security threats. The need for disposable and eco-friendly systems or devices for environmental monitoring has encouraged the development of new advanced technologies and more appropriate approaches, the ability to monitor the increasing number of samples (analytes) of environmental significance as rapidly and as cheaply as possible, and even the possibility of allowing on-site field monitoring (Mazhabi and Arvand, 2014). In this respect, biosensors have demonstrated a great potential in recent years and thus arise as proposed analytical devices for effective environmental monitoring programs.

Biosensor is portable analytical device composed of the sensing element of biological origin and a physico-chemical transducer. As per the International Union of Pure and Applied Chemistry (IUPAC), biosensor is a self-contained integrated device that is capable of producing specific quantitative or semi-quantitative analytical information using a biological recognition element, *i.e.* biochemical receptor, which is remained in contact direct spatial with a transduction element. Biosensors are used in the analysis of biomaterial samples, *i.e.* composition, quantity, structure and function, for diverse applications including food and environmental quality control, fermentation process control, agriculture, military and diagnostic sectors.

The aim of this paper is to describe the concepts behind the development of biosensor and update the most relevant applications in the area of environmental monitoring and detection of pollutants with suitable examples.

Biosensor equipment has the following components (Figure 1):

i. *Bioreceptor*: It is a sensitive element of biological origin, *i.e.* tissue, whole cells/ microorganisms, organelles, enzymes, antibodies and nucleic acids, Figure 2, or any biologically derived material or bio mimics). These sensitive elements can also be developed by applying of suitable biological engineering techniques (Koedrith *et al.,* 2015).
ii. *Transducer or the detector element:* It works in a physico-chemical way and converts the signal generating from the interaction of the analytes, *i.e.* sample, with the biological element into another signal that can be more easily measured and quantified. The most widely used various types of biotransducers are shown in Figure 3.
iii. *Signal processor*: it is primarily responsible for the display of the results in a user-friendly way.

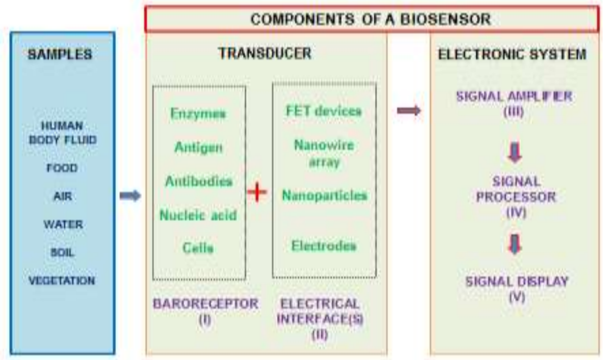

Figure 1: Elements of a typical biosensor.

Working of biosensor

The biological material is fixed by conventional methods, i.e. physical or membrane entrapment, non-covalent or covalent binding. A contact is made between the fixed biological material and the transducer. The analyte binds to the biological material to form a bound analyte which in turn generated the electronic response that can be measured. Sometimes the analyte is converted to a product which could be associated with the release of heat, gas (oxygen), electrons or hydrogen ions. The transducer then transforms the product associated changes into electrical signals which can be amplified, measured and displayed using the electronic system.

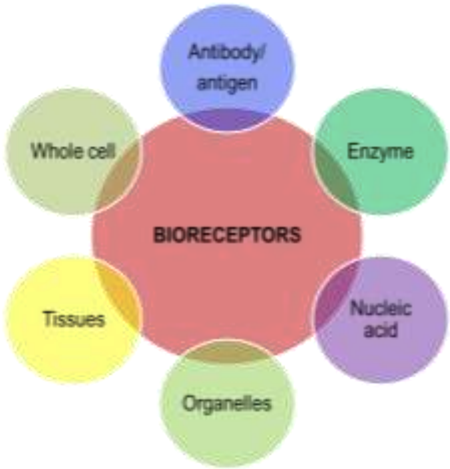

Figure 2: Potential biological recognition materials for development of a biosensor.

In the recent past, several biosensors have been designed using the various combinations of biorecptor and transducer. Biosensors can be classified according to the biorecptor (enzymes, immunoaffinity, DNA and whole microbial cells) or transducer (optical, mass-based, electrochemical, and thermal biosensors) used (Figure 3).

