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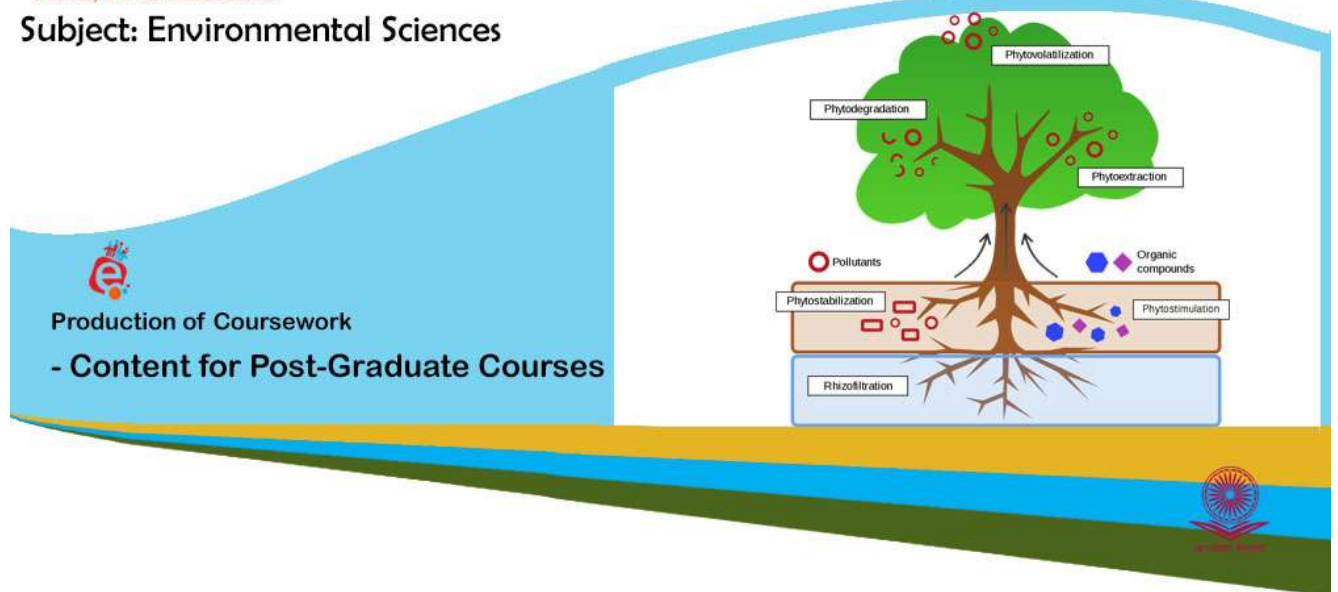
Paper No: 15 Environmental Microbiology & Biotechnology

Module: 26 Application of Biosensors in Environmental Monitoring and Recent Advances

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Description of Module	
Subject Name	**Environmental Sciences**
Paper Name	Environmental Microbiology & Biotechnology
Module Name/Title	**Application of Biosensors in Environmental Monitoring and Recent Advances**
Module Id	EVS/EP-XV/26
Pre-requisites	
Objectives	To learn the role of biosensors in environmental monitoring and new technologies in biosensor development
Keywords	Heavy Metals, Pesticides, Nanomaterials, Quantum Dots, Paper fluidics

Module 26: Application of Biosensors in Environmental monitoring and Recent Advances
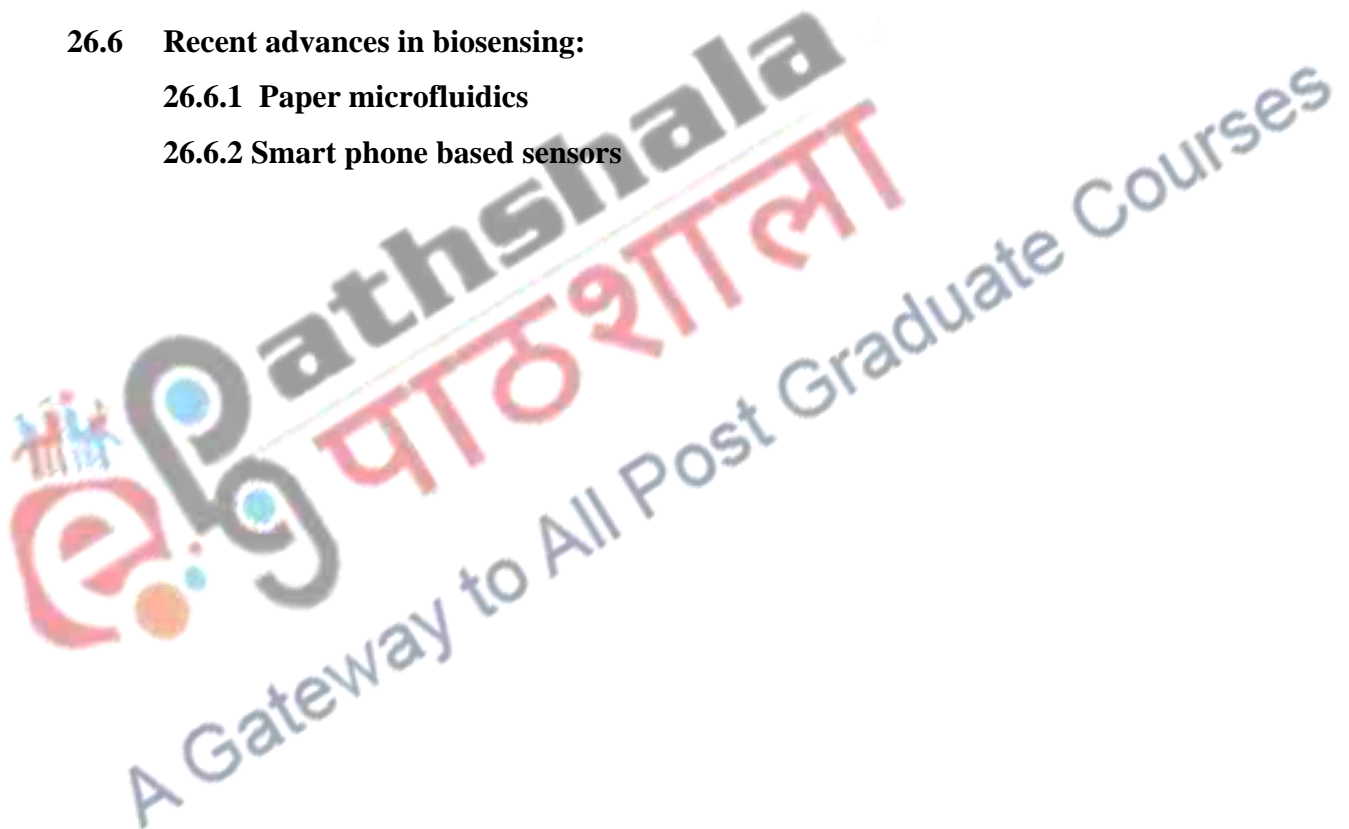
INTRODUCTION

