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Biosensors for monitoring environmental pollutants: A review

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ABSTRACT

Biosensors are used to identify and monitor different types of pollutants and its effect on the ecosystem. The nature of pollutants, their source and bioavailability can be identified with a high level of specificity and sensitivity with the help of the biosensors. Various biosensors used in pollutant studies for monitoring eutrophication, food safety, detection of pathogenic microorganisms, trace metals, pesticides, herbicides and organic wastes are analysed.

KEYWORDS

Bacteria;
Biosensors;
Pollutants;
Trace metals;
Whole cell biosensor.

INTRODUCTION

A biosensor is an analytical device, which converts a biological response into an electrical signal. It involves the interaction of a biological material / bioreceptor (such as proteins, enzymes, antibodies, lectins, nucleic acid, organelles, intact microorganisms, tissues, cell receptors) with an analyte to produce an electrical, optical or thermal signal. This signal is then converted by suitable transducer such as electrochemical sensor to current, voltage, conductance, capacitance or impedance or by an optical sensor to changes in wavelength, wave propagation, time, intensity, distribution of the spectrum, or polarity of the light. On the other hand, a piezoelectric sensor will convert the signal to mechanical vibration that is directly proportional to the amount of analyte present[14].

Biosensors are used in different areas like environmental pollution detection, clinical analysis, contaminants detection in food, health monitoring, medical health related targets, detection of pathogens and various industrial and agricultural applications[14].

Prior to biosensors, surface analysis techniques such as ion scattering spectrometry (ISS), secondary ion mass spectrometry (SIMS), Auger electron spectroscopy (AES), bulk analysis techniques such as atomic absorption and emission spectroscopy and neutron activation analysis, were used for monitoring pollution[84]. The disadvantages of these methods include primarily high costs and low detection limits. Biosensors being cheap, portable, user-friendly, low power consuming, highly sensitive and robust have gained wide acceptability. They also offer high degree of specificity that they can even distinguish between stereoisomers of the same compound[37]. One of the great advantage of using biosensors is the sample to be detected often requires little pretreatment and the bioavailable concentration of the toxic contaminant is measured rather than the total concentration.

In order to learn about the functions of the ecosys-

tem, interaction of its various components, extraction of raw materials from sea, biodiversity issues, habitat protection, ecotoxicology and pollution, studies have promoted the study of biosensors for marine environmental pollution[35]. Pollutants reach the marine environment through various ways like agricultural run offs, industrial discharges, domestic wastewater etc. Hence the range of contaminants is very diverse. They include pesticides, herbicides, surfactants, steroid hormones, anti-biofouling agents and plasticizers. Depending on the kind of contaminant various biosensors are developed some of which are discussed below[6].

1. Eutrophication

When fertilizers rich in nitrates and phosphates when applied to the fields, not all the added nitrogen and phosphorous is taken up by the plants. When it rains these minerals are leached from the soil and are thus transported into water bodies thereby increasing their salt and mineral contents leading to eutrophication. Plants and algal bloom associated with eutrophication consume a lot of oxygen for respiration, which in turn leads to the oxygen depletion in these water bodies and causes a serious threat to fish. For the detection of nitrate and nitrite in seawater as well as in fresh water which leads to eutrophication, microbial sensors are used. *Thiospaera pantotropha*, a denitrifying bacteria that reduces nitrate to nitrous oxide is immobilized in an optically transparent sol-gel matrix for the construction of amperometric sensor. Nitrate reductase isolated from *Alcaligenes faecalis* is immobilized in an electrogenerated polymer and is connected to the electrode acts as a nitrate sensor. This sensor is used for both nitrite and nitrate and has a detection limit of approximately 2.9 micro gram N-NO_3-/l[3]. The denitrifying bacteria *Agrobacterium radiobacter* is directly immobilized and made use in one of the nitrate sensors[40]. A sensitive conductometric biosensor for nitrate detection is configured by immobilizing the nitrate reductase from *Aspergillus niger* on a thin-film electrode. The enzyme is immobilized on the electrode by cross linking with bovine serum albumin in the presence of glutaraldehyde, Nafion cation-exchange polymer and methyl viologen[1,88,90]. Optical biosensor that contains controlled pore, glass beads with cytochrome *cd1* nitrite reductase immobilized on it. When nitrite binds, there is oxidation of enzyme and hence change in optical reflection[65].

