Real-Time Environmental Monitoring with Biosensors

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Introduction

Environmental pollution is widely regarded as one of the most persistent and severe threats to our planet. Every day, our world is exposed to harmful pollutants and chemicals from various sources, gradually deteriorating the ecosystem. The anticipated rise in global temperatures by up to 6°C by the year 2100 is expected to significantly impact organisms and ecological processes in both terrestrial and aquatic environments. Addressing these challenges requires urgent scientific intervention to develop effective and cost-efficient solutions.

According to the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is an integrated, self-contained device capable of delivering specific quantitative or semi-quantitative analytical data. This is achieved through the interaction of a biological recognition element with a transduction component. Biosensors are reagent-free analytical tools known for their high specificity and sensitivity, making them valuable in research areas such as food safety, clinical diagnostics, and environmental monitoring. A typical biosensor consists of three key components: a bio-recognition element, a transducer, and a signal processing unit. The bio-recognition element detects the target analyte within a sample, leading to physicochemical changes (Naresh and Lee, 2021). These changes are then converted into measurable signals by the transducer, which are subsequently processed into a readable format by the signal processing system.

Environmental applications of biosensors

1. Dissolved oxygen – Clark electrode

The Clark electrode measures oxygen concentration in a liquid using a catalytic platinum surface. Its development led to the first glucose biosensor, created by Clark and Lyons in 1962, which combined a Clark oxygen electrode with a counter-electrode. Like the Clark electrode, the glucose biosensor has a Pt electrode covered by a permselective membrane, but with immobilized glucose oxidase (GOx). As oxygen diffuses toward the electrode, GOx catalyses its conversion into hydrogen peroxide (H_2O_2) and gluconic acid. The reaction current depends on glucose and oxygen diffusion rates, with their concentrations on the analyte side being the key measured variables.

$$\boldsymbol{Glucose + O_2 \xrightarrow{Glucose\ oxidase} Gluconic\ acid + H_2O_2}$$

2. Biological oxygen demand

Biological oxygen demand (BOD) is a key indicator of organic water pollution, measuring the oxygen needed by aerobic microbes to break down organic matter. The conventional BOD test is time-consuming and requires expertise, whereas biosensors offer a quicker and more reliable solution.

i. **Ferricyanide-Mediated BOD Sensor**: A biochemical oxygen demand (BOD) sensor was developed using ferricyanide (FC) as a mediator, anchored on an ion-exchangeable polysiloxane synthesized via a sol–gel process. Ferricyanide was immobilized through ion association and used for electrode modification. FC efficiently transfers electrons between reduced bacterial enzymes and the electrode. During aerobic catabolism, electrons from organic substrate oxidation reduce FC to ferrocyanide (Jordan et al., 2013).

ii. **Hybrid Material for BOD Sensor**: An electrochemical BOD sensor was developed using an organic–inorganic hybrid material synthesized from silica co-polymerized with poly (vinyl alcohol) and 4-vinylpyridine (PVA-g-P(4-VP)). *Trichosporon cutaneum* strain 2.570 cells were then immobilized on this material. The hybrid material creates a biocompatible microenvironment, ensuring long-term cell viability, as confirmed by confocal laser scanning microscopy (CLSM). This biocompatible sensor can be used for BOD detection after proper arthroconidia activation.

3. Heavy metals

Bacterial biosensors have emerged as effective tools for detecting heavy metal pollution in the

environment (Table 1). These biosensors utilize genetically engineered or naturally resistant bacterial strains as sensing elements to identify the presence of toxic metals such as lead, zinc, mercury, cadmium, and copper.

Table 1. Biosensors used in detection of heavy metals and pesticides

Analyte	Recognition Biocatalyzer	Method
Heavy metals		
Hg, Cd, and As	Urease enzyme	Electrochemical
Cd	DNA, Phytochelatins	Electrochemical, Optical
Zn, Cu, Cd, and Ni	Enzyme	Optical
Hg (II) and Pb (II) ions	DNA	Optical
Cu(I) and (II) ions	Fluorescent protein	Optical
Pesticides		
Paraoxon	Alkaline phosphatase	Optical
Isoproturon	Antibody encapsulate	Fluorescence
Parathion	Parathion hydrolase	Electrochemical/ Amperometric
Carbaril	Acetylcolinesterase	Electrochemical/ Amperometric
Simazina	Peroxidase	Electrochemical/ potentiometric

i. **Enzyme-based biosensors for heavy metals**: Various enzymes are used to analyze heavy metal ions based on their activation or inhibition effects. Heavy metals act as activators when they serve as essential cofactors in metalloproteins. For example, a calorimetric biosensor for zinc ion detection in flow injection microanalysis was developed. It utilized alkaline phosphatase apoenzyme reactivation by Zn (II), an exothermic process. The immobilized enzyme detected Zn (II) within a 10 mM–1.0 mM range, with a 3-minute response time. The biosensor remained stable for up to two months and could be regenerated using 2,6-pyridine dicarboxylate solution (Ondes et al., 2021).