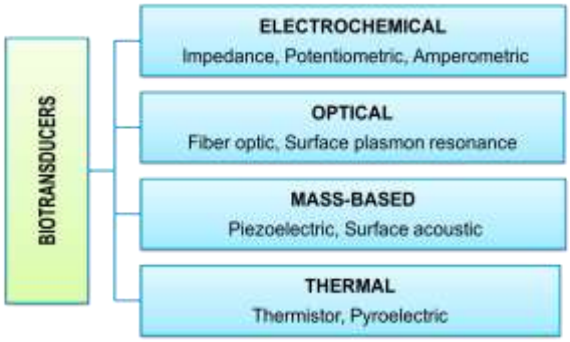

Figure 3: Types of biological detection elements for development of biosensor.

Most of the biosensors used for environmental application are made up of whole microbial cells, enzymes, antibodies and DNA as biorecptor and electrochemical transducer.

APPLICATION OF BIOSENSORS FOR ENVIRIONMENTAL MONITORING

Biosensor or biodevices can used as environmental quality monitoring tools in the assessment of physical, chemical and biological monitoring of pollutants. The major applications of biosensors are discussed below for detection, monitoring of various pollutants including heavy metals, organic and inorganic pollutants, toxins, antibiotics and contaminating microorganisms (Figure 4).

Heavy metals

Heavy metals, *i.e.* copper (Cu), cadmium (Cd), mercury (Hg), lead (Pb), zinc (Zn), *etc.,* are recently the major cause of serious environmental pollution problems. These are known for their high toxicity and bioaccumulation attribute in the food chain. A number of bacterial biosensors have been developed for the determination of heavy metals in different environmental samples using enzyme and DNA as biorecptor and optical and electrochemical as transduction system. An optical biosensor was by Durrieu and Tran-Minh (2002) to detect Pb and Cd by inhibition of alkaline phosphatase (AP) present on the outer membrane of microalgae (*Chlorella vulgaris*) used as a biorecptor.

Biochemical oxygen demand (BOD)

Biochemical oxygen demand is one of the significant parameters used in the estimation of the concentration of biodegradable organic pollutants present in a water sample. In routine practice, BOD determination of any sample is a time consuming process, *i.e.* 5 days, therefore it is not suitable for online process monitoring.

Its rapid determination of could be possible only by using BOD biosensors. Cells of recombinant *Escherichia coli* with *Vibrio fisheri* gene lux AE based biosensor was developed for measuring BOD by Nakamura and Karube (2003). Recently, a BOD biosensor has been developed using yeast with oxygen probe and able to detect organic pollutants within 15 minutes.

Nitrogen compounds

Nitrogen compounds, *i.e.* nitrates, are widely used by food manufacturing industries as preservatives (increase shelf-life) and chemical fertilizer industries as fertilizer (increase the fertility of the soil).

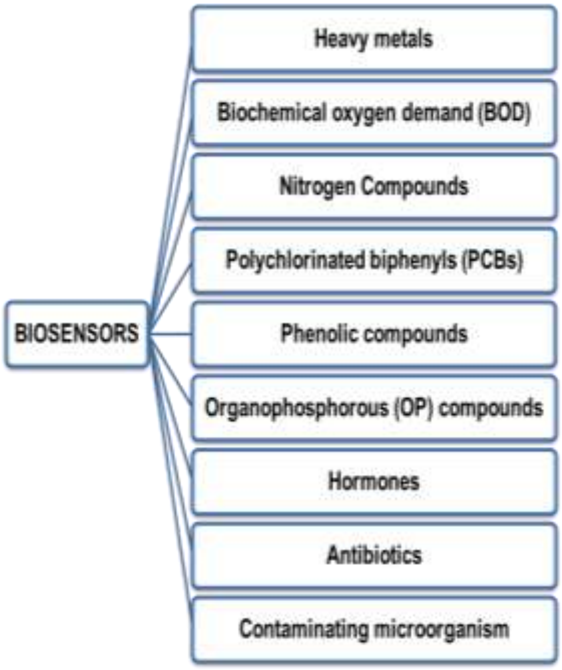

Figure 4: Different applications of biosensors for environmental monitoring.