Due to the advantages like easy cultivation, easy manipulation for sensor configurations and broad substrate range, yeast and filamentous fungal cells are also used as biosensors. *Trichosporon cutaneum* is used as sensor element in biochemical oxygen demand sensor. Apart from this two other *Candida* sp. isolated from wood pulp mill effluent is used to detect the biochemical oxygen demand of the waste stream. For the determination of middle chain alkane the flow injection analysis based sensor uses *Yarrowia lipolytica*, a psychotrophic yeast isolated from diesel oil-contaminated alpine soil[5].

2. Sea food safety

Monitoring of algal toxins in seafood becomes easy with the use of various biosensors. Shellfish are filter feeders that accumulate and metabolize toxins produced by microscopic algae in the form of dinoflagellates and diatoms. Those toxins responsible for Paralytic Shellfish Poisoning (PSP) are derivatives of saxitoxin. A tissue biosensor based on frog-bladder membrane and a sodium electrode- electrochemical immunosensor is used for saxitoxin a shellfish poison that blocks sodium channel. Screen- printed electrode (SPE) and electrochemical immunosensor using glucose oxidase are used for the detection of brevetoxin[12]. Alkaline phosphatase enzyme linked immunosorbent assays (ELISA's) and SPE are used for the detection of shellfish poisons like okadaic acid, brevetoxin, domoic acid, tetrodotoxin and gonyautoxin.

SPE has detection limits in the order of ng /ml and is also simple and cost effective due to its disposable nature. It also has an analyzing time of only 30 minutes.

Chemiluminescent immunosensor in mussel homogenate is widely used for Okadaic acid, a diarrheic shellfish poison[48]. A simple flow enzyme system that relies upon the thin-layer flow through cell placed into the measuring compartment of the luminometer is used here. The inner surface of the flow cuvette is immobilized with antibody and the second ligand (antigen) labeled with a peroxidase molecule moves through the flow cell. The intensity of chemiluminescence after the reaction of peroxidase with the substrates (p-iodophenol, luminol and hydrogen peroxide) gives the measure of the quantity of complex on the surface of the cell. Anti-OA monoclonal antibodies were labelled with horseradish per-

oxidase for their use in a competitive assay, in which the free antigen of the sample competes with immobilized OA. Low non-specific binding of antibodies is exhibited in this system in the presence of mussel homogenate depending on the polyethersulfone membrane availability[53].

Surface plasmon resonance biosensor is used for the detection of domoic acid[71]. On a gold chip suitable for a surface plasmon resonance, a homogeneous molecular imprinted polymer (MIP) film of domoic acid (DA) of thickness 40 nm is photografted. Optimization and proper grafting was achieved by using contact angle measurements and atomic force microscope imaging. There is a competition for binding between free DA and its conjugate labeled with horseradish peroxidase[45].

A whole cell biosensor was developed for finding the concentration of only middle-chain-length alkanes and some related compounds in water. The biosensor is induced with octane, a typical inducer of the alkane system. It was observed that the light emitted is directly proportional to the octane concentrations between 24 and 100 nM[72].

3. Detection of pathogenic organisms

Sensors based on nucleic acid hybridization detection have been developed[4]. For the detection of toxic algae using rRNA probes an instrument called environmental sample processor which is an electromechanical / fluidic system that collects discrete water samples, concentrates the microorganisms (particulates) and automates the application of molecular probes to detect various microorganisms and their gene products. For the detection of harmful algae, phytoplankton and their toxin, a rapid and reliable method called as PCR ELISA Dig Detection Kit is developed. Species-specific probes were designed for *Alexandrium minutum* and used in this biosensor[19].

Escherichia coli O157 in food and water samples is detected with a waveguide biosensor based on a fluorescent sandwich immunoassay[91].

Environmental sample processor is also used for the detection of *Escherichia coli* as a faecal contaminant in bathing water and also for detecting virus that causes disease in humans and fish[37,38]. An acoustic wave immunosensor integrated with 87.7 MHz oscillator is used for fast detection of *Escherichia coli* in water[18].

