

A Study on Recent Approaches in Biosensors for Detection of Water Defilement

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ABSTRACT

Water safety is a global health goal and the water borne diseases are a major crisis on health. Therefore, detection of microbial pathogens and different contaminants in water is the solution to the prevention and recognition of problems related to health and safety. Biosensors are being widely used for the detection of various contaminants in water. A biosensor is a self-contained integrated device capable of providing specific quantitative analytical information using a biological-recognition element, which is retained in direct contact with a transduction element. This paper summarizes recent advances made in detection and quantification of waterborne contaminants with different types of biosensors that offers capabilities for rapid, miniaturized on-line and in-situ analysis with minimal waste production.

Keywords: *Biosensors, Environmental Monitoring, Water Pollutant, Heavy Metal.*

I. INTRODUCTION

Pollution is one of the major issues of today's world. So there is an increasing need for effective tools to estimate the risks derived from the large number of pollutants released to the environment. Environmental toxicology is the qualitative and quantitative study of the adverse effects of anthropogenic and naturally occurring stressors. Initial aquatic ecotoxicology studies were based on acute toxicity measurements of vertebrates. However, these methods suffer some standardization problems, are expensive, time-consuming, and moreover, are associated with ethical problems. Hence, new technologies for aquatic ecotoxicological studies were launched. Biological tools like biosensors provide us with detection systems for signaling a potential damage in the environment. Early recognition will prevent eventual damage to environmental matrices. Ideally, early warning signals in ecosystems using sensing systems would not only tell us the initial levels of damage, but these signals will also provide us with answers for the development of control strategies and precautionary measures. Biosensors are mostly designed for routine analysis such as quality control. The development of these biosensors is a multidisciplinary effort. The impact of these biosensors is likely to be wide-ranging. Biosensors helps in detecting emerging contaminants like pharmaceuticals, personal care products (PPCPs), steroids, xenoestrogens and other endocrine disrupting compounds (EDCs), algal toxins, giardia (and other pathogens) and a variety of miscellaneous chemicals such as caffeine, cholesterol, etc (Rodriguez-Mozaz et al., 2007). The objectives of this paper are to discuss the recent advances made in detection and quantification of waterborne

contaminants with different types of biosensors.

1.1. Definition

A biosensor is an analytical device, which converts a biological response into an electrical signal. It consists of two main components: a bio-receptor or bio-recognition element, which recognizes the target analyte and a transducer, for converting the recognition event into a measurable electrical signal. The bio receptor recognizes the target analyte and the corresponding biological responses are then converted into equivalent electrical signals by the transducer. The amplifier in the biosensor responds to the small input signal from the transducer and delivers a large output signal that contains the essential waveform features of an input signal. The amplified signal is then processed by the signal processor where it can later be stored, displayed and analyzed.

1.2 Biosensors with biological effect-based analysis

Biosensors techniques utilizing enzymes, natural receptors, bacteria or cells can be used to rapidly identify toxicity and other biological effects in water containing different chemicals known as biosensing. The determination of toxicity provides an integrated picture of the overall impact on the environment. Research has been carried out where detection of arsenic is signaled as an easily detectable drop in pH and the chromogenic system. The endospores used can be stored and distributed in dried form without requiring freeze-drying or refrigeration (Joshia et al., 2008). Whole organisms can also be used to measure the potential biological impact of a water or soil sample. Sensors for other areas of ecotoxicology, such as genotoxicity and mutagenicity, have also been developed and have been described as “biosensors for environmental stresses”. Genotoxicity is associated with different compounds, such as phenols, chlorophenols, PCBs and PAHs, and can constitute an early warning screening parameter for possible cancer-inducing pollution activity. Mammalian cells, which are more complex than bacteria, can give a more sensitive response when compared to bacteria. In the particular case of pharmaceuticals, their environmental presence triggered a proposal to include an environmental risk assessment in the registration procedure for medical products. An ecotoxicological test battery has been designed for that.

1.3 Characteristics of biosensor

Biosensors offer sensitivity at small sample volumes and require minimal sample preparation. A large number of biosensors are available varying in biorecognition principle and/or transduction element. Direct sampling and analysis is possible, giving way to automation. By using specific biological recognition element, a compound can be selectively detected. Also, faster analysis and real-time detection can be done with minimal and non-contaminating waste. Biosensors help in determination of bio available pollutant content and toxicity testing. Availability of portable biosensor systems has enhanced applicability to early-warning and on-site monitoring. Biosensors are user friendly cost-effective equipment that can be used by non qualified personnel as well. Biosensors should be distinguished from bioassays or bio analytical systems, which require additional

processing steps, such as reagent addition and where the assay design is permanently fixed in the construction of the device. Biosensors are relatively cheap and fast, which make them ideally suited for routine testing and screening of samples. Biosensors have demonstrated a great potential in the past as analytical tools and avoids in many cases sample pre-treatment or with minimal sample preparation and even allowing on-site field monitoring.

