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Review Article

BIOSENSORS FOR THE ENVIRONMENTAL POLLUTION DETECTION AND MONITORING: A REVIEW

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Abstract

The discharge of dangerous contaminants like pesticides, chemicals and heavy metals into the natural ecosystem is a worldwide issue. Therefore, it is important to identify fast-moving and recyclable contaminants. Biosensors are highly sensitive devices for detecting environmental pollution. Various biosensor types have been developed to detect environmental contamination. Biosensor is the most recent breakthrough in environmental pollution detection and monitoring. Biosensors are widely used in the detection of pesticides, heavy metals, surfactants, biological oxygen demand, phenolic compounds, pharmaceutical compounds, and pathogenic organisms. This paper mainly focuses on the principle, working, characteristics, and uses of biosensors, which are constructed for the detection of pollution.

Keywords: Biosensors, pollution, detection, monitoring, heavy metals

1. INTRODUCTION

Rapid urbanization and advances in technology are produced high levels of chemicals and hazardous materials. These contaminants are discharged into the natural environment which disturbs the value of an environment (Bilal and Iqbal 2019). These contaminants are the main cause that involves human health complications and leads to poor social outcomes. Toxic drugs, heavy metals, chemicals, and water pollutants are the main causes of environmental contamination. Therefore, the need to get the operational devices for detecting the toxic substances and considering the threats which are linked with the discharge of big volumes of contaminants (Hernandez-Vargas et al. 2018). The conventional methods helped in the careful exploration of the physical or chemical characteristics of the environmental models, but these methods are not perfect, so it is a need for rapid and low-cost methods for the monitoring and detection of contaminants. The biosensor is the most capable tool for environmental research and environmental pollution detection (Gavrilaş et al. 2022). The IUPAC definition of a biosensor is “it is an independent integrated device that can provide numerical or semi-numerical information using biological markers (biochemical receptors), placed in direct contact with a magnetic field (Hashemi Goradel et al. 2018). Biosensor converts biological markers into electric markers and can be considered useful tools for chemical research in health, food, and environmental products. Biosensors can be used for ongoing research in the affected area (Khanmohammadi et al. 2020). They can also provide useful research qualities, like

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higher specifications and insights. Biosensors allow the determination of the specified chemicals and also determine their biotic effects, like toxins, genotoxicity and cytotoxicity (Meena et al. 2018). Compared to traditional systems, biosensors have many advantages, including mobility, miniaturization, the ability to detect contaminants and complex matrices with low sample preparation, as well as the speed and reliability of research. The usage of traditional approaches requires qualified staff to work in specialized laboratories (Zhang et al. 2020a). This review presents a brief overview of the class of biosensors and their applications in the monitoring and detection of environmental pollution.

2. Components of biosensors

The biosensor is made up of three sections, bio mediator, transducer and electronics or signal processor. The first one is bio mediator, which comprises biological or biologically derived materials, like microorganisms, nucleic acid, antibodies, enzymes and other biological genetically engineered elements (Ali et al. 2020). The second one is the transducer. It can be physiochemical, optical, piezoelectric, or electrochemical. The transducer turns the signals generated by the interaction between the analyte and biological element into the quantitative signals (Mowbray and Amiri 2019). The 3rd part is a signal processor which oversees exhibiting the results in a customer approach. Some biosensors necessitate the use of physical or chemical procedures to immobilize the bio mediator on the sensor surface, like metal, polymer, glass, or other materials (Zhang et al. 2018).

3. Principal of biosensors

Biosensors are based on the concepts of signal transduction and biometric identification of elements. As sensors or detectors for devices, any biological materials which can show the molecular interaction like enzymes, antibodies, and entire cells can be employed (Alvarado-Ramírez et al. 2021). But on the other hand, the specified inactivating enzyme is generally used as an ideal bioreceptor. The inactivating enzyme is positioned close to the transducer. The biochemical characteristics of the enzyme are altered as it binds to the examined substance. An electrical response is then generated as a result of this alteration (Justino et al. 2017). The

electro-enzyme technique is a biochemical method of converting enzymes into accompanying electrical signals via a transducer (Fig 1). In this situation, the electrical signal produced by the transducer is a direct reflection of the biological substance being measured (in this case the analyte and enzyme) (Bidmanova et al. 2016). For efficient evaluation and visualization, electrical impulses are often transferred to the visual screen.

