Enzymatic Biosensors: Advanced Tools for Soil and Plant Health Monitoring

Abstract

Soil health is the potential of the soil to support the productivity of organisms, preserve the environment, and improve the health of plants and animals. Healthy soil is vital for agricultural sustainability and environmental resilience. The presence of high concentrations of toxic chemicals (pollutants and contaminants) in the soil poses a significant risk to human health and the ecosystem. Plant health is influenced by various factors, including the surrounding environment. To minimize constraints such as low nutrient levels, ecosystem contamination/pollution, and pest and disease incidences in crops, and to maximize productivity and ensure agricultural sustainability, advanced detection and management strategies are urgently needed. Diagnosis, prediction, and monitoring are essential for managing agricultural practices to mitigate these harmful impacts. The development of improved biosensing devices has been driven by the demand for quick, accurate, sensitive, real-time, and fast instruments for screening and identifying contaminants. Specific enzymes are used as the biological sensing element in an enzyme-based biosensor, which is combined with a transducer to transform the signal from the enzymatic reaction into a quantifiable response proportionate to the analyte's concentration.Enzymatic biosensors need to possess high specificity, selectivity, reproducibility, stability, a low limit of detection, a low limit of quantification, and a rapid response time. They have a diverse array of uses, such as soil quality monitoring, water monitoring, food quality monitoring, pathogen detection, drug discovery, disease identification, and environmental research.

Keywords: soil health, enzymatic biosensor, plant health, environment, sustainability

1.Introduction

Soil health is described as the soil's capacity to support humans, animals, and plants as an essential living ecosystem. Intensive agriculture, practiced to meet the food demands of an ever-growing population, has a detrimental impact on soil health. Soil pollution refers to the occurrence of harmful substances (also known as pollutants or contaminants) in the soil at levels that are hazardous to the environment or human health.. Even when naturally occurring soil contaminants are not present at harmful levels, soil pollution is still considered to occur if these contaminants exceed their natural levels. Soil pollution is generally categorized into three types based on the source: pollution from solid waste and industrial effluents, pollution from agricultural soil, and pollution from urban areas. The primary sources of soil pollution in agricultural areas include herbicides, insecticides, fungicides, fertilizers, and heavy metals [22].

Pesticides are substances (or mixtures of substances), that are applied to eradicate or impede the growth of pests. Still, 'pesticide drift,' or the unintentional release of pesticides into the environment, raises various environmental concerns, including water and soil pollution [27]. Fertilization can influence the accumulation of heavy metals in the soil and plant systems. As plants absorb fertilizers from the soil, these substances can enter the food chain, leading to pollution of water, soil, and air. The significant use of chemical fertilizers and pesticides has been one of the primary methods of achieving crop productivity.

Heavy metals, particularly Arsenic (Ar), Mercury (Hg), Copper (Cu), Nickel (Ni), Cadmium (Cd), Zinc (Zn), Chromium (Cr), and Lead (Pb), contribute to heavy metal pollution in soil. The uptake of these metals by plants and their subsequent accumulation in the food chain poses potential risks to animal and human health [12]. Agriculture, which uses 70 percent of the world's water, significantly contributes to water pollution. Farms dump large volumes of sediments, organic debris, leftover pharmaceuticals, and agrochemicals into waterways. Nitrate from agricultural activities is now the most prevalent chemical contaminant in groundwater aquifers worldwide.

Plant health is influenced by a variety of factors, including the surrounding environment and the degree of protection from pests and diseases. Significant food losses have resulted from crop diseases caused by pathogens like bacteria, viruses, and fungus, which have been a constant problem in agriculture across the globe. To minimize yield constraints like low nutrient levels, ecosystem contamination and pollution, reducing crop disease and pest incidence, as well as to optimise yield and provide agricultural sustainability, effective detection and management techniques are required [30].

Diagnosing, predicting, and monitoring are critical for managing agricultural practices aimed at mitigating these challenges. Traditional chromatographic and spectroscopic methods are typically employed to detect constraints in soil and water ecosystems, known for their high sensitivity and selectivity. These techniques, however,

are time-consuming, labour-intensive, necessitate several sample preparation procedures, involve hazardous chemicals, and call for trained operators. The development of improved biosensing devices has been driven by the demand for fast, precise, sensitive, accurate, and real-time sensors to screen and detect contaminants. When tracking the present state of soil and water samples to detect contaminants including pesticides, potentially hazardous substances, pathogens, toxins, and endocrine-disrupting chemicals, biosensssors play a critical role [10].

2.Biosensor and their components

An analytical tool known as a biosensor is one that uses immobilised biological material with the ability to selectively interact with an analyte to produce an output that can be measured, analysed, or produced chemically, electrically, or physically. It is an integrated, self-contained tools made to provide particular analytical data, either semi-quantitative or qualitative. The system makes use of a transduction element in close spatial proximity to a biological recognition element. The output signals are directly correlated with the analyte concentration in the reaction [15].