The aim of biosensors is to provide accurate and reliable detection of analytes in complex matrices such as air, water, serum, industrial effluents etc. As a consequence it finds diverse applications in the field of drug delivery, disease detection, environmental monitoring, soil quality monitoring, Food quality monitoring, detection of toxins of defence interest, water quality management, prosthetic devices etc. Among all applications of biosensor, environmental monitoring includes maximum studies and out of them 71% are focused on detection of pesticide residues and 21% for heavy metals. It is the utmost concern of regulatory authorities of several countries that has given importance to biosensors for environmental pollutants. Apart from these water quality monitoring is the critical parameter that needs major concern and encompasses a lot number of biological oxygen demand (BOD) biosensors. The present chapter is dedicated to discuss all these environmental applications.
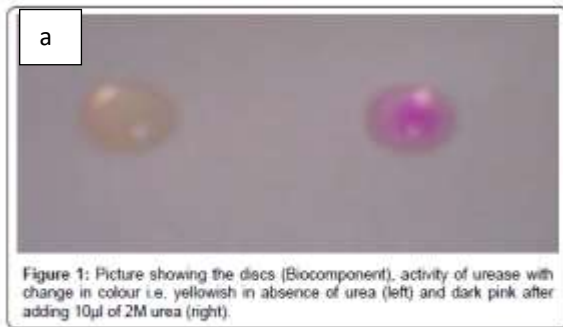
26.1 Heavy metal biosensors

Heavy metals are among the serious pollutants of our environment. They have been found to be toxic to living forms at very minute concentrations. As they are non-biodegradable, they pose serious health hazards to humans and deleterious to our environment also. The conventional techniques to analyse heavy metals are too expensive and laboratory bound. As a remedy biosensors have come up with advantages of real time and economical monitoring in addition to better sensitivity and specificity. Based upon the inhibitory or activating action of heavy metals on enzymes and proteins, a number of biosensors have been developed. Apart from enzymes, antibodies, recombinant whole cells, DNA, DNAzyme etc. have been employed as bioreceptors. Depending upon the type of biocomponent employed heavy metal biosensors can be categorized as per scheme 1.
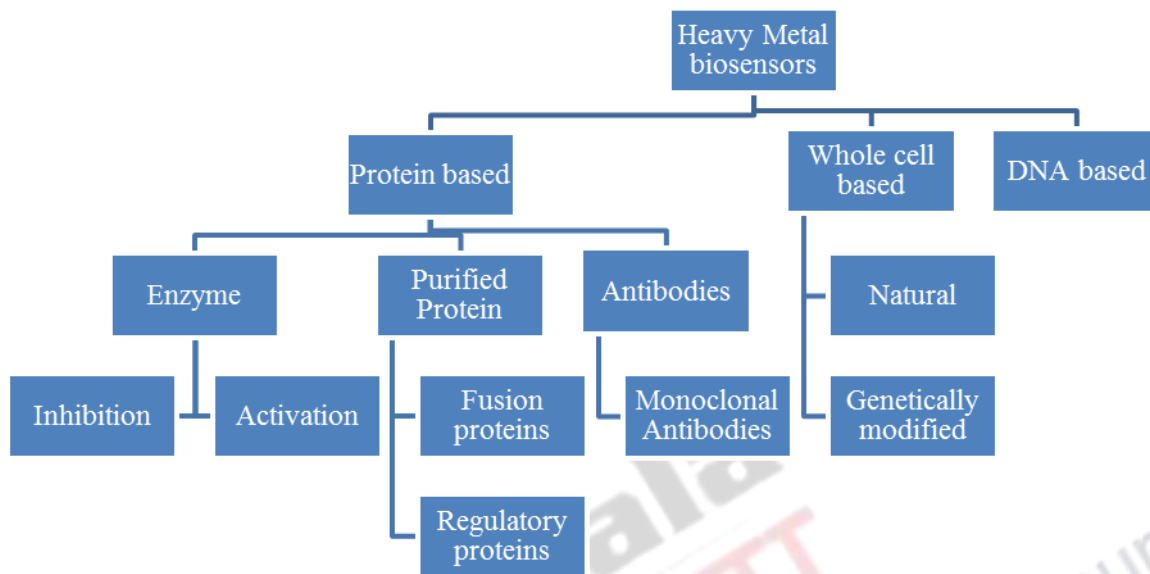
Scheme 1: Categorization of Heavy Metal Biosensors