4. Working of biosensors

The coupling of sensitive biological materials with a transducer is capable of converting the biological material into an electrical signal. The transducer's outcome might be voltage or current, it depends on the type of the enzyme (Ma et al. 2021). The outcome is a current, which should first be converted to a voltage equivalent. The amplitude of the output voltage signal is frequently quite low, and it overlapped the noise exposure signal. Therefore, before being routed via a low-pass RC filter, the signal is increased. This signal amplification and filtration procedure are handled by the signal processing unit, also known as the signal conditioning unit (Bhattarai and Hameed 2020). Analog signals are generated by the signal processing unit's output. This is the same as the biological amount that was measured. Although on the LCD panel the analogue signal is visible directly, it is transmitted to the microcontroller for the conversion to a digital signal (Bi and Han 2019). The reason for this is that digital signals are simple to examine, process, and store.

5. Types of biosensors

5.1 Electrochemical biosensors

Electrochemical biosensors have the benefits of low budget, the convenience of use and ease of manufacture. Electrochemical biosensors detect the electrical characteristics of a biological system (Hernandez-Vargas et al. 2018). Electrochemical biosensors primarily comprise enzymes, although numerous detection techniques are employed in their creation. Electrochemical biosensors are constructed with three electrodes. For maintaining a constant voltage, a reference electrode (Ag/AgCl) is used, a counter electrode or auxiliary electrode to connect to the electrolyte solution and the third one is the working electrode (Qian et al.

2021). Biosensors that monitor the current, potential, or charge build-up, as well as the medium's conductive qualities, are known as amperometry, potentiometric, and conductivity measurement biosensors. Impedance and resistor are measured using the impedance measuring biosensors (Tajik et al. 2021). The field-effect biosensor's mechanism is to detect current using the potentiometric effect on the gate electrode. A whole-cell electrochemical biosensor was reported to be utilized to determine the extreme biotoxicity in wastewater (Gao et al. 2017).

5.2 Amperometric biosensor

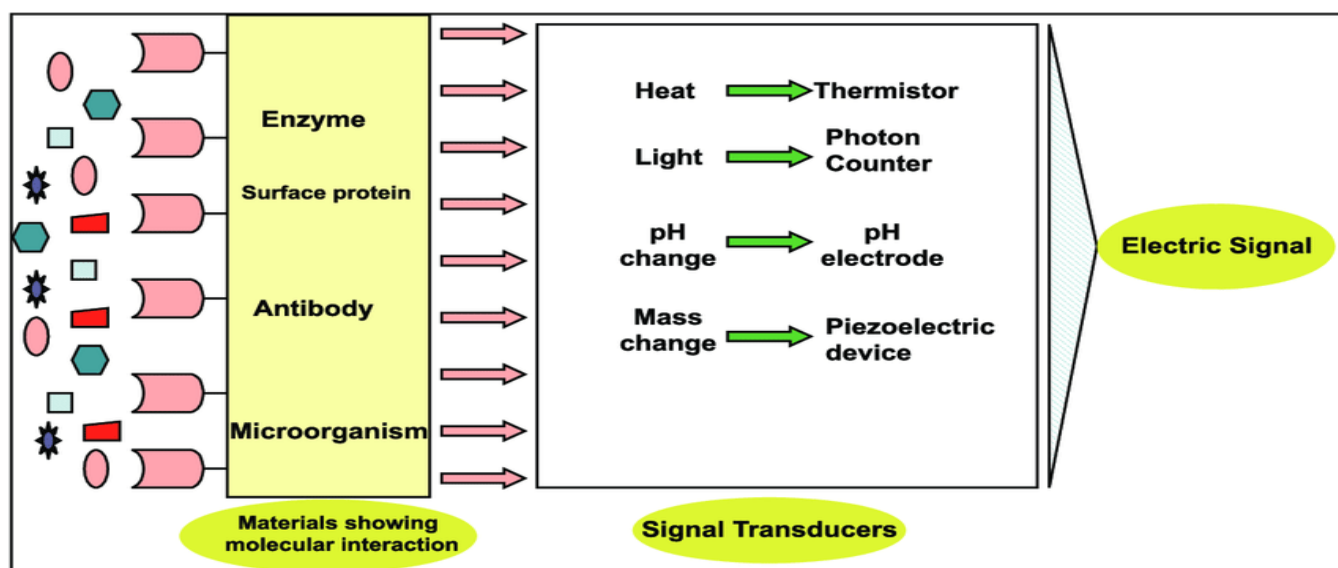


Fig 1: Principal of biosensors (Vargas-Bernal et al. 2012)