Various biosensors have been developed for the detection of *Salmonella typhimurium*. Magnetoelastic resonance sensors that contain polyclonal antibodies immobilized on magnetostrictive platform or onto piezoelectric transducers can detect *Salmonella typhimurium* by a shift in the resonant frequency of sensor[26,39,54].

4. Pesticides and herbicides

There are number of biosensors for the detection of pesticides. Acetylcholinesterase (AChE) is an enzyme involved in signal transduction in the central and peripheral nervous system. Organophosphorus and carbamate pesticides inhibit cholinesterase and are therefore powerful insecticides. For the detection of pesticides various electrochemical biosensors are used[79]. For herbicides, photosynthetic bacteria coupled to electrochemical transducers are used. Immunosensors with optical detection are used for the detection of isoproturon in natural water[46] and also for the detection of paraquat[47] and irgarol[58] have been developed.

A whole cell biosensor using marine algae *Spirulina subsala* coupled to a Clark-type oxygen electrode is used for chlorophenols, pesticides and surfactants. The advantage of wholecell is that it gives information concerning bioavailability and potentially measures physiological responses, which are relevant to marine processes[79].

Pesticides like Dichlorvos, Parathion and Azinphos can be determined down to concentrations of 1×10^{-17}M, 1×10^{-16} M and 1×10^{-16} M, respectively with the help of novel sonochemically fabricated, bioengineered acetylcholinesterase and polyaniline carbon/cobalt phthalocyanine biosensors[42]. For the detection of Dichlorvos, a mediator-free amperometric biosensor for screening organophosphorus pesticides (OPs) in flow-injection analysis (FIA) system has been developed. Here the acetylcholinesterase enzyme (AChE) immobilized is prepared by in Al_2O_3 sol-gel matrix screen-printed on an integrated 3-electrode plastic chip[67].

For detecting low concentration (like 50 μg/L) of herbicides like linuron and atrazine that interact with photosynthetic electron transfer chain, bacterial biosensors incorporating the *Cyanobacterium synechoccus* as biocatalyst is developed[84].

The biosensor for the detection of metals, solvents,

crop protection chemicals, and other organic molecules consists of two *Escherichia coli* heat shock promoters dnaK and grpE fused to the *lux* genes of *Vibrio fischeri*. This biosensor when encountered by those pollutants produce light. For the detection of pentachlorophenol, the outer membrane mutation tolC is introduced[83].

For the detection of atrazine, 3-(3,4-dichlorophenyl) -1,1-diethylurea (DCMU), toluene and benzene, algal biosensor is prepared by entrapping unicellular microalga *Chlorella vulgaris* in an alginate gel or a polyion complex membrane. This gel or membrane is then immobilized on the surface of a transparent indium tin oxide electrode[69].

Immobilized *Chlorella vulgaris* microalgae entrapped on a quartz filter is used for the continuous assessment of herbicide like atrazine, simazine, isoproturon and diuron detection is done with algal biosensor. The fibre optic biosensor is constructed with five removable algal membranes. The membrane's position is maintained in front of the tip of optical fibre bundle by an electronically controlled motor. When the incident light hits the upper part of the algal membrane fluorescence is produced and is sent to fluorimeter for measurement. It was observed that it could detect photosystem II herbicides at sub-ppb concentration level[86]. Various biosensors configured for the detection of different types organophosphorous pesticides/ insecticides are described in TABLE 1. All of these biosensors are based on immobilized acetylcholinesterase enzyme.

5. Anti-biofouling agents

Marine structures like leisure craft and hulls, that are prone to biofouling use Organotin compounds such as tributyltin (TBT) as anti-fouling paints. The quantification TBT from sediments is a big problem[13] and this TBT causes direct toxicity, shell thickening in oysters, a decline in recruitment of their juvenile stages, and endocrine disruption[37]. A flow- through sensor based on bacterial bioluminescence based bioassay, by immobilizing the bacteria on a disposable chip and detecting the luminescence with the help of an optical biosensor is recently developed[77]. Recently a biosensor using two different wild type *Halomonas* sp and *Bacillus pumilis* were used in the analysis of TBT. A plot of respiration of protoplast of *Halomonas sp* as a function of TBT concentration shows positive sigmoid response and the plot of respiration of protoplast of *Bacillus pumilis* as a function of TBT concentration shows a shift from negative to positive[52].