Among all bioreceptors, enzymes have been widely used for heavy metal sensing. This is because the inhibiting or activation effects of heavy metals on enzymes catalytic efficiency or interaction with formed product. Heavy metals could also interact with the enzyme – substrate complex and thereby change the final outcome of the biochemical reaction. In this way enzymes are the best source for measuring the toxicity efficiency of a metal. A list of enzymes used for development of heavy metal biosensors is tabulated in table 1. As indicated urease, lactate dehydrogenase and many such enzymes respond to more than one metal and hence been reported in numerous investigations. The basic principal of enzyme inhibition based heavy metal biosensor is the interaction of heavy metals with the thiol group of cysteine residues in the protein. Some investigators have observed that metals like Cu^{2+} and Ag^{2+} binds to nitrogen of histidine and oxygen of aspartic acid and glutamic acid residues in the active site of enzymes such as urease. Whereas metals such as Pb^{2+} binds to thiol of cysteine residues present in the flap which results in loss of mobility and activity of urease. All heavy metals exert similar effects on enzymes and thus can be targeted in electrochemical or optical transducer based biosensors. Here is an example of enzyme inhibition based optical biosensor for Cd^{2+} ions. In this study a urease producing microbe *Bacillus badius* was

immobilized on nylon membrane at the tip of a fibre optic with a pH indicator phenol red (figure 1b). The urease tend to catalyze urea into ammonium and carbonate ions that results in increase in pH of the system and color change of the indicator from yellow to pink as illustrated in the figure 1a. The presence of Cd^{2+} in the system leads to urease inhibition and consequently concentration dependent slowing down of color change.

Figure 1: Picture showing the discs (Biocomponent), activity of urease with change in colour i.e. yellowish in absence of urea (left) and dark pink after adding 10µl of 2M urea (right).

Figure 2: Different parts of fiber optic tip, arrow indicating the position of plastic disc (biocomponent) used for the study, adjustment of disc in the fiber optic tip.

Figure 1: Optical biosensor for detection of Cd2+ ions (a) colour of sensing mixture before and after the urease activity, b) Assembly of biocomponent at the fibre optic tip. (Verma et al. 2011)

Table 1: Enzyme based heavy metal biosensors

Enzyme	Heavy metal	Lowest detectable conc. (Ic50)	Device
Alkaline phosphatase	Zn	10 µM	Calorimeter
L - glycerolphosphate oxidase	Hg	20 µM	Amperometer with Clark electrode
Pyruvate oxidase	Hg	50 nM	Amperometer with Clark electrode
L- lactate dehydrogenase	Hg	1 µM	Amperometer with Clark electrode
	Ag	0.02 µM	
	Cu	0.5 µM	
	Zn	5.0 µM	
	Pb	0.2 µM	
Peroxidase	Hg	0.02 µM	Calorimeter (oxidation based)
Urease	Hg	1.0 µM	Fiber optic
	Cu	3.94 µM	
	Ag	0.18 µM	optical
	Cd	2.67 µM	

		Zn	3.05 µM	

The most interesting phase in the area of biosensor development for heavy metals is the construction of genetically modified organisms (recombinants) that may respond to very low levels of heavy metals. Recombinant microbes have been constructed by fusing the copper inducible promoter of yeast with lac Z gene of *E. coli*. Such recombinants give rise to blue colonies in the presence of copper in growth media. Similarly *lac Z* gene may be replaced by luciferase gene, green fluorescent protein (GFP) or some other reporter genes that produce some kind of signal in the presence of proteins. The best example of this is the construction of recombinants for arsenic. The analysis is based on the natural instinct of micro- organisms to combat toxic metals like As (V) and As (III) which is used to construct a recombinants that produce some signal (fluorescence or luminescence) in response to As (III) (figure 2). The general micro-organism harbor an *ars operon* that contains a regulatory gene and some structural genes. One of the structural gene is ArsC that codes for the enzyme arsenate reductase responsible for reduction of arsenate into arsenite into the cell and sequestration of the latter one outside the cell. The arsenate is able to enter the cell through phosphate pump but the cells inherent metal resistance mechanism pumps it out after reduction. The protein produced by the regulatory gene blocks the expression of the structural genes as repressor, but in the presence of arsenite its repressor function is blocked that enable expression of structural genes. To create a recombinant responsive to arsenite the structural genes could be replaced with the reporter genes such as luciferase, GFP or *lac Z* genes to produce consequent luminescence, fluorescence or color change response respectively.