A biosensor comprises several essential components: the analyte, biochemical receptor, transducer, electronic device, and display [16]. Biochemical Receptor: This element works with the analyte to transform chemical modifications into an electrical signal. The physical element known as the transducer is responsible for amplifying the biochemical signal that the receptor detects, converting it into an electrical signal, and displaying it in a format that can be used. Electronic Circuit: This related component consists of a display unit, a CPU or micro-controller, and a signal conditioning device.

The principles underlying biosensors involve both signal transduction and biorecognition of an element. Various biological materials such as enzymes, antibodies, nucleic acids, hormones, organelles, or whole cells can serve as sensors or detectors within these devices. Typically, a specific deactivated enzyme acts as the desired bio-receptor. This enzyme is positioned near the transducer. When the analyte being tested binds to the specific enzyme (bio-receptor), it induces a change in the enzyme's biochemical properties. This change is then translated into an electronic response through an electroenzymatic process. Electroenzymatic processes involve converting enzymatic changes into corresponding electrical signals with the aid of a transducer. The electrical signal generated directly reflects the biological components being measured (i.e., the analyte and enzyme in this case). Finally, this electrical signal is usually converted into a physical display for proper analysis and interpretation [10].

3. Enzymatic Biosensors: These biosensors utilize enzymes as biological sensing elements. Enzymes are complex macromolecules that act as biological catalysts. When an analyte interacts with the enzyme, it triggers an enzymatic reaction. The resultant signal is then transformed by a transducer into a measured response that is proportionate to the concentration of the analyte. This signal can manifest in various forms such as thermal release, proton concentration, electric current, temperature change, light absorption, or other measurable parameters [3]. Enzymatic biosensors are highly valued for their specificity, selectivity, broad analyte detection range, flexibility in application, and the purity of available enzymes.

The characteristics of enzymatic biosensors, as outlined by Gavirlas*et al* [10], include:The terms "sensitivity" and "selectivity" describe how sensitive a biosensor is to an analyte concentration in units. Selectivity refers to the bioreceptor's capacity to identify a particular analyte even in complicated samples, which improves the biosensor's analytical capacity over a range of samples. Stability: The stability of a biosensor is essential to its long-term and continuous operation. Precision and Accuracy: When measuring the same sample more than once, precision is represented by the standard deviation of the biosensor responses, whereas accuracy is determined by comparing computed values against actual values (% recovery). Reproducibility: This quantifies, in terms of relative standard deviation, the consistency of biosensor responses over multiple experimental configurations. The link between the analyte concentration and the biosensor's output signal is known as linearity. Response Time: Usually expressed as the amount of time needed for the device to reach 95% of its ultimate response, this term describes the amount of time it takes the biosensor to register a specific change in input signal. Limits of Quantification (LOQ) and Detection (LOD): The lowest concentration of analyte that the biosensor can accurately detect and measure is represented by LOD and LOQ, which also characterise the biosensor's sensitivity. Analyte concentrations as low as ng/mL or pg/mL can be detected by biosensors, indicating their capacity to identify traces of analyte [17].

Extremozyme

Extremozymes are a unique class of enzymes derived from extremophiles, microorganisms that thrive in harsh environments such as high salinity conditions, acidic lakes, cold regions, and deep-sea hydrothermal vents. These enzymes are highly adapted to function efficiently under extreme environmental stressors, demonstrating exceptional stability and activity in conditions that would denature regular enzymes. Due to their remarkable properties, extremozymes hold significant potential in various applications, particularly in the design of biosensors and analytical techniques for environmental monitoring.

Extremozymes like laccases, tyrosinases, alkaline phosphatases, and aldehyde dehydrogenases exhibit a wide range of catalytic capabilities, making them suitable for detecting pollutants in challenging environments. For example, laccases and tyrosinases can oxidize a variety of organic and inorganic compounds, while alkaline phosphatases and aldehyde dehydrogenases catalyze reactions that can be utilized for monitoring heavy metals and other contaminants. These extremozymes' resilience to temperature, pH, and other environmental factors makes them ideal for biosensors, which need to perform consistently in fluctuating conditions.The integration of extremozymes into biosensor platforms offers improved stability and efficiency for the detection of pollutants in water, soil, and air, enhancing the reliability of these systems in real-world applications [35].

3.1 Enzymes active at low temperature

Microorganisms that thrive in cold-adapted environments include bacteria, fungi, and actinomycetes, as well as micro-eukaryotes such as archaea. These cold-adapted organisms are capable of growing optimally at temperatures around 15 °C, with a maximum growth temperature typically not exceeding 20 °C. They produce cold-active enzymes that function efficiently at low temperatures, which are crucial for their survival and metabolic processes. Examples of these cold-active enzymes include:

- **Cellulase**: Breaks down cellulose into simpler sugars.
- **Protease**: Hydrolyzes proteins into peptides and amino acids.
- **Pectinase**: Degrades pectin, a component of plant cell walls.