II. CLASSIFICATION

On the basis of the bio-recognition principle, biosensors are classified into various categories. A bio receptor can be a tissue, micro organism, organelle, cell, enzyme, antibody, nucleic acid and bio mimic etc. and the transduction may be optical, electrochemical, thermometric, piezoelectric, magnetic and micromechanical or combinations of one or more of the above techniques. Some of them will be reviewed in this section.

2.1. Plant and animal tissue based biosensor

Biocatalysts, such as specialized tissues from higher animals and plants, have been incorporated into various electrochemical transducers to construct biosensors for the detection of important analytes including drugs, hormones, toxicants, neurotransmitters and amino acids. Nerve cells in animals and phloem cells in plants share one fundamental similarity that they possess excitable membranes through which electrical excitations can propagate in the form of action potentials. It is conceivable that action potentials are the mediators for intercellular and intracellular communication in response to environmental irritants. Plants quickly respond to changes. Once initiated, electrical impulses can propagate to adjacent excitable cells. The change in transmembrane potential creates a wave of depolarization or action potential, affecting the adjoining resting membrane. Most plant tissue-based biosensors are based on electrochemical detection, usually amperometric or potentiometric. However, optical techniques, such as chemiluminescence or fluorescence, have recently appeared providing higher sensitivities and faster response times.

Work has been done on primary-source freshwater drinking samples from the Clinch and Tennessee Rivers using tissue based detection system that uses naturally occurring aquatic photosynthetic tissue as the sensing material for detection of chemical antagonists in the water. Sensor readout is based on well-known principles of fluorescence induction by living photosynthetic tissue. They successfully detected algae in every sample and readily monitored changes in the characteristic fluorescence induction curves when the samples were exposed to various pollutants. The unique aspect of this approach to real-time water quality monitoring is that unlike conventional sensing devices, this sensor material is external to the detecting instrument and is continuously refreshed (Rodriguez Jr et al., 2002). Another invention done on water quality sensors for detecting the presence of at least one chemical or biological warfare agent includes: a cell; apparatus for introducing water into the cell and discharging water from the cell adapted for analyzing photosynthetic activity of naturally occurring, free-living, indigenous photosynthetic organisms in water (Greenbaum et al., 2003).

Another work has been done in which a kinetic model was developed to describe the processes of herbicide diffusion into plant tissues and binding to the active sites. DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], a commonly used herbicide, was used as a test chemical and its diffusion into plant leaves and binding to

plastoquinone B (Q(B)) sites were analyzed by using the model (Guo et al., 2010).

The main advantages of using plant tissues in biosensors are high stability, high level of activity, long lifetime, high reproducibility of the experimental results, availability, cheaper price, less time consumption and its diversity. However, they suffer from low specificity, due to the presence in the tissue of enzymes others than the one of interest, and long response times, due to the diffusion barrier (Campàs et al., 2008).

2.2. Microbial whole cell based biosensor

Whole cells can be used as biosensors if they have transducer property along with the bio receptor element. Generally, cells capable of sensing are modified to incorporate the transducer capacity. Certain parameter such as bioavailability, toxicity and genotoxicity can be assayed using whole cells only. They provide estimation for pollutant bioavailability. The use of whole cells as biocatalysts has several advantages as compared to isolated enzymes, the most important being increased stability and protection from interfering substances. Consequently, microbial biosensors are preferred for measurements in contaminated samples. Whole cell bioassays can be classified as turn off assay- degree of inhibition of a cellular activity that is continuous; or turn on assay – activation of a certain process by the target pollutant. Table 1 includes few studies regarding the use of microbes as biosensors.