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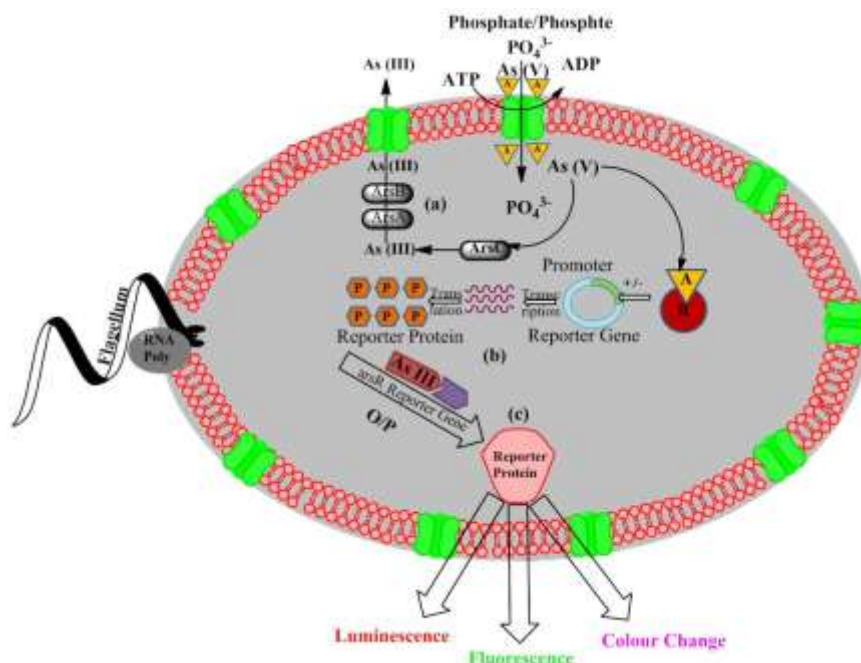
Figure 2. Schematic representation of Whole cell biosensor for (a) Arsenic [As (V) and As (III) transport by Phosphate channel (b) Role of As (III) in signal transduction and (c) Illustration of reporter protein analysis, either through luminescence, fluorescence or colour change. (Kaur et al.2015)

Heavy metals are the major target in water and soil quality analysis and have been detected through recombinants in many investigations. Some recombinant whole cell based biosensors developed for heavy metals are tabulated in table 2.

Table 2: Recombinant whole cell based biosensors for heavy metals

Species	Type of transducer	Heavy metals and media	Lower limit of detection	Detection range	Immobilization Technique

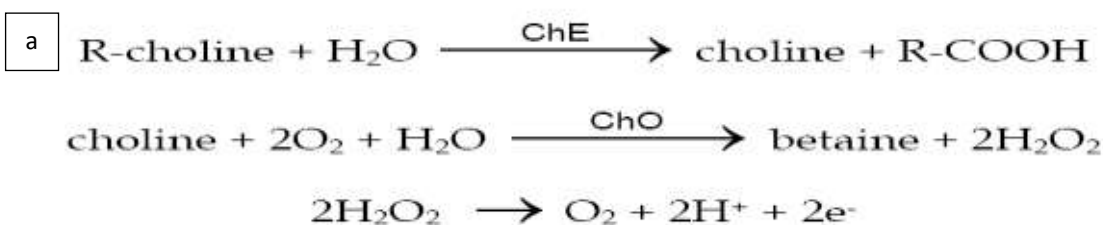| *Escherichia coli* with *goITSB* operon from *Salmonella enterica* and *lacZ* reporter gene	Optical-Colorimetry	Gold (Au) in soil	$2\ \mu g\ L^{-1}$	$20 – 1000\ \mu g\ L^{-1}$	No immobilization. Cell suspension used
E. coli with *ars* regulatory element and *Photobacteria luxCDABE* operatorpromoter	Optical-Luminescence	Arsenic (As) in water	NA	$0.74 - 60.00\ \mu g\ L^{-1}$	No immobilization. Cell suspension used
Caulobacter crescentus with GFPuv reporter gene under the control of *Caulobacter urca* gene	Optical-Fluorescence	Uranium (U) in soil and water & food	$0.5\ \mu M$		No immobilization. Sprayed directly onto soil or water surfaces.

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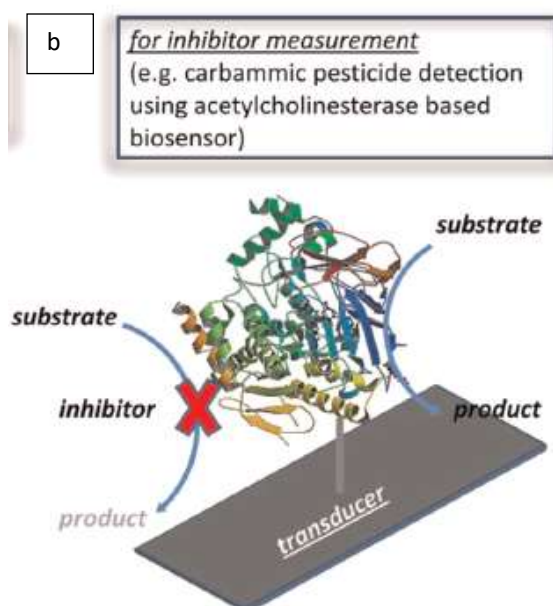| *B. sphaericus* | Electrochemical-Potentiometry | Ni in water | - | 0.002 - 0.040 | Physical adsorption onto filter paper. |
| *Pseudomonas putida* with *cadR* promoter fused to *lacIq* and *gfp*, with additional tac promoter and *cadR* transcribed divergently | Optical-Fluorescence | Cd in water | 0.01 µM | | No immobilization. Cell suspension used |

3.2 Pesticide biosensors

Pesticides, due to their high yield capacity, are majorly used in agricultural fields to enhance crop production. Pesticides of various classes have been designed to persist in the environment over a longer duration after their application. Apart from their agricultural benefits, pesticides also impose acute toxicological effects various life forms. Their accumulation in the living system may prove to be detrimental if at higher concentration. Majorly pesticides are classified into three categories: Organochlorines, Organophosphates, and Carbamates. Organochlorine hydrocarbons (e.g. DDT) operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit

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continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bio accumulate.