3.2 Enzymes active at high temperature

Extreme habitats like hot springs, hydrothermal vents, and geothermal conditions are inhabited by organisms that thrive in high-temperature environments. These organisms produce thermostable enzymes, which retain their functionality and stability under extreme heat. Examples of such enzymes include:

- **Amylase**: Breaks down starches into sugars, maintaining activity at high temperatures.
- **Laccase**: Catalyzes oxidation reactions, particularly in the degradation of lignin and phenolic compounds.
- **Xylanase**: Degrades xylan, a component of plant cell walls, into simpler sugars.
- **Cellulases**: Hydrolyze cellulose into glucose, crucial for the breakdown of plant material.

3.3 Enzymes adapted to saline condition

Enzymes and proteins adapted to high salinity environments offer significant practical advantages in biotechnology, particularly in biosensing applications where traditional enzymes may rapidly lose activity due to high salt concentrations. These salt-tolerant enzymes maintain their functionality and stability in saline conditions, enhancing the effectiveness of various biotechnological processes. Examples include:

- **Superoxide dismutase**: Catalyzes the conversion of superoxide radicals into oxygen and hydrogen peroxide, protecting cells from oxidative damage even in high-salt environments.
- **ACC deaminase**: Breaks down 1-aminocyclopropane-1-carboxylate (ACC), a precursor of ethylene, influencing plant stress responses and growth, and remains effective in saline conditions.
- **Proteases**: Enzymes that hydrolyze proteins into peptides and amino acids, and can function optimally in high-salt environments.

3.4 Enzymes active in acidic condition

Acid-stable enzymes are highly valuable in industrial and environmental applications due to their resilience in low pH conditions. Their stability in acidic environments is attributed to their ability to manage electrostatic interactions on their surface. By reducing the density of positive and negative charges, these enzymes minimize electrostatic repulsion among charged groups, which can otherwise destabilize protein structures in acidic conditions. This adaptation enables them to maintain their activity and functionality in harsh, acidic environments, providing insights into their resilience and making them ideal for applications where low pH poses a significant challenge.

4. Enzyme Immobilization Techniques in Enzymatic Biosensors

Enzyme immobilization is a critical step in biosensor design, offering advantages such as repeated use over time, easier enzyme recovery, increased stability, and easier separation of products from enzymes.During immobilization, enzymes undergo physical and chemical changes that must be carefully considered. Factors such as enzyme stability, catalytic activity loss due to conformational changes, and the strength of enzyme attachment to the solid support are critical in each immobilization method. Various approaches have been investigated for developing biosensors based on immobilized enzymes.

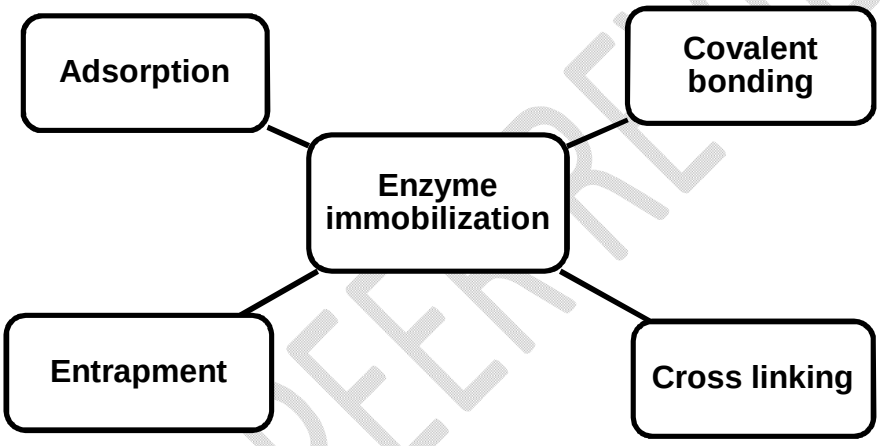

Fig .1 Enzyme Immobilization Techniques

4.1. Adsorption: This technique relies on low-energy interactions between the bioreceptor and the substrate. Weak forces such as hydrogen bonds, van der Waals forces, and salt linkages facilitate enzyme attachment. Although these interactions are reversible and may cause molecules to detach from the matrix, they have minimal impact on enzyme performance, making adsorption a simple and widely used method for biosensor construction.

4.2. Entrapment: In this method, biomolecules are trapped within a polymerized matrix. The entrapment approach provides control over the thickness of the polymer layer, allowing for optimization based on the required sensitivity and performance of the biosensor. Electrical load measurements are often used to regulate this process, ensuring that biomaterials remain embedded within the polymer network.

4.3. Covalent Bonding: This technique involves immobilizing enzyme functional groups directly onto a support matrix through covalent bonds. Covalent bonding offers robust attachment, preventing the enzyme from detaching from the matrix. This method is advantageous when long-term stability and enzyme retention are crucial, particularly in harsh environmental conditions.

4.4. Crosslinking: Crosslinking creates a network of interconnected enzyme molecules, forming a rigid structure that enhances the biosensor's stability. By crosslinking enzymes together, this method increases resistance to

environmental stressors and prolongs the functional lifespan of the biosensor, making it ideal for applications requiring high stability and durability.

Each immobilization technique offers specific advantages and challenges, influencing the overall performance and durability of enzymatic biosensors [25].