The excess uses of these compounds can cause adverse effects on human health and also contaminating the surface and groundwater, later which can be toxic for aquatic environment. Nitrogen compounds can react irreversibly with hemoglobin hence, decreasing oxygen carrying capacity. To determine the concentration of nitrogen compounds in water samples, several biosensors have been developed by researchers. Some of them are discussed below:

i. Amperometric based biosensor has been developed by Chen *et al.* (2007), using an enzyme (cytochrome c nitrate reductase) obtained from *Desulfovibrio desulfuricans* for determination of nitrate.

ii. Another rapid, highly sensitive and stable conductimetric enzymatic based biosensor has been described by Wang *et al.* (2006) for the estimation of nitrate in water.

Polychlorinated biphenyls (PCBs)

PCBs are toxic organic compounds, universal environment pollutants; hence several countries banned their production long time ago. Such compounds are highly lypophilic nature, therefore abundant chances of accumulating in the food chain. Numerous biosensors have been developed to detect PCBs in the environment including:

i. Nucleic acid (DNA) based biosensor with chronopotentiometric detection (Marrazza *et al.*, 999).
ii. Immunosensors with fluorescence (Endo *et al.,* 2005).
iii. Electrochemical sensors (Del Carlo *et al.,* 1997).

Phenolic compounds

Phenols and their derivatives, *i.e.* chlorophenol are distributed commonly in the environment. Such compounds are mainly used in the production of dyes, drugs, plastics, pesticides, detergents, *etc.* Since, phenolics having high toxicity and possible accumulation in the environment and therefore, their detection and monitoring is essential to protect the environment. Most commonly used biosensors for detection and monitoring of phenolics are:

i. Amperometric biosensor with enzyme (tyrosinase) as bioreceptor for selective detection of phenol in effluent (Parellada *et al.*, 1998).
ii. A flow-injection chemiluninnescence fiber-optic biosensor detection of chlorophenols (Tschmelak *et al.,* 2006).

Organophosphrous (OP) compounds

Organophosphorous compounds are a group of organic chemicals that are commonly used as insecticides, herbicides and pesticides in modern agriculture for controlling pests, weeds and vectors. Pesticides are the substances meant for preventing, destroying or repelling of pest. Pesticides are most widely distributed in water, soil and food. Toxicity and persistence of pesticides in the environment are a matter of concern. Enzyme based sensors are most extensively used for detection of these compounds (Sara *et al.,* 2006). Immunological based amperometric biosensors have also been developed for detection pesticides in water (Wilmer *et al.,* 1997). For detection of herbicides like phenylureas and triazines (inhibit photosynthesis), amperometric and optical transducers have been developed and employed. Dioxins are polychlorinated compounds discharged as byproducts during several chemical processes involving chlorine. These are potentially toxic and carcinogenic substances for human beings due to a major impact on the environment due to an accidental entry in the food chain. The surface plasmon resonance (SPR) biosensor has been designed for detection of dioxins (Shimomura *et al.,* 2001).

Hormones

Increasing human population and intensive farming continuously adding natural and synthetic hormones residues *i.e.* estradiol, estrone and ethylnilestradiol, in the environment which are adversely affecting human health due to increased incidence of genital cancer and reduction in human sperm counts. Hormones along with other organic pollutants have been detected with advanced automated optical immunosensors in surface water samples.

Antibiotics

Extensive and non-medical uses of antibiotics in the environment are worrying since they promote antibiotic resistance. The occurrence of such antimicrobial compounds also raises food safety issues due their possible entry in the food chain. During past years, several types of biosensors have been developed to determine the quantity of antibiotics in food and different biological samples. For example, Akkoyun *et al.* (2000) developed an optical immunosensor for determine sulfamethazine in animal urine.

Contaminating microorganisms

Wastewater plays a significant role in the transmission of various diseases causing agents (pathogens) like bacteria, viruses, protozoa, fungi, *etc.,* which are usually found in polluted or untreated water. These organisms are imposing worldwide public health problems. Therefore, effective monitoring of water supply is required to reduce the impact of the waterborne pathogens on public health.

Introduction of new technologies such as biosensors has been employed for rapid detection and real-time monitoring of contaminating microorganisms at source of entry. Numerous types of biodevices including nucleic acid and immunoaffinity based sensors provided not only rapid detection of contaminating organisms but also their toxins (biotoxins).