Biosensor technology used for the direct, fast, and easy determination of various pesticides (organophosphates, organochlorides, carbamates, etc.) and has been achieved by integrating various biocomponents with different transducers. Majorly organophosphate detection was initially done by enzyme-based biosensors which monitored the inhibition of acetylcholinesterase (AchE) by various neurotoxins. Majority of the pesticides are neurotoxic compounds and irreversibly inhibit the enzyme AchE, an essential enzyme for the functioning of the central nervous system in humans and insects. This inhibition leads to the accumulation of the neurotransmitter acetylcholine in nerves which interferes with muscular activities and the functioning of vital organs, produces serious symptoms, and may even lead to death. The working principle of AchE based pesticide biosensors rely on the catalytic activity of the enzyme to produce choline which can be further hydrolysed to hydrogen peroxide. The estimation of generated peroxide can be done amperometrically through an enzyme electrode (figure 3a and b). The pesticide inhibits AchE resulting in decreased production of choline and hydrogen peroxide, consequently lower current is observed in amperometric measurements. Higher the pesticide concentration, lower the current produced at the enzyme electrode.

a $\quad$ R-choline + H_2O $\xrightarrow{\text{ChE}}$ choline + R-COOH

$$\text{choline} + 2O_2 + H_2O \xrightarrow{\text{ChO}} \text{betaine} + 2H_2O_2$$

$$2H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

Figure 3 a: Bioassay principle of AchE based pesticide detection, b)Assembly of enzyme at the working electrode.

A number of other enzymes such as urease, organophosphorus hydrolase (OPH), organophosphorus acid anhydrolase etc. have been used for pesticide detection. In addition various immunosensors and aptamer based biosensors have also been reported. The use of nanomaterials, quantum dots (QD) for signal enhancement has also been a regular practise. For example a QD based biosensor for pesticide is reported where the production of hydrogen peroxide by the enzymatic action is related to quenching of the fluorescence of QD. The presence of pesticide would lead to less H_2O_2 production consequently less quenching of the QD fluorescence. So, in this case higher pesticide would lead to higher fluorescence as illustrated in figure 4.
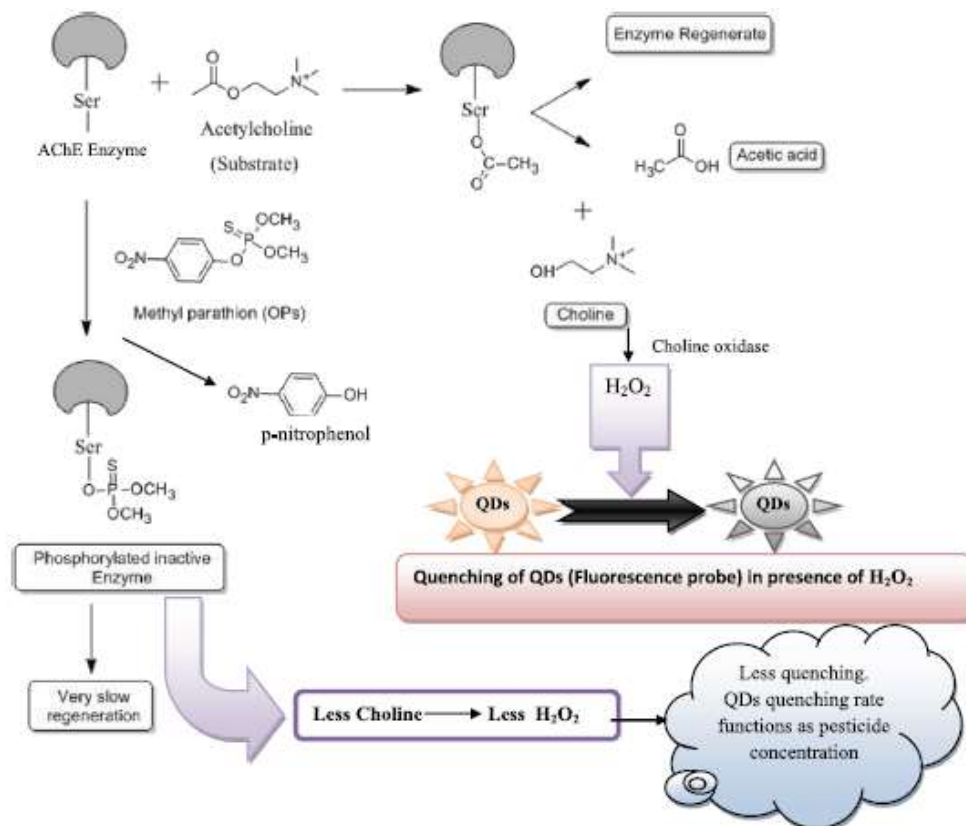
Figure 4: Quantum Dot based optical biosensor for pesticide. (Verma and Bhardwaj, 2015)

The detection of pesticides is not only important in drinking water but also in all agricultural products consumed by us. Their presence in soft drinks has been an issue always and biosensors have developed a significant place in such monitoring assays. Table 3 illustrates few pesticide biosensors and their detection limits. Another example of biosensor for pesticide may be discussed here for better understanding. Methyl parathion pesticide is extensively used in the field of agriculture despite its high toxicity and contributes a major share in terms of restricted use in India. OPH catalyzes hydrolysis of methyl parathion pesticide into detectable product p-nitrophenol (PNP) and generates two protons as a result of the cleavage of the P-O bond. Products that are chromophoric and/ or electroactive can be detected by colorimetric and electrochemical methods, and are exploited to develop biosensors for detection of methyl parathion pesticide. The analyte can be determined, as the rate of product

formation is directly proportional to the concentration of the analyte. As the OPH is a periplasmic enzyme, whole cells can be immobilized directly on the matrix and integrated with transducers for biosensor development.