5. Advancements in Soil Nitrate and Phosphate Detection

Nitrate (NO^{3-})is an essential source of nitrogen (N) for plant growth, its high solubility in soil solutions may also affect the quality of air and water. To optimize nitrogen fertilizer use, farmers now rely on soil NO3- readings. The objective is to avoid both excessive inputs that negatively impact profitability and the environment, as well as inadequate inputs that limit crop output. However, because these procedures rely for physical soil sampling in the field followed by laboratory analysis, they frequently produce erroneous results and cause delays of five to ten days before data is available. The creation of quick and accurate techniques for measuring soil NO^{3-} could improve fertiliser management, resulting in higher profits and better environmental circumstances. Thus, the development of sensitive, small, and reasonably priced nitrate sensors is imperative[1].

In another study, Kahveci *et al* [17] developed a fluorescent biosensor to detect phosphate ions. This biosensor utilizes ALP (Alkaline phosphatase), where phosphate ion acts as a competitive inhibitor. Hydrolysis of PNPP (p-nitrophenol phosphate) by ALP produces PNP (p-nitrophenol), which quenches the fluorescence of NPs, resulting in fluorescence turn-off. The presence of phosphate ions inhibits ALP activity, preserving the fluorescence of the system. The biosensor exhibited a linear response up to 5 mM of phosphate ion concentration, with a limit of detection (LOD) of 0.1 mM, demonstrating its capability to detect phosphate inhibitors effectively.

6. Advancements in Heavy Metals Detection Techniques

Heavy metals can alter enzyme-substrate complexes, thereby influencing the outcome of biochemical reactions. However, the effectiveness of enzyme-based biosensors over the long term can be significantly impaired by potent inhibitors. Inhibitory interactions with enzymes can be reversible, characterized by rapid association and dissociation rates through non-covalent interactions, or irreversible, leading to permanent enzyme inactivation. Some heavy metal ions can integrate into enzyme structures and act as cofactors, thereby enhancing enzyme functions. For instance, metalloproteins activate enzymes by serving as essential cofactors. Conversely, irreversible inhibitors bind to enzymes in a manner that prevents the formation of enzyme-substrate complexes. In enzyme biosensors, inhibition of enzyme activity occurs when metal ions interfere with thiol or methylthiol groups present in the active sites of enzymes [39].

Zeng *et al* [42] presented a novel inhibition-based glucose oxidase (GOx) biosensor aimed at detecting chromium (VI) in environmental samples. The sensor consists of an electropolymerized aniline membrane with ferrocene acting as an electron transfer mediator, all assembled on a platinum electrode. Glucose oxidase is cross-linked to the membrane through the use of glutaraldehyde.The presence of chromium (VI) inhibits the enzyme's activity, causing a weaker response to glucose. This inhibition allows the sensor to detect lower concentrations of chromium, reducing the detection limit to 0.49 mg L^{-1}. By taking advantage of the inhibitory effect of chromium (VI) on GOx, this biosensor provides a sensitive and effective tool for monitoring chromium contamination in environmental samples, offering a novel approach to pollutant detection in complex environments.

A multilayer paper-based instrument with colorimetric detection for Ni, Fe, Cu, and Cr as well as electrochemical detection with a focus on Pb and Cd was presented by Rattanarat*et al* [28]. The colorimetric layer's sensitivity in visual detection methods was highlighted as it displayed its lowest detection limit for Cr at 0.12 µg. The electrochemical layer, on the other hand, demonstrated excellent sensitivity and was suitable for accurate electrochemical analysis in environmental monitoring scenarios, with detection limits as low as 0.25 ng for Pb and Cd.

Nie *et al* [26] developed a paper-based electrochemical biosensor for the detection of Pb (II) ions, capitalizing on the inherent advantages of paper as a substrate. Paper platforms offer benefits such as flexibility, low cost, disposability, and the ability to transport fluids via capillary action without the need for external power sources. In their design, the biosensor integrated an electrochemical detection system with a paper substrate, allowing for portable, user-friendly operation. The biosensor was functionalized with gold nanoparticles, enhancing sensitivity toward Pb (II) ions. This sensor demonstrated effective lead ion detection in aqueous solutions, showcasing the potential for field-deployable applications in environmental monitoring. The paper-based approach highlighted the

possibility of creating inexpensive, portable sensing devices for real-world use, addressing the need for on-site, rapid testing of heavy metal contamination in water and potentially contributing to broader environmental health and safety monitoring efforts.

Alkaline phosphatase conductometric biosensors were built utilising interdigitated gold electrodes and enzyme membranes in a work by Berezhetskyy*et al* [4]. These biosensors were created especially to measure the levels of heavy metal ions in water, which limit the action of certain enzymes. The immobilised phosphatase's residual activity was assessed in Tris-nitrate buffer with Mg^{2+} ions acting as activators, but without metal pre-incubation. The immobilised phosphatase was shown to be more hazardous to several tested metals in the following order: Cd^{2+}> Co^{2+}> Zn^{2+}> Ni^{2+}> Pb^{2+}. The research revealed that the biosensor's sensitivity to various heavy-metal ions was roughly 0.5 ppm for Cd^{2+}, 2 ppm for Zn^{2+} and Co^{2+}, 5 ppm for Ni^{2+}, and 40 ppm for lead ions.