These biosensors rely on the electron's mobility (i.e., current) via enzyme-catalyzed oxidation-reduction. There is normally a steady voltage between electrodes that can be measured. The substrate or product can transport electrons to the electrode's surface to be oxidized or reduced during an enzymatic reaction (Lopez et al. 2017). This alters the measured flow of current. The current is proportional to a substrate concentration. The most basic type of amperometric biosensor is Clark Oxygen Electrode, which evaluates O₂ depletion. Using glucose oxidase to measure glucose is a great illustration (Vlamidis et al. 2017). The emitted electrons move directly to the electrodes in first-generation amperometric biosensors, which can cause some practical problems. Second-generation amperometric biosensors have been developed in which mediators receive electrons and transfer them to electrodes (Othman and Wollenberger 2020).

Hydroquinone determination is reported to be done by using amperometric biosensors based on recombinant bacterial laccase CotA. The creation of a laccase-dependent amperometric biosensor will provide a new tool for substance detection in harsh conditions, particularly for industrial contaminants (Zhang et al. 2020).

5.3 Blood glucose biosensor

This is an excellent example of an amperometry biosensor widely used by diabetics around the world. The blood glucose biosensor resembles a bell and is equipped with a single easily removable electrode along with ferrocene and glucose oxidase derivatives acting as mediators

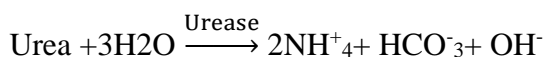
(Scognamiglio and Arduini 2019). To equally disperse a blood drop, the electrodes are enveloped with a hydrophilic gauze fabric. The shelf life of cheap and disposable test strips enclosed in aluminium foil is approximately 6 months (Liu et al. 2019). To determine the freshness of fish, amperometry biosensors have been designed. In comparison to other nucleotides, the concentration of inosine and hypoxanthine shows the fish's freshness and how long it has been dead (Mandpe et al. 2020). To this end, biosensors have been developed that use electrode-immobilized nucleoside phosphorylase and xanthine oxidase. It is reported that Rapid methods for measuring lactose in milk have been developed utilizing blood glucose biosensors (Amamcharla and Metzger 2011).

5.4 Potentiometric biosensors

Changes in the ion concentration are measured using selective electrodes in these biosensors. Because many enzymatic activities emit or accept hydrogen ions, the pH electrode is mostly used for ion selection (Karimi-Maleh et al. 2021). Ammonia selective electrodes and carbon dioxide selective electrodes are also essential. The output voltage difference between the reference electrode and the main voltage electrode can be detected and measured, and it is proportionate to the concentration of the substrate (Kaur and Prabhakar 2017). The enzyme sensitivity to the concentration of ions like NH_4 and H^+ is the major constraint of the potentiometric biosensor. To minimize the size of the voltage biosensor, ion-selective field-effect transistors (ISFETs) are less-cost components that are utilized. During open-heart surgery to measure the pH of the heart muscles, ISFET biosensors are used (Wang and Hu 2016).

5.5 Biometric biosensors

Many activities in biological systems lead to alterations in ionic species. And, these ionic species change the electric conduction which can be measured. The urea biosensor that employs deactivated urease is an example of a metric biosensor (McGoldrick and Haláček 2020). Urea catalyzes the following reaction:



The above-mentioned reaction equation is related to a rapid shift in the concentration of ions, that can be utilized to regulate the levels of urea. In hemodialysis and renal surgery urea biosensor have been widely employed with considerable success (Koltunov et al. 2018).

5.6 Thermometric biosensors

Heat produces during many biological interactions, which serves as the basis for thermal biosensors. It is composed of a heat insulation box and heat exchanger. In a reactor, this reaction occurs that has a thin layer of the enzyme (Gupta and Kakkar 2020). As the substrate diffuses with the other substrate, the product forms and generates heat. Thermistors determine the temperature differences between the substrate and the product. Biothermal sensors can detect minor temperature changes (Chauhan and Upadhyay 2020). Blood

cholesterol levels are estimated using a thermal biosensor. The enzyme cholesterol oxidase oxidized the cholesterol, as a result, heat is produced that can be measured. These biosensors can also measure penicillin G, glucose, uric acid, and urea (Zheng et al. 2022). The thermal biosensor is used in an enzyme-linked immunoassay (ELISA) and emerges a new technology called thermal ELISA (TELISA).