Table 3. Pesticide biosensors with detection limits

Biosensing element	Pesticide detected	Transducer	Limit of detection
OPH	Methyl parathion	Amperometeric	3.4×10^{-9}
OPH	Paraxon	Electrochemical	1.2×10^{-7}
Anti-atrazine antibody	Atrazine	Electrochemical	4.6×10^{-12}
Methyl parathion hydrolase	Methyl parathion	Electrochemical	1.0×10^{-9}
AChE	Paraoxon	Optical	1.0×10^{-11}
AChE	Carbaryl	Electrochemical	1.4×10^{-6}
AChE	Methyl parathion	Amperometric	2.7×10^{-9}
Anti-atrazine antibody	Atrazine	Amperometric	5.0×10^{-11}
Anti-carbofuran antibody	Carbofuran	Electrochemical	1.3×10^{-8}
AChE	Chlorpyrifos	Amperometric	5×10^{-12}
AChE	Malaoxon	Amperometric	5×10^{-10}
AChE	Methyl parathion	Amperometric	2.5×10^{-6}

Heptan	Endosulfan	Electrochemical	1.2×10^9

3.3 Biosensors for phenolic compounds

Phenolic compounds have been recognised as toxic substances and endocrine. These include a large variety of analytes having significance in health care and environmental pollution monitoring. Phenolics constitute a large group of pollutants, which originate from a variety of industrial processes such as manufacture of plastic, paper, dyes, drugs and pesticides. This definition has been used by the scientific community to classify certain chemicals of natural or synthetic origin which are capable of interfering with the endocrine system, modulating it or mimicking natural hormones. The result of this interaction for humans and wildlife is the induction of serious pathologies such as developmental abnormalities and carcinogenesis. For these reasons, the determination of phenolic compounds in environmental matrices, including tap and surface water, has become a matter of great concern and scientific interest. Catechol is one such phenolic derivative which is readily absorbed by the gastrointestinal tract, causing vasoconstriction, renal tube degeneration, and decrease in liver function, cancers, and neurodegenerative diseases. Tyrosinase or laccase-based enzyme electrodes have been designed for the selective determination of phenolic compounds in environmental matrices. Their functioning is based on the reductive amperometric detection of the produced quinone species. The reaction can be schematized as equation (1) according to which the enzymatic *o*-hydroxylation of phenolic compounds to catechols is followed by dehydrogenation to *o*-quinones.

$$\text{Phenol} \xrightarrow{\text{Enzyme} + O_2} \text{Catechol} \xrightarrow{\text{Enzyme} + O_2} \text{o-quinone} + H_2O$$
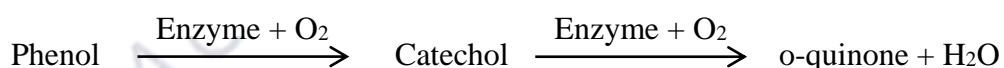
A number of laccase based optical and electrochemical biosensors have been developed for phenolic compounds, which are tabulated in table 4 with detection limits and response time.

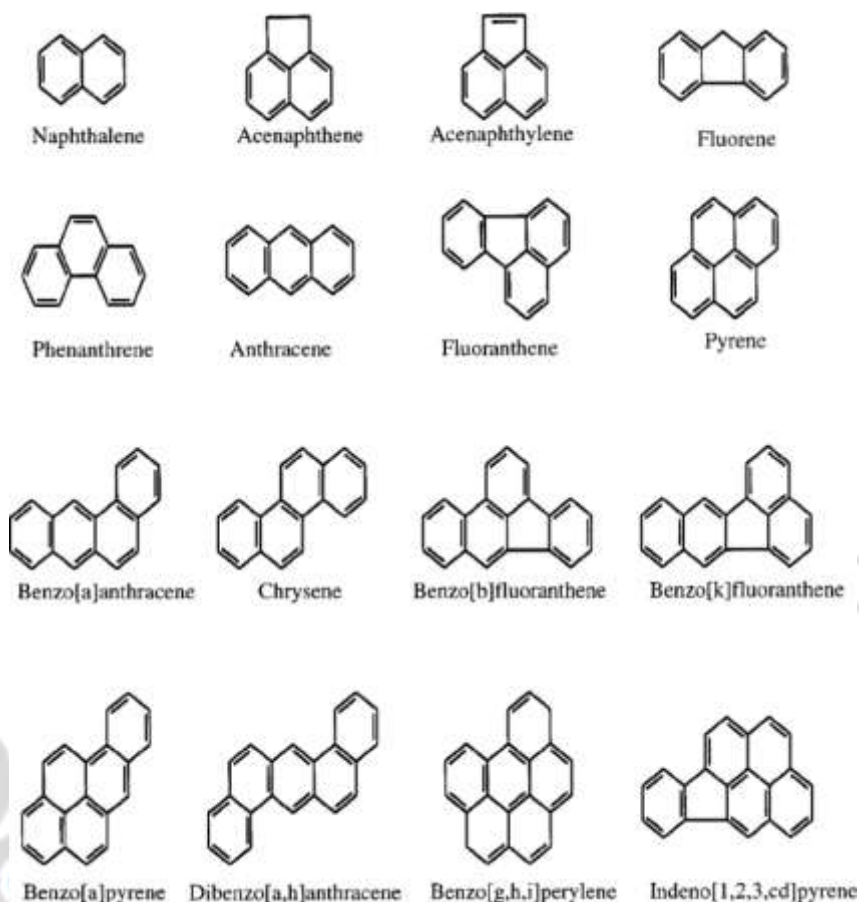**Table 4: Laccase based biosensors for phenolic compounds**