Ilangovan *et al* [13] designed a conductometric biosensor using a thick film interdigitated electrode and a sol-gel-immobilized-urease enzyme for the detection of heavy metal ions. This biosensor operates by measuring the variation in urea concentration, which directly correlates with enzyme activity. The immobilization of urease in the sol-gel matrix enhanced the sensor's stability and sensitivity. Between 1 mM and 15 mM, the biosensor exhibited a strong and linear response to changes in urea content, indicating its reliable performance in detecting urea concentration variations.

Table 1 Enzyme-Based Heavy Metal Detection Biosensors

Enzymes	Heavy Metals	Lowest detectable concentration	Device
Alkaline phosphatase	Zn	10 µM	Calorimeter
L-glycerolphosphate Oxidase	Hg	20 µM	Amperometerwithclark electrode
Pyuruvateoxidase	Hg	50 nM	Amperometerwithclark electrode
	Hg	1 µM	Amperometerwithclark electrode
	Ag	0.02 µM	
	Cu	0.5 µM	
	Zn	5.0 µM	
	Pb	0.2 µM	
Peroxidase	Hg	0.02 µM	Calorimeter
Urease	Hg	1.0 µM	Fibreoptic
	Cu	3.94 µM	
	Ag	0.18 µM	Optical
	Cd	2.67 µM	Calorimeter

7. Pesticide Residue Detection Methods

Enzymatic biosensors detect pesticides by either measuring the inhibition of enzyme activity caused by pesticides or directly monitoring the compounds produced or consumed during enzymatic reactions. These biosensors provide a sensitive and specific method for pesticide detection in environmental samples.

7.1. Cholinesterase-Based Biosensors: Asal *et al* [3] highlighted that pesticide detection using enzyme biosensors primarily revolves around cholinesterase (ChE) inhibition. Organophosphates and carbamates, common pesticide

classes, inhibit ChE activity, preventing the hydrolysis of acetylcholine. This inhibition is used as a key mechanism in biosensors to detect the presence of these pesticides. By measuring the decrease in enzymatic activity, these biosensors can sensitively quantify pesticide levels in various environmental and food samples. ChE-based biosensors are highly effective for monitoring pesticide contamination, offering rapid and selective detection, crucial for environmental protection[36].

7.2. Tyrosinase-Based Biosensors: Atrazine and carbamate insecticides are two substances that inhibit tyrosinase. It has been established that a multitude of electrochemical biosensors based on tyrosinase activity inhibition exist [7]. However, because tyrosinase is susceptible to interference from a wide range of substrates and inhibitors, poor selectivity poses a problem to tyrosinase biosensors. Furthermore, the intrinsic instability of tyrosinase limits the stability of biosensors based on the enzyme. Guan *et al* [11] reported that tyrosinase, an enzyme used in biosensors, exhibits remarkable resistance to high temperatures and organic solvents, making it highly suitable for pesticide detection in challenging conditions. Unlike other enzymes that may denature or lose activity under such conditions, tyrosinase remains stable and functional. This resistance is particularly advantageous in pesticide biosensors, where organic solvents are often required to dilute pesticide samples, and high temperatures might be encountered in field testing or industrial environments. The stability of tyrosinase allows for continuous and reliable monitoring of phenolic pesticides and related compounds, providing a robust and efficient method for environmental testing. Its resilience to harsh conditions enhances the longevity and effectiveness of biosensors, making it a preferred enzyme for applications where stability under extreme conditions is essential.

7.3. Alkaline Phosphatase (ALP)-Based Biosensors: Alkaline phosphatase (ALP) is a versatile enzyme known for its broad substrate specificity and optimal activity in alkaline pH conditions. Due to its ability to catalyze the hydrolysis of phosphate esters, ALP has been widely utilized in biosensors for detecting various pesticides and hazardous substances. ALP can be inhibited by numerous substances, which affects its enzymatic activity and provides a basis for detection in biosensor applications.

Tehri *et al* [37] highlighted the development of ALP-based biosensors employing diverse enzyme substrates to detect different types of pesticides. These biosensors are designed to identify organochlorine, organophosphorous, carbamate, and other toxic substances by monitoring changes in ALP activity. The transduction techniques used in these biosensors convert the inhibition or alteration of ALP activity into measurable signals, such as colorimetric, electrochemical, or fluorescent outputs.

Mazzei *et al* [21] developed electrochemical alkaline phosphatase (ALP)-based biosensors for detecting environmental contaminants like malathion and 2,4-dichlorophenoxyacetic acid (2,4-D). These biosensors used enzyme substrates such as ascorbate-2-phosphate, phenyl phosphate, or 3-indoxyl phosphate to generate measurable electrochemical signals upon interaction with the target analytes. Additionally, the researchers created an ALP-based biosensor for broader environmental screening. This sensor trapped ALP within a hybrid sol-gel/chitosan matrix that was applied to the electrode surface via screen printing. The hybrid coating stabilized the enzyme and enhanced its activity, allowing for effective immobilization and reliable detection of contaminants. The resulting biosensor demonstrated the potential for sensitive, selective, and rapid detection of a range of environmental pollutants, showcasing a promising tool for on-site environmental monitoring and pollution control.