Table 1: Microbial biosensors for water contaminant detection

S.No.	Application	Micro organism	Reference
1.	Nitrous oxide, nitrite, nitrate	Immobilized denitrification bacteria	Larsen et al., 1997
2.	Chlorophenols, pesticides	Spirulina subsalsa	Tonnina et al., 2002
3.	Atrazine, simazine, isoproturon and diuron(herbicides)	Chlorella vulgaris	Vedrine et al., 2003
4.	Organotin compounds	Recombinant luminescent bacteria	Durand et al., 2003
5.	Cadmium, zinc, mercury , chromium	Recombinant luminescent bacteria	Ivask et al., 2002
6.	Cadmium	Chlorella vulgaris	Chouteau et al., 2004
7.	Halogenated hydrocarbons	Rhodococcus sp. DSM 6344	Peter et al.,1996
8.	Cd(NO ₃) ₂ , HgCl ₂ , Benzalkonium chloride, Sodium Dodecylsulfate	Saccharomyces cerevisiae	Campanella et al.,1997
9.	Aqueous toxicity	Vibrio fischeri	Jennings et al.,2001
10.	Mitomycin C	E.coli tolC mutants	Davidov et al., 2000 ; Norman et al.,2005

11.	N-methyl-N 0-nitro-N-nitrosoguanidine (MNNG), naladixic acid, formaldehyde	E.coli tolC mutants	Norman et al.,2005
12.	Cerulinin	E.coli tolC mutants	Bechor et al.,2002
13.	Water toxicity	Photobacterium leiognathi	Ulitzer et al.,2002
14.	Toxicity testing	Janthinobacterium lividum	Cho et al.,2004
15.	Dioxins, endocrine-disrupting chemicals, and ionizing radiation	Luciferase and GFP in various whole cells	Ikebukuro et al.,1996
16.	p- Nitrophenol	Pseudomonas sp.	Prakash et al.,2008
17.	p-Nitrophenol	Moraxella sp	Mulchandani et al.,2002
18.	2,4-DNP	Rhodococcus erythropolis	Emelyanova and Reshetilov, 2001
19.	Radioactive waste	Deinococcus radiodurans	Brim et al.,2000
20.	Biocides	Bioluminiscent E. coli	Fabricant et al.,1995
21.	Naphthalene, salicylate	Pseudomonas fluorescens	Heitzer et al.,1994
		HK44	
22.	Groundwater toxicity	Salmonella	Baumstark-Khan et al., 2005
23.	Acrylamide, acrylic acid.	Brevibacterium sp	Ignatov et al.,1997
24.	Chlorinated and brominated hydrocarbons	Rhodococcus sp.	Hutter et al.,1997

2.3. Antibody and enzyme

In surface, ground, or drinking water other than regular pollutants, hormones, pesticides, endocrine disrupting compounds (EDCs) and antibiotics are also found to have an adverse and toxic effect on humans at low nanogram per litre levels. The first issue related to EDCs is removal of steroids from wastewater treatment process. In spite of several reported cases, EDCs did not draw much attention, because of the trace level concentration of detected EDCs and the lack of information on their significance in toxicity. EDCs are known as a class of chemicals which have xenobiotic and exogenous origins while mimicking or inhibiting the natural action of the endocrine system in animals and human, such as synthesis, secretion, transport, and binding.

One of the effective methods to determine EDCs is usage of biologically based assays. The biological methods are intended to measure the levels of individual EDCs, based on the assumption that the target compound has been identified as an EDC and much is known about its chemical properties. However, traditional toxicity tests may not always be suitable for certain water samples. Several mechanisms are involved in the biological assays

to determine EDCs, such as cell proliferation, ligand binding, luciferase induction, vitellogenin induction, or antigen–antibody interactions (Chang et al., 2009).

Cell proliferation utilizes the estimation for cell growth and reproduction in different samples. Ligand binding quantifies the number of specific estrogens binding sites. Luciferase induction measures the amount of luciferase induced from estrogens receptors and response elements with luminescence after cell lysing and the addition of luciferin. They maintain the homeostasis, reproduction, metabolism, development, and/or behaviour of living species. Vitellogenin induction quantifies the amount of vitellogenin in the plasma of female fish liver after extraction, which is secreted as a response to estrogens. In addition, the production of vitellogenin in male fish can be seen as an indication of endocrine disruption. Biologically based assays may be applied with whole organisms, cellular, or non-cellular materials, such as antibodies or estrogens receptors. Along with bioassay, immunoassay have become an important tool as automated immunosensor which is based on the principle of total internal reflection fluorescence (TIRFs) and antigen-antibody non covalent binding interaction, that can measure several organic compounds (antibiotics, hormones, pharmaceuticals, EDCs, pesticides) in parallel. Thereafter, the TIRF-based biosensor setup was used to determine the steroidal hormone testosterone (Tschmelak et al., 2005) and estrogens (Tschmelak et al., 2004) at real world samples without sample pre-treatment or sample pre-concentration.