Species	Transducer	Analyte	Limit of detection

Trametes versicolor	Optical/absorption	Phenol	3.27 μM
Trametes versicolor	Optical fiber	Epinephrine	3.5 pg/mL
Rhus vernicifera	Amperometric	Catechin	0.003 μM
Pleurotus ostreatus	Amperometric	Catecholamines:	7.9 μM
Coriolus hirsutus	Amperometric	Dopamine	10 nM
Rigidoporus lignosus	Amperometric	1,4-hydroquinone	2 μM
Aspergillus oryzae	Voltammetry	Rutin	0.0623 μM
Aspergillus oryzae	Voltammetry	Adrenaline	0.293 μM
Trametes versicolor	Amperometric	Gallic acid	0.587 μM
Trametes versicolor	Amperometric	Catechol	0.67 μM

3.4 Biosensors for polycyclic aromatic hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants in aquatic environments. These contaminants are generated through oil spills, manufactory processes, and industrial wastes or naturally through the incomplete combustion of coal, oil, gas, and wood waste. Most of these compounds are noted as carcinogenic and mutagenic (structures illustrated in figure 1). Therefore, detection of these pollutants by a sensitive and inexpensive method in air, water and food stuffs is very important. Generally the biosensors developed for these pollutants are based on their DNA damage effect that results in reduction in guanine oxidation signal in the presence of PAH. The selected DNA from calf thymus or bovine thymus is immobilised on the working electrode and the guanine oxidation is recorded in the presence and absence of analyte.

Figure 5: Chemical structure of 16 PAH listed by US EPA as priority pollutants

(https://www.intechopen.com/books/air-pollution/polycyclic-aromatic-hydrocarbons-in-the-urban-atmosphere-of-mexico-city)
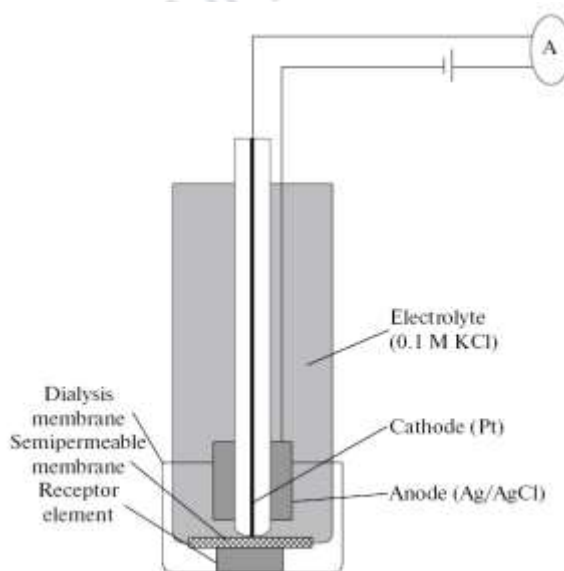
3.5 BOD biosensors

Biological Oxygen demand (BOD) is the parameter to accesses organic pollution of waste water after treatment. It is estimated by determining the amount of oxygen required by aerobic microorganisms for degrading organic matters in wastewater. Conventional BOD method is the well-known BOD_5 which needs 5-day incubation at $20°C$ in the dark. The BOD of a waste water can be defined as the

amount of oxygen expressed in milligrams per liter required by the microorganism for the biodegradations of the degradable carbonaceous organic matter present in the water through their biochemical, bioprocess, and under the following reaction conditions: temperature 20°C, five-day retention time, and darkness to avoid the presence of microscopic algae that produce oxygen by photosynthesis thus interfering with the result. Because the saturation conc. for oxygen in water at 20°C is approximately 9 mg/L dilution of the sample with BOD free, oxygen-saturated water is necessary to measure BOD values greater than just a few mg/L. BOD of a diluted sample is calculated as:

$$BOD = DO_i - DO_f/P$$

where DO_i and DO_f are initial and final dissolved oxygen concentrations (mg/L) and P is the decimal fraction of the sample in 300 mL bottle. Fast determination of BOD could be achieved by biosensor-based methods. A common feature of these sensors is that they consist of Clark electrode with microbial films that can bio-oxidize the organic substrate to be quantified, sandwiched between a porous cellulose membrane and a gas-permeable membrane as the biological recognition element as illustrated in figure 6.

Figure 6: Clark electrode based BOD biosensor

First BOD biosensor was developed by immobilization of *Trichosporon cutaneum* on the oxygen electrode. Some BOD sensors have been developed and marketed by various manufacturers in both, biofilm and bioreactor-type configurations. Most commercially available BOD sensors are flow-type systems that can be more easily automated but generally require high maintenance to prevent fouling and clogging (figure 7). The response is usually a change in concentration of dissolved oxygen or other phenomena such as light emission.
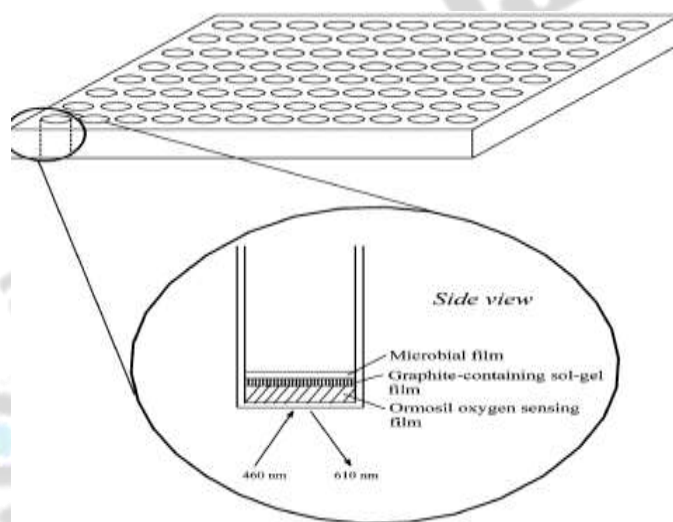
Figure 7: High- throughput detection of BOD by microplate based biosensor