5.7 Optical biosensors

Optical biosensors are based on optical measurement (absorption, emission, and chemical light). Fiber optics and photovoltaic converters are used (Yoo and Lee 2016). Optrode consists of two phrases optical and the electrode is commonly used. Enzymes and antibodies are used as transducers. Optical biosensors offer harmless non-electrical remote detection of materials (Kaur and Singh 2020). Reference sensors are not required in these biosensors, because the comparison signal is created through a light source correspondent to the sample sensor.

5.8 Fiber optic lactate biosensors

Its function is to determine the cooling effect of O_2 on fluorescent dye to quantify variations in molecular O_2 concentration. The lactate monooxygenase enzyme catalyzes this reaction illustrated in the Fig 2 (Halilović et al. 2019).

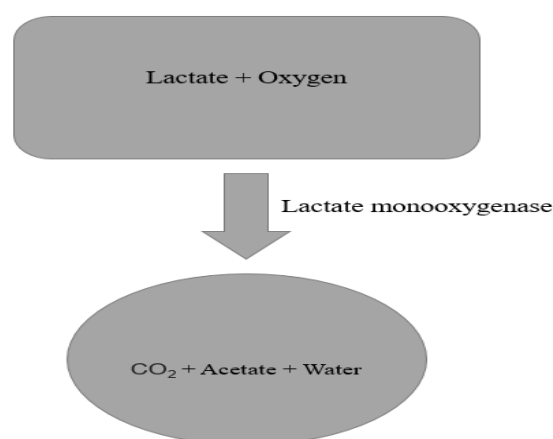


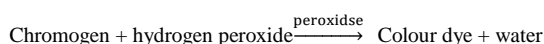
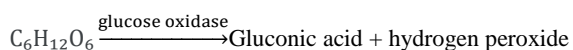
Fig 2: Reaction mechanism of the fiber optic lactate biosensor

The quantity of fluorescence produced through the coloured film depends on oxygen. On fluorescence, O_2 has a cooling effect. The cooling effect decreases when lactate concentration in reaction increases, due to

which O₂ is consumed (Halilović et al. 2019). As a result, measurable fluorescence output increases.

5.9 Optical biosensors for blood sugar

For monitoring diabetes, blood glucose testing is important. This is accomplished using a simple reagent-impregnated paper strip technique. Glucose oxidase, horseradish peroxidase and chromogen are all included in the paper strip (Rachim and Chung 2019). The following reaction occurs.



A portable reflectometer is used to measure the colour intensity of pigments. Glucose strip manufacturing is a large industry worldwide. To determine various blood and urine parameters, calorimetric test strips are composed of fiber covered with suitable reagents and enzymes (Khansili et al. 2018).

5.10 Luminescent biosensors for the detection of urinary tract infections

Luminescent biosensors are used to detect microorganisms in urine that cause urinary tract infections. An inactive luciferase enzyme is used in luminescent biosensors (Davenport et al. 2017). Microorganisms emit ATP after degradation, as demonstrated in the following reaction (fig 3). Electronic devices can be used to measure the amount of light generated (Kumar et al. 2016).

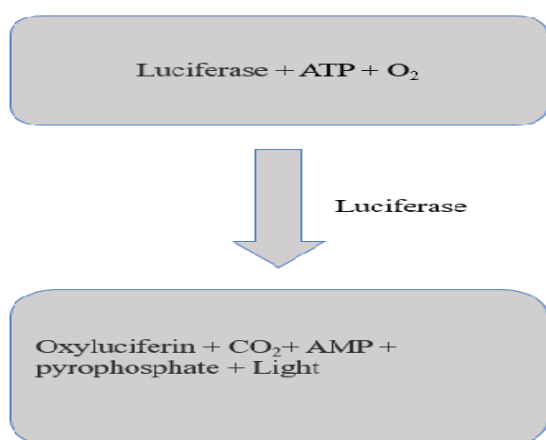


Fig 3: Reaction equation in luminescent biosensors

5.11 Piezoelectric biosensors

These biosensors work based on audibility (acoustic vibration). These biosensors are made

of piezoelectric crystals. Positive and negative charge crystals oscillate at different frequencies (Skládal 2016). Alteration of resonance frequencies that occurs due to absorption of some molecules on the crystal surface can be measured using electronic devices. The molecules that attach to the crystal surface include enzymes with substrates or gas inhibitors (Kaur et al. 2018).