7.4. Peroxidase-Based Biosensors: Hydrogen peroxide (H_2O_2) first oxidises peroxidase enzymes, and phenolic substances subsequently decrease them. Compounds I and II are the two enzyme intermediates involved in this process. The resultant oxidation of phenolic compounds produces quinones or free radical products, which can be reduced electrochemically on the electrode surface. Peroxidase enzyme activity has been observed to be inhibited by both organic and inorganic substances [32]. Mocceli*net al* [23] developed a biosensor specifically designed for the detection of the carbamate insecticide thiodicarb. This biosensor is based on the inhibition of peroxidase, an enzyme involved in catalyzing reactions that typically produce measurable signals, such as changes in current or color. When thiodicarb is present, it inhibits the activity of peroxidase, reducing the enzyme's ability to facilitate these reactions. By monitoring this inhibition, the biosensor can sensitively detect and quantify thiodicarb levels in various samples. This peroxidase inhibition approach offers a simple and effective method for pesticide detection, contributing to environmental monitoring and food safety efforts.

7.5.Acid Phosphatase-Based Biosensors:Some pesticides have the ability to reversibly inhibit the enzyme acid phosphatase (AP). A bi-enzymatic biosensor for the electrochemical detection of pesticides, including malathion, methyl parathion, and paraoxon, has been developed by combining AP with glucose oxidase (GOD). The two enzymes were connected to a commercial electrode for measuring H_2O_2.

Butmee*et al* [5] studied into the application of an enzymatic biosensor based on acid phosphatase inhibition to detect glyphosate. The enzymatic reaction between acid phosphatase and its enzyme substrate, disodium phenyl phosphate, produced the current. As glyphosate's suppression of acid phosphatase rose, it also increased the biosensing response. When glyphosate solution was added in different amounts, the proposed AP biosensor's current-time curve gradually decreased. Reduced enzymatic activity towards the substrate was observed at higher doses of the inhibitor. A reduced inhibitory effect was obtained because not all glyphosate molecules were consistently attached to the enzyme, even though most active sites were occupied by a very high quantity of the inhibitor.

7.6. Organophosphorous Hydrolase-Based Biosensors: Organophosphorous hydrolase (OPH), a well-characterized metalloenzyme sourced from *Pseudomonas diminuta*, plays a crucial role in the hydrolysis of organophosphorus compounds. This enzyme is capable of breaking down various phosphorous-containing bonds, including P-O, P-S, and P-CN bonds. During the hydrolysis process, OPH produces two protons, which can be detected using electrochemical methods. This detection is based on measuring the change in pH or proton concentration as a result of the enzymatic reaction.ChE-based biosensors, on the other hand, are known for their higher sensitivity and lower detection limits, making them more effective for detecting minute quantities of pesticides and other contaminants. Despite these challenges, OPH-based biosensors remain valuable tools for environmental monitoring and remediation, particularly for applications where cholinesterase-based sensors may not be suitable [6].

Silva *et al* [34] developed a nanobiosensor using an atomic force microscopy (AFM) tip for detecting the herbicide metsulfuron-methyl. The AFM tip was functionalized with the enzyme acetolactate synthase, sourced from yeast and bacteria. This functionalization process involved the gaseous evaporation of 3-aminopropyltriethoxysilane, followed by exposure to a glutaraldehyde solution, triethylamine, and an acetolactate synthase-enriched enzyme extract.Incorporating this enzyme onto the AFM tip made the nanobiosensor highly sensitive to metsulfuron-methyl, offering a new and precise method for detecting herbicides at the nanoscale, with promising applications in environmental monitoring and agricultural safety.

Table 2. Characteristics of Enzyme-Based Biosensors for Pesticide Residue Detection

Target analyte	Detection technique	Enzyme immobilization technique	Detection limit(M)	Reference
Cholinesterase				
Chlorpyrifos	Piezoelectric (Amperometric)	Entrapment	4×10^{-9}	Ion *et al* [14]
Paraoxon	Electrochemical (Amperometric)	Cross linking	7.3×10^{-9}	Wang *et al* [40]
Dichlorvos	Electrochemical (Amperometric)	Entrapment	7×10^{-12}	Gan *et al* [9]
Trichlorfon	Electrochemical (Potentiometric)	Adsorption	1.94×10^{-11}	
Dimethoate	Amperometric	Adsorption	2.4×10^{-6}	
Alkalinephosphatase				
Chlorpyriphos	Amperometric	Crosslinking	1.3×10^{-7}	Scongnamigilo*et al* [33]
Paraxon	Chemiluminescen Ces	Covalent bonding	3×10^{-9}	LeDoux [20]
2,4 D	Amperometric	Crosslinking		Touloupakis*et al* [38]