6. Organic wastes

A fully automated immunoassay using monoclonal antibodies chemically conjugated with bacterial magnetic particles forms the basis of one of the sensors of

TABLE 1: Sensors for organophosphorous pesticides/insecticides

Compound	Sensor type	Description
Dichlorvos and paraoxon	Liposome-based nano-biosensors.	Increase in the pesticide concentration leads to decrease in enzyme activity and hence decrease in the fluorescence[82]
Aldicarb and carbaryl	Acetylcholinesterase biosensor with Prussian blue-modified screen-printed electrodes.	Acetylcholinesterase biosensors show high sensitivity towards aldicarb and carbaryl[2].
Chlorphyrifos	Fiber-optic sensor	Competitive binding of pesticide(inhibitor) to the enzyme acetylcholinesterase against the substrate (acetlythiocholine iodide)[59].
Paraoxon	Localized surface plasmon resonance sensor	The sensor is immersed in acetylcholine chloride. Presence of paraoxon prevents acetylcholine chloride reacting with acetylcholinesterase[44].
	Butyrylcholinesterase biosensor[2].	This sensor along with prussian blue-modified screen-printed electrodes shows high sensitivity for paraoxon[2]
Chlorpyriphos-oxon and chlorfenvinphos.	Acetylcholinesterase - based inhibition sensor.	This biosensor immobilizes genetically engineered acetylcholinesterase (B394) to the iminodiacetic acid preactivated magnetic microbeads based on Ni-His affinity[28]
Monocrotophos, malathion, metasystox and Lannate.	Screen-printed electrodes with immobilized acetylcholine esterase	In the presence of acetylcholine substrate, the pesticide deactivates acetylcholine esterase[21].

bisphenolA[49]. On a suitable surface plasmon resonance sensor, a thin film of gold is laid and bisphenolA-ovalbumin (BPA-OVA) conjugate is immobilized on it by amine coupling method. The BPA sample containing anti-BPA antibody when introduced into the sensor system, both the BPA and anti-BPA competitively binds to BPA-OVA on the chip[50].

A whole-cell biosensor is developed by using the phenol-inducible Po promoter from *Pseudomonas sp*. CF600 as a contaminant-sensing gene and *luxCDABE* operon of the *Pseudomonas fluorescens* OS8 as a reporter gene. This biosensor can be used in ground waters and semi-coke leachates[52,24].

A bacterial biosensor for benzene, toluene and xylene is constructed by plasmid incorporating the transcriptional activator *xylR* from the TOL plasmid of *Pseudomonas putida mt-2*. The XylR protein binds a subset of toluene-like compounds and activates transcription at its promoter, Pu. Under the control of XylR and Pu, the *luc* gene for firefly luciferase is placed and the reporter plasmid was constructed. On transformation of the *Escherichia coli* cells with this plasmid vector, luminescence is induced from the cells in the presence of benzene, toluene, xylene, and similar molecules[89].

The redox dye, resazurin a good indicator of bacterial respiration gets reduced to a fluorescent product resorufin. The presence of contaminants like toluene increases the resorufin concentration and in turn the fluorescence is enhanced. This principle is used in Resazurin respiration biosensor[78].

Sodium dodecyl sulfate (SDS) can be detected within range of 0.25-0.75 mg 1^{-1}.by using a *Pseudomonas rathonis* T-based amperometric biosensor[61]. Certain strains belonging to *Pseudomonas* and *Achromobacter sp* due to their inherent property to degrade anionic surfactants and also substrate specificity, are used in the construction of biosensors[75].

Biosensor constructed for the detection of aromatic hydrocarbon consists of *Escherichia coli* K-12 cells that carry xylS gene from *Pseudomonas putida* fused with luciferase operon of *Vibrio harveyi*[70,72]. Bioavailable benzene, toluene, ethylbenzene, and related compounds in aqueous solutions were detected using a green fluorescent protein-based biosensor was constructed using *Pseudomonas fluorescens*[55,73]. Biosensors containing cells of *Pseudomonas cepacia* immobilized on the calcium alginate gel and integrated to a simple flow calorimeter was found to respond to aromatic compounds[76].