5.12 Whole-cell biosensors

They are useful in multistep or cofactor stages that require interaction. Living or dead microbial cells can be used in these biosensors (Gui et al. 2017). The table 1 includes a list of species chosen for study, as well as the analyses and types of biosensors used.

5.13 Immune biosensors

A biosensor that integrates a biological recognition mechanism with a transducer that provides a detectable signal in response to variations in the concentration of a specific biomolecule is known as an immune sensor (Eivazzadeh-Keihan et al. 2017). One of the components of the test reaction is covalently fixed in the matrix and the other reaction partners (analytes) are transferred into the solution by the sensor. Biosensors that use antibodies are called immune ligands. Safety measurement technologies are very new and were first used in food analysis in the mid-1990s (Meena et al. 2018). Many advances have been made in downsizing safety sensors in recent years. Portable safety sensors for on-site sensing are now available commercially, and some of them can detect many biomolecules simultaneously (Senturk et al. 2018).

6. Environmental monitoring

Pollutants in the environment are hazardous to human health. Organic chemicals and inorganic compounds are the two types of contaminants found in the environment. Hormones, Pesticides, PCBs, dioxins, bisphenol A, phenols, alkyl benzene sulfonates, surfactants, and alkylbenzene sulfonates are linear hydrocarbons, antibiotics and poisons are polycyclic aromatic hydrocarbons (McConnell et al. 2020). Metals, mineral phosphates, and nitrates are examples of inorganic contaminants. There are the following pollutants which can be detected by biosensors:

6.1 Pesticides

Pesticides are found everywhere in the environment because of their widespread agricultural use to increase the yield and productivity of crops. Pesticides cause disorders of the nervous, respiratory and immune systems and are carcinogenic (Bucur et al. 2018). Pesticides are categorized based on their chemical makeup: carbamates, synthetic pyrethroids, organochlorine, mineral pesticides and organophosphate. Organic chlorine control agents cause negative changes in the fish's reproductive system and therefore can affect the ecosystem (Arduini et al. 2019). Enzyme-based biosensors which are specifically focused on the inhibition of choline oxidase and acetylcholinesterase are the most commonly used biosensors for pesticide detection. The inhibition of acetylcholinesterase in the nerves leads to the accumulation of the dangerous neurotransmitter acetylcholine (Karadurmus et al. 2021). Pesticides are identified by measuring enzyme inhibition and the components involved in enzymatic processes.

2019). Although some minerals are required for various biological processes like enzymatic reactions, in some cases they can catalyze enzyme inhibition. For example, activating or inhibiting enzymes are used to develop biosensors for heavy metals. Heavy metals including cadmium, nickel, cobalt and copper can be detected using the inhibition of glucose oxidase (GO) as a biosensor (Aloisi et al. 2019). To detect metal, optical biosensors rely on the activity of a reporter gene controlled by an induction promoter. This strategy is known as continuous measurement; the signal level of the reporter increases with the concentration of the contaminants (Odobashić et al. 2019). The most often utilized reporter genes in bioassay systems are green fluorescent protein, β -galactosidase, and luciferase. In the table 2 study of heavy metals including their detection limit, transducer and biorecognition elements is given.