ii. **Antibody-based biosensors for heavy metals**: An inhibition immunoassay resistant to interference from metal ions has been used to detect cadmium. It employs anti-cadmium (2A8 1G5) monoclonal antibodies that selectively bind to Cd–EDTA complexes but not to free EDTA. These antibodies detect Cd(II) in the 70–500 ppb (0.06–4.45 mM) range (Khosraviani et al., 1998). More recently, monoclonal antibodies have been developed for cadmium–EDTA, cobalt–DTPA, and lead–CHXDTPA complexes, achieving enhanced sensitivities of 0.25, 10, and 6.0 nM, respectively.

iii. **DNA-based biosensors for heavy metals**: A novel approach in biosensor development includes non-protein-based sensors, such as a calorimetric Pb (II) sensor using DNAzyme-directed gold nanoparticle assembly. The "8-17" DNAzyme serves as the sensing element, exhibiting high selectivity for Pb(II). In the absence of Pb(II), DNAzyme, its substrate, and gold nanoparticles aggregate, producing a blue color. However, Pb(II) prevents aggregation, resulting in a red color. This sensor detects Pb(II) within a 100 nM–4.0 µM range (Saidur et al., 2017).

4. Pesticides

The EPA defines pesticides as substances used to prevent, repel, or eliminate pests. They are among the most widespread pollutants, found in soil, water, air, plants, and food. Due to their toxicity, the EU has set limits on their concentration, capping individual pesticides at 0.1 µg L^{-1} and total pesticides at 0.5 µg L^{-1} in drinking water. Enzymatic biosensors, which inhibit specific enzymes, are widely used for detection. Parathion, a broad-spectrum pesticide, is highly toxic and can be fatal even in small amounts through air or skin absorption.

5. Persistent organic pollutants (POPs)

Many mono- and polyaromatic hydrocarbons, including their chlorinated derivatives, are persistent organic pollutants (POPs). These include polychlorinated biphenyls (PCBs), dioxin-like compounds, and polycyclic aromatic hydrocarbons (PAHs), which are widespread ecotoxicants. Bacterial biosensors often utilize reporter proteins with autofluorescence. Fluorescent protein-expressing bacteria can be detected via fluorimetry,

epifluorescence microscopy, or flow cytometry at the single-cell level. Modern biosensors frequently incorporate multiple reporter genes encoding different fluorescent proteins. Other reporter proteins, such as β-galactosidase from *E. coli* (encoded by lacZ), degrade synthetic substrates into coloured products detectable calorimetrically. Many biosensors are based on the lacZ gene (Table 2).

Table 2. Microbial biosensors used in detection of POPs

Bacterial strains	Type of registered signal	Detected compound	Pollutant detection limit
P. putida pPG7lux	Luminescence	Naphthelene	0.1 µM (water), 0.03 µM (gas phase)
P. fluorescens HK44	Luminescence	Naphthelene	12-120 µM
Burkholderia sartisoli RP037	Fluorescence	Phenanthrene	0.3 mg/L (Crystallline)
Sphingomonas sp. L-132lux	Luminescence	Fluorene	200 µg/L (1.2 µM) in water
E. coli hpbR	Luminescence	27 congeners of OH-PCBs	10-5-10-9 M
P. fluorescens F113	Fluorescence	3-monoCB, CBA	< 10 µM (CBA), 10 µM
P. Fluorescens F113L1180gfp		PCBs (total content in soil)	1534 ppb

6. Pathogenic microorganism

Pathogenic microorganisms, including bacteria, viruses, and protozoa, pose serious public health risks and must be removed from potable and polluted water. Biosensors offer faster alternatives, with immunosensors using fluorescence, surface plasmon resonance, quartz crystal microbalance, and impedance for detection (Barreiros's dos Santos et al., 2013). DNA-based biosensors provide greater specificity and sensitivity. Aptamers, synthetic oligonucleotides with high target specificity, enhance pathogen detection. Mycobacteria, responsible for tuberculosis and leprosy, can be identified in environmental samples using a microfluidic culture-based biosensor that exploits their paraffinophilic nature. Recently, a potentiometric biosensor capable of detecting a single CFU mL^{-1} of *Staphylococcus aureus* was developed, enabling near real-time detection (Hernandez et al., 2014).

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