A surface plasmon resonance based sensor was designed by Koubova *et al.* (2001) for real-time monitoring of *Salmonella enteriditis* and *Listeria monocytogenes* in liquid samples.

CHALLENGES IN BIOSENSOR DEVELOPMENTS

In spite of several prospective uses of biosensing technology in numerous sectors, *i.e.* health, medical, agriculture, space research, technology, *etc.,* there are certain safety issues concern with the use of such nanotechnology based technology on the environment and health are still need to be discussed.

Some key limitations and risk associated with environmental application of nanotechnology are as follows:

i. *Environmental safety:* the components of biosensors are made up of nanomaterials, hence the exposure of such materials to human beings and accumulation in agri-food chain is possible. The present knowledge on the nanotechnology is still in infancy stage; therefore, it is not possible to forecast the impact of the nanoparticles on the human health and the environment.
ii. *Alleviating poverty:* it has been concluded from various reports that the nanotechnology may significantly contribute to the alleviating poverty, etc. (Mukhopadhyay, 2014).
iii. *Non-target interaction:* the interaction of nanoparticles with the non-target sites, which lead to certain environmental and health issues (Claudia *et al.,* 2014).
iv. *Disposal issues:* these are due to use of nanomaterials, which may cause negative impacts on ecosystems.
v. *Cost*: At the initial stage, biosensor involves the high cost of production.
vi. *Sensitivity and specificity*: most of the sensors are tested in either distilled water or buffer. Real-time monitoring information was not sufficiently available (lacking of on-site data) and sometime we are not receiving accurate data due to the presences of certain interfering compounds.
vii. *Durability*: existing biosensors have limited life span and cannot withstand adverse environmental due to the sensitive nature of biological material used in construction of the sensor (Campas *et al.,* 2007).
viii. *Awareness*: there is a lack of public awareness in developing countries.

FUTURE PROSPECTIVES

Numerous laboratory prototype biosensors have been reported which capable in the measurement of a fairly broad spectrum of environmental contaminants. However, specific requirements of biosensor must be met for each field or farm level monitoring. Some of the key requirements are listed below.

i. *Operational cost:* low as possible for each analysis.
ii. *Assay time:* within one hour
iii. *Sensitivity:* parts per billion
iv. *Specificity:* specific to one biomolecule or closely associated group of compounds.
v. *Matrix:* biodegradable with minimal use of natural resources.
vi. *Working format:* in situ, reversible.
vii. *Portability:* minimal manpower without need of external power to operate.
viii. *Detection range:* minimum two orders of magnitude.
ix. *Personal training:* easy to operate, should not require extensive training prior to operation.
x. *Maintenance:* minimal cost.

Therefore, there is an urgent need to explore the applications of novel degradable biomaterial and broad spectrum environmental sensing can be used in the environmental surveillance program. We should focus on construction of novel eco-friendly biomaterial based biosensors, whole cell biosensor (Aisyah *et al.,* 2014), polymer based microsystems and hybrid biofilm based biosensors (Salgado *et al.,* 2011). Besides the several challenges in developing cost-effective and more reliable bosensor, *i.e.* optical biosensor has an immense potential to provide the early warning in addition to detection of pollutants in the environment (Long *et al.,* 2013). It is expected that recent advances in nanotechnology, miniaturization based progresses in biosensing technology for environmental surveillance will have a promising and bright future (Koedrith *et al.,* 2015).

CONCLUSION

Environment safety requires technologies that are sensitive, selective, cheaper, user and eco-friendly and portable with minimal power consumption. Biosensors are biophysical devices which can be capable in detection of diverse environmental pollutants, including phenolics, pesticides, toxins and hormones. While most of the biosensors meant for detection and monitoring of environment pollutants are practiced merely fulfilling these requirements and sometimes not giving the accurate information due to certain limitations, *i.e.* time consumption and interference by other compounds. Therefore, further research is required to improve the performance of the biosensor in real samples in the field or farm levels. Since, most of the biosensor system has been tested only with distilled water or buffered solutions. In the context, biosensors for potential environmental monitoring must continue to show progress in the areas including genetically modification of enzymes and whole microbial cells by applying genetic engineering technologies, use of novel and more selective and disposal bioreceptors to improve analytical performance, improvement of recognition element immobilization, transducers and sensor interfaces.

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Source of Financial Support: Nil
Conflict of interest: None. Declared.