			4.9×10^{-10}	
Tyrosinase				
Carbaryl	Amperometric	Crosslinking	5×10^{-6}	Guan *et al* [11]
Aldicarb	Amperometric	Adsorption	5×10^{-6}	
Thiodocarb	SquareWave Voltammetry (SWV)	Entrapment	1.58×10^{-7}	
Organophosphorous hydrolase				
Paraoxon	Amperometric	Crosslinking	0.314×10^{-6}	Zamaleeva*et al* [41]
Paraoxon	Amperometric	Entrapment	15×10^{-8}	
Ethyl Parathion	Amperometric	Covalentbonding	$<3.4 \times 10^{-9}$	

8. Advancements in Crop Disease Detection

Polymerase chain reaction (PCR), immunofluorescence (IF), fluorescence in-situ hybridisation (FISH), enzyme-linked immunosorbent assay (ELISA), flow cytometry (FCM), and gas chromatography-mass spectrometry (GC-MS) for direct detection methods are some of the laboratory-based techniques that have recently made significant advances in crop disease detection. Hyperspectral techniques, fluorescence imaging, and thermography are examples of indirect methods that are also used.

Fang and Ramasawmy [8] emphasized the role of biosensors as a major advancement in the early identification of crop diseases, particularly through the use of highly selective bio-recognition elements like enzymes. Enzymatic biosensors are designed to detect specific volatile organic compounds (VOCs) produced by crops when they are infected by pathogens. These VOCs often include alcohols and aldehydes, such as cis-3-hexen-1-ol and trans-2-hexanal, which are emitted by stressed or diseased plants.

Infected plants release various volatile organic compounds (VOCs), such as methyl salicylate, monoterpenes, diterpenes, sesquiterpenes, isothiocyanates, and aromatic acids [33]. Methyl salicylate, in particular, has been identified as a marker for plant infections, including those caused by *Tetranychusurticae* in lima beans, *Fusarium* in maize, and *Phytophthora* in pepper. To selectively detect methyl salicylate, a bienzyme-based amperometric biosensor has been developed.

List 1 : Biotic Stress Causing Agents that increase VOC emissions

Volatiles	**BioticStressCausingAgentsthatincrease VOCemissions**
cis-3-hexen-1-ol	*Botrytis cinerea, Spodoptera litoralis, Lirimyzahuidobrensis, Spodoptera exigua, Manductasexta, Macrosiphum euphorbiae, Helicoverpaarmigera*
trans-2-hexanal	*Botrytiscinerea,Spodopteralitoralis,Lirimyzahuidobrensis,Manducta sexta, Helicoverpaarmigera*
Methylsalicylate	*Botrytis cinerea, Spodoptera litoralis, Tetranychusurticae, Manductasexta, Macrosiphum euphorbiae, Tobacco mosaic virus*

Biosensors designed for in-field detection of plant pathogens offer advantages such as low cost, minimal expertise requirement, rapid detection of target pathogens, high specificity, and sensitivity. For example, a nanoparticle electrochemical biosensor demonstrated greater sensitivity in detecting Pseudomonas syringae compared to conventional PCR. This biosensor could diagnose infected plants even before symptoms of the disease became apparent [19].

9. Advancements in Water Pollutant Detection

Khadro*et al* [18] reporteda nitrate biosensor that employed a methyl viologen mediator in conjunction with *Aspergillus niger* nitrate reductase (NR) and a Nafion cation-exchange polymer contained in a plasticised PVC membrane placed atop interdigitated electrodes. With an operational range of 4×10^-3 to 50 mg/L, and a linear calibration range from 4×10^-3 to 8 mg/L, this biosensor demonstrated a sensitivity of roughly 1.48 µS/L per mg and a detection limit of 1.2 µg/L. Over a two-month period, the sensor showed good stability when stored at 4°C in 20 mM phosphate buffer (pH 7.5).

Zhylyak*et al* [44] explored a urease-based conductometric biosensor for detecting heavy metal ions in water, focusing on enhancing the enzyme's stability and effectiveness. The biosensor utilizes urease, an enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide, which affects the conductivity of the solution. The presence of heavy metal ions inhibits urease activity, leading to changes in conductivity that can be measured to determine ion concentration.

10. Enzymatic Nanobiosensors in Soil and Plant Health

Zhang *et al* [43] developed advanced enzymatic nanobiosensors designed for the selective detection of both organophosphorus and non-organophosphorus pesticides. These nanobiosensors demonstrated enhanced sensitivity, making them effective for identifying very low concentrations of pesticide residues. Specifically, they were able to detect paraoxon, an organophosphorus pesticide, at concentrations as low as 0.5 µM. Similarly, they could identify carbaryl, a non-organophosphorus pesticide, at a detection limit of 1 µM. The high sensitivity of these nanobiosensors enables precise monitoring of pesticide levels, crucial for environmental safety and agricultural practices. Naresh and Lee [24] confirmed the effectiveness of these sensors in accurately detecting pesticide residues, highlighting their potential for widespread application in pest management.