6.3 Pharmaceuticals

In recent times one of the major water pollution challenges is improper disposal of hospital

Table 1: Particular list of some organisms with the type of biosensors and analytes

Organism	Type of biosensor	Analyte	References
Cyanobacteria	Amperometric (mediated)	Herbicides	(Tucci et al. 2019)
Escherichia coli	Potentiometric (Carbon dioxide)	Glutamate	(Jia et al. 2022)
Desulfovibrio desulfuricans	Potentiometric (SO_3^-)	Sulfate	(Asif et al. 2022)
Sarcina Flava	Potentiometric (ammonia)	Glutamine	(Guo et al. 2014)
Lactobacillus arabinosus	Potentiometric (hydrogen ion)	Nicotinic acid	(Šakinytė et al. 2021)
Nitrosomonas sp	Amperometric (oxygen)	Ammonia	(Raud et al. 2013)
Proteus morganii	Potentiometric (Hydrogen sulfide)	Cysteine	(Hassan et al. 2007)
Lactobacillus fermenti	Amperometric (mediated)	Thiamine	(Shimomura-Shimizu and Karube 2009)
Various organisms	Amperometric (oxygen)	BOD	(Verma and Rani 2021)

6.2 Heavy metals

Mining and many other industrial operations increases the number of heavy metals in the environment. Heavy metals are non-biodegradable and build up over time in the environment (De Benedetto et al.

waste. Common water-based drugs are beta-blockers, antihypertensive drugs, anti-inflammatory drugs, analgesics, psychiatric drugs and antibiotics (Qian et al. 2021). Therefore, it is very important to identify these compounds to prevent humans and other organisms from their threatening effects.

Enzyme-based biosensors include tyrosinases, lactases, and peroxidases and are very important biosensors for the detection of drugs (Campaña et al. 2019). HPR- based biosensors can be used to detect Levetiracetam, which is an epileptic drug. Another example is the enzyme lactase, which is used to detect methyl dopa, an antihypertensive drug (Sridharan et al. 2022).

cresol were detected using a polyphenol oxidase-based biosensor with detection limits of 0.96, 1.5, 2.03 and 1.38 AM-1 / cm² (Forzato et al. 2020). On customized magnetic nanoparticles, a phenol biosensor with tyrosinase immobilization was developed, which can sense phenols in concentrations of 7-10M. Horseradish polyphenol

Table 2: Heavy metals with their detection limit, transducer and biorecognition elements

Heavy metals	Detection limit	Transducer	Biorecognition element	References
Chromium (VI)	0.2mg/L	Amperometric	Cytochrome C ₃	(Zhang and Li 2021)
Cadmium (II) Copper (II)	10 µM	Optical	Urease	(Tagad et al. 2016)
Copper (II) Cadmium (II) Zinc (II) Mercury (II)	10 ⁻¹⁵ M	Potentiometric	Metallothionein SmA (Escherichia coli)	(Patel et al. 2020)
Mercury (II)	0.05 µM	Potentiometric	Urease	(Gumpu et al. 2017)
Mercury (II)	5 × 10 ⁻⁹ M	Luminescence	Escherichia coli S30 extract	(Upadhyay and Verma 2015)
Lead (II) Copper (II) Cadmium (II)	0.01-10mM	Conductometric	Urease	(Ilangovan et al. 2006)
Copper (II) Nickel (II) Cadmium (II) Cobalt (II)	0.2 µM 3.3 µM 2.4 µM 2.1 µM	Amperometric	Glucose oxidase	(Patel et al. 2020)
Mercury (II)	7.4 × 10 ⁻⁶ M	Amperometric	Urease	(Kuralay et al. 2007)
Mercury (II)	0.49 µg/L	Amperometric	Glucose oxidase	(Ayenimo and Adeloju 2016)
Cadmium (II) Mercury (II) Copper (II) Silver (II)	5 µM 2.5 µM 5 µM 0.05 µM	Amperometric	Glucose oxidase	(Saenchoopa et al. 2021)

6.4 Phenolic compounds

In the mining, paints, plastics, and pharmaceutical industries, phenols and their derivatives are extensively used. They are major water pollutants due to their toxicity. Different types of biosensors have been developed to detect the presence of phenols (Hashim et al. 2021). Many biosensors are dependent on enzyme tyrosinase. Tyrosinase is a copper-containing enzyme that catalyzes the simultaneous oxidation of phenols to catechol and then to quinone in the absence of O₂. Phenol biosensor with tyrosinase immobilization was developed on customized magnetic nanoparticles, which can sense phenols in concentrations of 7-10M (Ge et al. 2020). Horseradish polyphenol oxidase, peroxidase and tyrosinase have been used to make phenol biosensors. Phenol, m-cresol, catechol and p-

oxidase, peroxidase and tyrosinase have been used to make phenol biosensors (Wee et al. 2019). Phenol, m-cresol, catechol and p-cresol, were detected using a polyphenol oxidase-based biosensor with detection limits of 0.96, 1.5, 2.03 and 1.38 AM-1 / cm². Non-standard biosensors for the detection of phenol and its derivatives were designed using modified horseradish peroxidase electrodes with detection limits of 0.22 M, 0.93 mm and 0.5 mm (Antunes et al. 2018).