3.6 Recent advances in biosensing

26.6.1 Paper Microfluidics based devices

The advent of nanotechnology, microfluidics and screen printed technologies have equipped biosensors with enhanced sensitivity, low production cost and commercial aspect. A step forward towards development of easy and handy technology is the integration of paper microfluidics with biosensor technology. In this case the channels of microfluidics are replaced with paper support which provides similar flow of liquids as micro channels. Channels on paper are made by imprinting

hydrophobic materials outside the channels to allow liquid flow inside the paper channel only. Different reservoirs/inlets for sample, bioreceptor and other reagents may be formed with a common mixing channel and detection chamber at the end. One such example is the detection of arsenic through paper microfluidics as illustrated in figure 8. In this case a Y – shaped channel was imprinted on paper with both the arms carrying inlets or different moieties. One of the inlets was for thioctic acid and thioguanine coated gold nanoparticles that served as the arsenic binding reporter. Through second inlet arsenic containing sample was introduced. The direct interaction of modified gold nanoparticles and arsenic in the long arm of Y channel resulted in aggregation of gold nanoparticles and hence change in colour from blue to red. A direct relationship of arsenic concentration in the sample could be deduced through colour change rate and intensity.
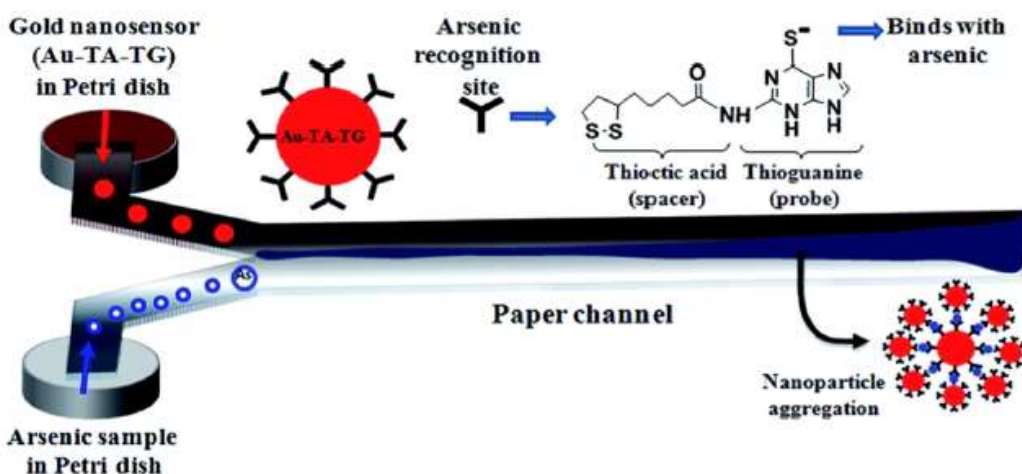

Figure 8: Heavy Metal detection with paper microfluidics (Nath et al. 2014)

Such paper based devices provide the benefits of easy, cost effective and rapid analysis of pollutants along with on-site monitoring. Due to these reasons, paper based devices are highly encouraged in clinical diagnosis also where fast response time is the most desirable attribute of any point –of- care tool.

3.6.2 Smart phone based sensors

Smartphones have come up as new era of biosensing due to their portability and high efficiency. The continuous improvement of smartphone electronics and the development of new apps have stimulated research into the use of smartphones as biosensors. By using the smartphone photo camera as a 'smart detector', almost all the optical-based methods have been integrated, including absorbance, reflectance, fluorescence, surface plasmon resonance (SPR), bio-chemiluminescence, and electrochemiluminescence. Generally colorimetric assays for environmental pollutants such as potassium and chlorine have been developed using smartphones and a good detection limit has been achieved. The smart phones are enabled with cartridge adaptors to keep sample and perform the analysis as illustrated in figure 2a and b. The biosensor aspect has normally been applied to clinical diagnosis but there is a lot potential of smart phone based biosensors for environmental monitoring. The synergistic combination of biotechnology, electronics and nanotechnology are in the process of developing potable future devices. Such a device has been developed and commercialized by iHealth Labs for glucose monitoring and it's called iHealth Smart Gluco – monitoring system (figure 2c). The company has fabricated a small portable device that may be connected wirelessly to a smartphone through an app that can monitor, store and transmit the blood glucose data in zero time. The device is similar to traditional glucometer with a screen printed three electrode strip as the sensing platform. The simple interface of devise with the smart phone has enabled the patients with capacity of large data storage of the medical history. The device takes 5seconds and only 0.7 µl of blood for glucose monitoring.

Figure 2: a) Smart phone accessories iHealth Smart Gluco – monitoring system

(http://www.ilounge.com/index.php/news/comments/ihealth-announces-wireless-smart-gluco-monitoring-system)

More such devices are in progress that incorporates the sample port and all other required accessories as counter part of smart phone, but, application of such devices for environmental monitoring is in infancy till now.

Concluding Remarks

Biosensors are rapidly capturing the markets of clinical diagnosis and environmental monitoring due to their obvious merits. They have been widely used the detection of heavy metals, pesticides, phenolic compounds, poly aromatic compounds are various other pollutants. Enzyme inhibition has been the preferable working principle against heavy metals and pesticides. Microbial consortia containing different microbes are employed in BOD biosensors to extend the substrate specificity and stabilize a long term stability of the biosensor. Recent advances in biosensor technology includes paper based microfluidic devices and smart phone based applications which will provide a new horizon to this technology and lead to affordable commercial biosensors for monitoring purposes.