Rigo *et al* [29] developed a cantilever nanobiosensor functionalized with urease enzyme using self-assembled monolayers. This nanobiosensor utilizes urease as a biocatalytic receptor to detect heavy metals. Urease interacts with heavy metals through various mechanisms, including enzyme inhibition, oxidative stress, or disruption of antioxidant metabolism. The chemical groups within the enzyme's active site are specifically responsive to heavy metal ions, leading to detectable changes in the enzyme's activity. The cantilever’s sensitivity allows for precise measurements of these interactions, providing an effective method for identifying and quantifying heavy metal contamination. This approach enhances the sensor’s ability to monitor environmental pollutants and ensure safety in various applications

Nanowires, recognized for their high surface-to-volume ratio and outstanding electrical properties, have gained growing interest as sensors for detecting plant diseases like cucumber mosaic virus (CMV) and papaya ring spot virus (PRSV). For their use as biosensors, nanowires are modified on the surface with amino group solutions, which allow the attachment of enzymes or other biological receptors.This modification allows the bioreceptors to bind effectively to the nanowire surface, creating a sensitive interface capable of capturing target viruses [2].

Table. 3. Enzymatic Nanobiosensors for Agricultural Applications

Nanomaterials	Nano-bioconjugation approach	References
Carbon nanotube	Layer-by-layer electrostatic assembly of carbon nanotubes and several enzymes on the transducer surface	Zhang*etal* [43]
Tip of atomic force microscopy	Adsorption of acetyl coenzyme physically A carboxylase on the transducer's surface	Sarkar*etal* [31]
Zirconium, carbon nanotube, and magnetic nanoparticles combined into a nanocomposite	Acetylcholinesterase covalently immobilised on transducer surface	Gan*etal* [9]

11. Applications of Extremozyme-Based Biosensors

11.1 Heavy metal detection

Toxic metals found in water, soil, sediments, and rocks are often introduced into the environment through human activities such as mining, the use of vehicles, industrial waste disposal, the dye manufacturing industry, fertilizers, and batteries. One approach to detecting arsenic (As) contamination has been the development of electrochemical biosensors specifically designed for As(III) detection. These biosensors were created by immobilizing chimaeras on gold electrodes. The immobilized chimaeras can bind both As(V) and As(III); however, only As(III) can be selectively identified. This distinction occurs because, after undergoing electrochemical reduction in acidic conditions, specifically in 1 M (HCl) solution on the surface of the gold electrodes, As(III) is readily reduced to elemental arsenic (As(0)), making it detectable by the biosensor. In contrast, As(V) does not undergo this reduction under the same conditions, allowing for the selective identification of As(III). This method takes advantage of the distinct electrochemical behavior of arsenic in different oxidation states, facilitating accurate detection in environments impacted by toxic metals, which pose a significant risk to environmental and human health due to their persistence and potential for bioaccumulation.

11.2 Pesticide detection

Enzyme-based biosensors have emerged as alternative analytical tools to traditional chromatographic methods for pesticide detection, alongside antibody, aptamer, and molecularly imprinted polymer (MIP)-based biosensors. However, fewer approaches leverage the catalytic action of enzymes that degrade organophosphates, such as glutathione transferase, phosphotriesterase, and organophosphorus hydrolase. Examples of pesticide detection biosensors include: (i) an electrochemical sensor employing MoS_2 nanosheets, gold nanoparticles (AuNPs), and acetylcholinesterase, which achieved a limit of detection (LOD) of 3.5×10^{-11} M for paraoxon; (ii) a MIP sensor for detecting fenamiphos, utilizing a core-shell Co3O4@MOF-74 nanocomposite, with an LOD of 3.0×10^{-12} M, 60 days of stability, and reusability up to 50 times; (iii) an aptasensor designed for malathion detection, featuring a cationic polymer and AuNPs, with an LOD of 6×10^{-14} M; and (iv) an electrochemical sensor that integrates manganese dioxide nanosheets as a nanozyme, achieving an LOD of 6.6×10^{-10} M for paraoxon.

11. Conclusion

Enzymatic biosensors are a pivotal advancement in analytical technology, utilizing enzymes as biorecognition elements to detect a wide array of target molecules. Their core strength lies in the high specificity of enzymes

towards particular substrates, which allows for selective detection in complex environments. These biosensors function by converting the enzymatic reaction of the target analyte into a measurable signal, such as electrical, optical, or thermal changes. This process ensures both sensitivity and precision, making them highly effective for applications in diverse fields such as environmental monitoring, medical diagnostics, food safety, and agricultural assessment.

Despite their numerous advantages, enzymatic biosensors also face challenges, such as enzyme stability and potential interference from other substances. However, ongoing research in enzyme immobilization techniques and nanomaterial integration is continually addressing these limitations. There is a significant demand for specific, rapid, highly sensitive, low-cost, portable, and home-use compatible sensors that offer reliable and efficient performance. Therefore, combined efforts are needed to develop a validated next generation of advanced biosensors that meet market requirements. In conclusion, enzymatic biosensors offer a promising and versatile platform for precise detection and monitoring, with continued advancements paving the way for broader applications and improved efficiency in critical areas of health, environmental protection, and industry.

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