6.5 Surfactant

Surfactants are the active component of cleaner products and represent a large range of organic contaminants. They are resistant to biodegradation in the presence of branched hydrocarbons. The anionic surfactants are preferred for use as compared to cationic surfactants (Ragavan et al. 2018). Surfactants

are made up of sulfosuccinates, alkane sulfonates, sodium dodecyl Sulphate, methyl ester sulfonates and foronate. Using strains of *Achromobacter* and *Pseudomonas* microbial-based biosensors have been produced that can decompose anionic surfactants. The detection was made by measuring a fall in dissolved oxygen concentration caused by SDS-activated cellular respiration (Nguyen and Jang 2021). The designed biosensor has a detection limit of less than 1 μM of SDS. Amperometry biosensors for the detection of SDS and foronate were also built utilizing *Pseudomonas rathonis* T. Detergents are detected using microorganisms with a detection limit of 0.25 to 0.75 mg / L (Wang et al. 2018).

6.6 Pathogenic microorganisms

Bacteria, protozoa and viruses are pathogenic microorganisms that pose a major health risk and they should be removed from drinking and polluted water. The detection of these microbes is not just vital for environmental reasons, but also important for food quality control and clinical diagnosis (Wang et al. 2022). Biosensors have played an immense role in the detection of traditional pathogen identification. Immune sensors have been developed to detect pathogens in various biological samples using phenomena of surface plasmon resonance, fluorescence, impedance and crystal oscillator microbalance (Zhao et al. 2018). The detection of DNA is more specific than sensitive immune sensors. Aptamers are a form of synthetic oligonucleotide that has been utilized in pathogen detection biosensors and they bind to specific target molecules with higher sensitivity (Yi et al. 2020). Biosensors are based on paraffin-like properties that are unique to mycobacteria. Recently, potentiometric titration biosensors have been developed to sense a single CFU / ml *Staphylococcus aureus* (Ali et al. 2021).

6.7 Biochemical oxygen demand

The biochemical oxygen demand (BOD) is an important and commonly used measure for determining the extent of organic water pollution. The amount of oxygen required by aerobic bacteria to break down the organic matter in wastewater is referred to as BOD (Verma and Rani 2021). The traditional method for calculating BOD is a five-day procedure that requires knowledge and experience. Biosensors

provide an alternate way for estimating BOD, with the advantage of being a quick and accurate method. Biochemical oxygen demand biosensors are mainly composed of biological membranes and oxygen electrodes, that can measure the respiratory action of cells (Sonawane et al. 2020). Firstly, microbial biosensors for estimating BOD were developed by Calve in 1977. Later, several biosensors with improved properties were developed. Yeasts, especially those that belong to the genus *Debaryomyces* are the most preferred biomaterials to develop BOD biosensors due to their resistance to harmful environmental factors. To prevent the drawbacks associated with microbial membrane biosensors like low stability, new biosensors have been developed by using immobilized microbial cell (IMC) beads freely suspended in an aqueous solution (Xiao et al. 2020). Cellular biosensors have also been developed with reaction times of 10-15 minutes to monitor BOD levels in factory sewage samples that process concentrated rubber latex. Amperometry biosensors have been developed for quick and easy estimation. Unlike live cells, heat-sterilized microbial culture complexes can also be used to develop BOD biosensors, improving sensitivity, stability, and reproducibility (Cui et al. 2019).

7. CONCLUSION

Biosensors have various applications in the detection of different types of environmental pollutants. The development of sensitive, specific, and cost-effective biosensors is essential to detect small molecules. Biosensors have multiple types which are used in environmental monitoring. Biosensors are involved in the detection of pesticides, heavy metals, surfactants, phenolic compounds, BOD and pathogenic organisms at very low concentrations. Biosensors are less time consuming with efficient results. Biosensing is an eco-friendly technique than other traditional pollutants methods. In future, this technique will get more attention from researchers due to its beneficial advantages.

8. ACKNOWLEDGEMENT

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9. CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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