2.4. Nucleic acid based biosensor

Nucleic acid-based biosensors are finding increasing use for the detection of environmental pollution and toxicity. A nucleic acid-based biosensor employs as the sensing element an oligonucleotide, with a known sequence of bases, or a complex structure of DNA or RNA. Nucleic acid biosensors can be used to detect DNA/RNA fragments or either biological or chemical species. In the first application, DNA/RNA is the analyte and it is detected through the hybridization reaction (this kind of biosensor is also called a genosensor). In the second application, DNA/RNA plays the role of the receptor of specific biological and/or chemical species, such as target proteins, pollutants or drugs (Palchetti et al., 2008). New trends in nucleic acid research include development of aptamers and aptazymes as affinity ligands and potential coupling to transduction technologies (Mascini et al., 2005). Deoxyribonucleic acid (DNA) biosensors (genosensors) have been exploited for their inherent physico-chemical stability and suitability to discriminate different organism strains. The main principle of detection among genosensors relies on specific DNA hybridization, directly on the surface of a physical transducer (Teles et al., 2008). Surface plasmon resonance and piezoelectric sensing are reported as transduction principles for DNA-based devices (Minunni, 2003). DNA also showed the possibility of detection of the *E. coli* O157:H7 EDL933 species by using 20-mers (5'- TAATATCGGTTGCGGAGGTG-3') sequence of Gene (Bahsi et al., 2009).

Genus *Mytilus* are intertidal filter-feeders commonly used as biosensors of coastal pollution. Mussels adjust their functions to ordinary environmental changes, e.g. temperature fluctuations and emersion-related hypoxia, and react to various contaminants, accumulated from the surrounding water and define a potential health risk for sea-food consumers. Despite the increasing use of mussels in environmental monitoring, their genome and gene

functions are largely unexplored. The transcriptional footprints and discriminating capacity of different mussel tissues have to be taken into account in the microarray analysis.

In the digestive gland, numerous gene probes (101) discriminated biologically relevant doses of two contaminant mixtures and about half of them appear potential markers of real exposure to heavy metals and persistent organic pollutants (Venier et al., 2006). Moreover, among nucleic acids, aptamers represent a new promising recognition element for biosensor development. Recent understanding of the structure–function of nucleic acids, specifically RNA, has opened new perspective in the development of new analytical and diagnostic methods. The coliform *Escherichia coli* were used as a model fecal indicator. DNA probe-coated magnetic beads in combination with the electrochemical monitoring of the oxidation state of guanine nucleotides should allow for direct detection of bacterial RNA (LaGier et al., 2005). In vitro evolution from random sequence libraries makes it possible to build nucleic acids that specifically recognise and bind to virtually any kind of target, such as ions, metabolites, drugs, toxins, peptides and proteins.

The quickly growing area of genomics, ribonomics, proteomics and metabolomics requires the development of high-throughput and massive-parallel analysis of biological samples. In this regard, biosensor technology coupled to nucleic acids could represent a successful approach to the functional genomic area. DNA sensors are being used to detect salmonella enteric using keypad user interface to operate a nucleic acid sensor with fluid handling and real-time polymerase chain reaction (PCR) capabilities. The progress of the Human Genome Project has generated substantial interest in the use of nucleic acid hybridisation technologies to detect and identify organisms and mutations. Biosensors and micro-array chips that are based on detection of hybridization/interaction of short strands of nucleic acids offer platforms for applications such as screening of genomes, detection of pathogenic organisms, and efficient searching of compound libraries for detection of potential therapeutic agents.

III. CONCLUSION

Biosensors for potential environmental applications continue to show advances in areas such as detection of heavy metals, biocides, pollutants, microorganisms and various polyaromatic compounds. Also, water toxicity testing, mutagen analysis and BOD estimation is facilitated by use of biosensors. The use of genetically modified AChE in biosensors has significantly increased their sensitivity to inhibition by OP pesticides. Furthermore, genetic modification shows the potential for selection of enzyme variants that are specific for a range of individual compounds. Recently, genetically engineered microorganisms based on fusing of the lux, gfp or lacZ gene reporters to an inducible gene promoter have been widely applied to assay toxicity and bioavailability. Novel gene fusions have been constructed that maybe used to detect response against a wide range of physical and chemical stressors.

One of the major challenges for this area will be the development of environmental applications related to ecosystem and human exposure to genotoxins. Biosensor techniques for potential environmental applications have continued to show sustained advances in a wide range of areas. It is also likely that these advances will play an important role in the development of biosensor systems for the environmental market. Nevertheless,

until biosensors achieve operational characteristics similar to the simple pH electrode in terms of durability, sensitivity, selectivity, extended concentration range, achievable response time and resistance to befouling, they will continue to experience significant obstacles to widespread acceptance and use for environmental monitoring. We believe, with current advances in biosensor and progress in modern biotechnology, biosensors will have a promising and bright future.

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