A rapid, easy, specific and highly sensitive technique called as DRESSA, is available for detection of dioxin and dioxin-like chemicals. This contains dioxin-responsive element fused to a minimal viral promoter. This is then subcloned into an expression plasmid upstream of a secreted alkaline phosphatase (SEAP) gene[33]. Dioxins, polychlorinated biphenyls (PCB) and pesticides are examples of very harmful endocrine disrupting chemicals. A technique based on competitive immunoassay using surface plasmon resonance (SPR) is used for the detection of PCB, 2,3,7,8-Tetrachloro dibenzo ml^{-1} respectively[68]. Biosensors based on recombinant mouse hepatoma cell line has been developed for compounds like furans, biphenyls and polyhalogenated dioxins[56]. Immunosensor chip fabricated by MEMS technology is available for the determination of coplanar polychlorinated biphenyls down to the range of 0.1 ppt in 30 seconds. Co-PCB antibody immobilized polysterene beads were used in the microflow immunosensor chip[23].

Sol-gel-derived array DNA biosensors with ethidium bromide as sensing probe is used for -p-dioxin and atrazine with in detection limits of 2.5, 0.1 and 5 ng the detection of polycyclic aromatic hydrocarbons (PAH) in water and can detect naphthalene and phenanthrene at a concentration of 0 - 10 mg/L[20,60,34].

7. Heavy metal waste

Contamination of soils by heavy metals is a worldwide problem and various techniques for monitoring heavy metal contamination and remediation are explored [9,64].

There are different ways by which the mercury concentration in the contaminated soil can be detected. Two of the sensors for mercury detection are protein based biosensor and bacterial biosensor. The whole-cell biosensor makes use of *lux* genes from *Vibrio fischeri*. This *lux* gene is fused with a mercury-inducible *mer* gene and is introduced in *Escherichia coli* (CM2624). The resulting strain emits light in the presence of mercury ions. The result obtained is directly proportional to the concentration of mercury ions in the soil[9]. Vibrio fischeri is also used as teat organism for the detection of various bioavailable toxicants. For the detection of

bioavailable concentration of lead and cadmium, recombinant luminescent bacterial sensors that uses *Vibrio fischeri* as a test organism is also used. The sensitivity limit is 0.3 ppb Cd[31,32,87,27,66]. The *isi*AB promoter was fused with *luxAB* genes taken from *Vibrio harveyi* and was introduced into the *Synechococcus* sp. strain PCC 7002. Luminescence confirms the free ferric ion availability[10].

In chromate whole-cell biosensor and mercury whole-cell biosensors a number of promoter regions have been identified as sensing elements, sequenced and cloned as promoter cassettes. *lux*CDABE reporter system determines the specificity of the promoter cassettes. Two independent clones containing the *copSRA* promoter region of the *Alcaligenes eutrophus* CH34 *cop* operon and the tetracycline marker were constructed in plasmid pUC18/*Sfi*I. An *Sfi*I cassette containing the mercury responsive elements from Tn501 was also constructed and was used for mercury detection.

In the construction of copper whole-cell biosensor the pMOL30 copper resistance operon from *Alcaligenes eutrophus* was located, sequenced and analysed [17]. Catalytic DNA sensor with fluorescent detection is used for the detection of lead[62].

The metallothionein from the cyanobacterium *Synechococcus* PCC 7942 and the MerR regulatory protein from transposon Tn*501* allow the detection of non-specific metal binding down to 10M concentrations of Hg (II) and Cu (II) in pure solution. The fusion proteins GST-SmtA and MerR dissolved in phosphate buffered saline were immobilized on gold rods pretreated with thioctic acid and coupled with 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC). When exposed to Hg (II), Cu (II), these ions go and bind to these proteins and a large conformational change takes place. (Figure 1). This change can be directly measured with the help of a suitable transducer[4]. GST-SmtA biosensor that shows change in capacitance is used here. This biosensor is most sensitive to Cu(II) at concentration less than 10^{-11} M and most sensitive to Hg(II) concentration greater than 10^{-7} M[8,17].

A whole-cell sensor using the alkaline phosphatase activity of the algae *Chlorella vulgaris* and interdigitated conductometric electrodes are used in detecting of cadmium ions[5]. The bioavailability of cadmium and lead can be analyzed by a biosensor using two kinds of bacterium- one is sensor bacteria that reports the presence of the toxicants by increasing the luminescence which is being controlled by protein, specifically recognizing this toxicant and the second is luminescent control bacteria that belongs to the same type and same host strain of the sensor bacteria. This luminescent control bacteria lacks the metal recognizing protein and the corresponding promoter. Both these bacteria are fused together and introduced into a host strain. *Bacillus subtilis* BR151 (pTOO24) is used as sensor bacteria and *Staphylococcus aureus* RN4220 (pTOO24) is used as luminescent control bacteria. The luminescence obtained is directly proportional to the concentration of the ions[29,57].

The two wild strains of *Halomonas* sp and *Bacillus pumilis* are used in biosensor is used in the analysis of cadmium (similar to TBT analysis mentioned in section 6 of this paper)[52].

Aminolevulinic acid dehydratase (ALAD) a metalloprotein along with metallic co-factors shows sensitivity towards toxic metals. *Pseudomonas* ALADs can be used as a biosensor for some heavy metal exposed in aquatic environments. Nickel increased the ALAD activity of *Pseudomonas putida, Pseudomonas pseudoalcaligenes* and *Pseudomonas aureginosa* ATCC 27853. Manganese also increased the enzymic activity in *Pseudomonas putida* and *Pseudomonas aureginosa*. There is an increase in the enzymic activity of both *Pseudomonas pseudoalcaligenes* and *Pseudomonas putida* with increasing lead concentration[22].

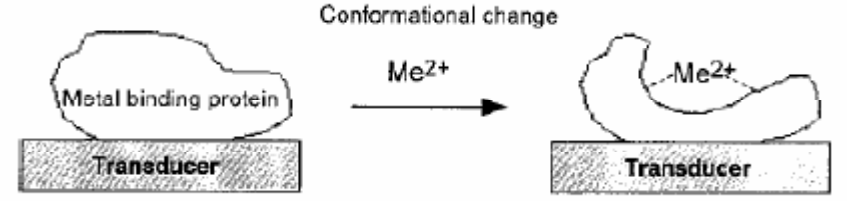

Figure 1: Protein-based biosensor concept for measuring conformational change upon binding of heavy metal ions[7]

Fluorescence quenching-based siderophore biosensor that makes use of Parabactin extracted from cultures of *Paracoccus denitrificans* is used for the detection of ferric ions. This biosensor in integrated with a flow cell system[16].

Mercury and Arsenic are detected using recombinant sensors that consist of the host *Escherichia coli* MC1061 carrying the pmerRluxCDABE plasmid in the case of mercury sensor and parsluxCDABE plasmid in the case of Arsenic sensor[30]. A Whole-cell bacterial biosensor is used for the detection of Arsenic. The bacterial biosensors for this purpose are engineered by pairing a *luc* operon reporter gene from firefly *Photinus pyralis* encoding the enzyme luciferase, that produces a detectable cellular response and a contaminant-sensing gene that detects the contaminant and in turn triggers the reporter gene. The contaminant-sensing gene is the arsenic resistance gene in gram-negative bacteria that usually remains inactive in the absence of As(III). In the presence of As(III) in the environment a series of changes occur and various proteins like ArsB, ArsC, ArsD, ArsR are produced[74].

Haloarcheal whole-cell biosensors for the detection of cadmium and arsenic have also been recently developed[11].

CONCLUSION

From the above examples it is clear that biosensors have a wide range of application. The real use of these biosensors is when it is applied on field. The main problem in biosensor technology includes complexity of environmental samples and diversity of compounds. Advancements in material fabrication technique, signal transduction method, powerful computer softwares[63] and use of Micro-Electro Mechanical System (MEMS) where there is integration of micron sized sensors, actuators and associated electronics on a single silicon substrate have attempted to solve these problems. Research is underway in constructing biosensors by using nanomaterials to improve its performance and sensitivity, use of bio-mimetic sensors that is based on the techniques such as molecular imprinting and combinatorial chemistry for environmental monitoring, multi-analyte determination sensor[36,63]. Multidisciplinary approach in Biosensor research will equip us with highly sensitive and specific biosensors.

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