Journal of Engineering, Science and Technological Trends (JESTT)

Vol. 3, Issue 2, July 2026
DOI: https://doi.org/10.64060/JESTT3i25

Review

Role of Pectinases and Carbohydrate-Degrading Enzymes in Biosensing Applications

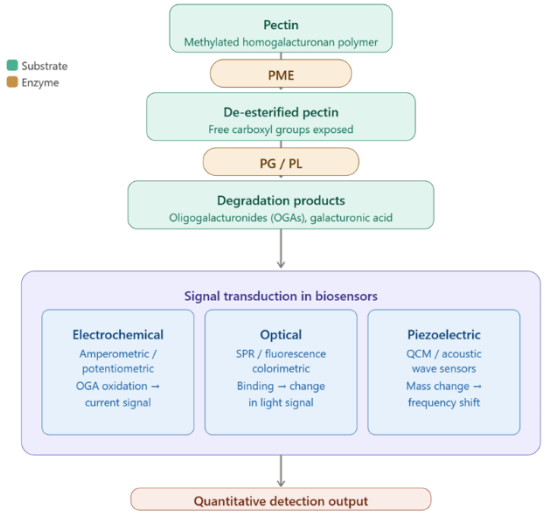
Rahmat Ullah[1*]

[1]Department of Biochemistry, Government College University Faisalabad, Pakistan
* Corresponding Email: rahmat6034125@gmail.com (R. Ullah)

Received: 23 April 2026 / Revised: 19 June 2026 / Accepted: 07 July 2026 / Published online: 10 July 2026

ABSTRACT

Enzyme-based biosensors have emerged as powerful analytical platforms for transforming specific biochemical interactions into measurable signals, enabling rapid, sensitive, and selective monitoring across healthcare, food safety, environmental analysis, and industrial biotechnology. However, current enzyme biosensing research has predominantly focused on oxidoreductases, while carbohydrate-active enzymes (CAZymes) remain comparatively underexplored despite their exceptional substrate specificity and catalytic diversity. This review provides a comprehensive overview of carbohydrate-degrading enzyme-based biosensors, highlighting their fundamental mechanisms, sensing strategies, and future technological opportunities. Particular emphasis is placed on pectinases, including pectin methylesterase, polygalacturonase, and pectin lyase, which offer unique capabilities for detecting pectin degradation, plant physiological changes, food quality deterioration, and environmental processes. The catalytic principles of these enzymes, their production approaches, and their integration with electrochemical, optical, piezoelectric, and colourimetric transduction systems are systematically discussed. Furthermore, advanced immobilisation approaches involving nanomaterials, microfluidic architectures, and hybrid enzyme–nanozyme systems are examined to address challenges associated with enzyme instability, sensitivity, matrix interference, and reproducibility. Emerging concepts including artificial intelligence-assisted biosensing, wearable sensing platforms, multiplex detection, and sustainable biodegradable sensor designs are also explored as pathways toward next-generation intelligent biosensors. Despite significant progress, practical translation remains limited by substrate variability, large-scale manufacturing challenges, and insufficient standardisation. Future advances in enzyme engineering, smart data analysis, and integrated sensing technologies are expected to establish robust, portable, and environmentally sustainable biosensing platforms. This review provides critical insights into the development of advanced CAZyme-based biosensors and their potential role in shaping future intelligent analytical systems.



Keywords: Biosensing; Carbohydrate-Active Enzymes; Enzyme Engineering; Nanomaterials; Pectinase

1. Introduction

The recent advancements in sensors depend greatly on effective interaction between humans and machines, with biosensors being very important in this situation as they are used in transforming biochemical information into statistical data related to biological health, environment and food [1],[2]. However, the use of pectinase and other carbohydrate-degrading enzymes in practical application continues to face problems like insufficient enzyme stability, insufficient sensitivity, loss through

the matrix, and repeatability in actual samples [3],[4]. Therefore, it is important to design efficient biosensors that take into account pectin and other carbohydrate-sensitive enzymes [5],[6]. Biosensors refer to devices that utilise biological reactions to generate an electric signal [7]. These reactions are normally brought about by the use of bioreceptors that are mainly enzymes, antibodies, or nucleic acids together with transducers [8],[9]. Biosensors have a wide range of applications in different fields such as food safety, health care, and the environment, all owing to their specificities and sensitivities [10]. Common examples of biosensors include biosensors for blood glucose monitoring and paper-based biosensors that are inexpensive and can be used portably to detect biomarkers in food, medical samples, and environmental samples [11]. Biosensors are generally constructed using biological components such as antibodies, DNA/RNA, enzymes, and receptors combined with supportive matrices including hydrogels, polymers, nanoparticles, and conductive surfaces to provide a medium for signal generation and stability [12],[13]. In addition, other materials such as biopolymers (e.g., pectin), carbon nanotubes, conducting polymers, graphene, and metallic nanoparticles are often used in immobilising matrices and transducers of biosensors [14],[15]. In order to achieve sustainability, enzyme biosensors are designed from biodegradable and renewable sources in order to mitigate any harm to the environment without compromising the performance of these biosensors, which is characterised by high sensitivity and selectivity in detecting the target analyte [16],[17]. In this regard, enzymes play a significant role in acting as specific catalysts in biosensors and thus the ability to detect analytes such as glucose and contaminants as well as markers in biological studies [18]. The use of environmentally friendly materials such as cellulose and other biopolymers facilitates the design of cost-effective, disposable, and environmentally sustainable biosensors [19],[20].

The first biosensor was introduced in 1962 by Leland C. Clark Jr. and Champ Lyons, who invented an amperometric oxygen electrode based on the enzyme glucose oxidase for measuring glucose concentrations in the blood [21]. This development became the basis of contemporary glucose monitoring and opened the way for developing enzyme biosensors. In 1977, the term "biosensor" emerged when researchers invented the selective arginine electrode using biological components as recognition elements [22]. The first enzyme biosensor functioned by immobilising enzyme glucose oxidase at an electrode [23]. As glucose was being transformed in the presence of glucose oxidase, hydrogen peroxide appeared, whose amount proportionally depended on glucose concentration [24]. A simple electrochemical measurement system helped make biosensors very convenient in medical diagnostics, including monitoring diabetes [25]. Nowadays, glucose biosensors are still one of the main types of enzyme biosensors, and the two enzymes used for glucose determination are glucose oxidase (GOx) and glucose dehydrogenase (GDH) [26]. Recently, there has been significant interest in developing green and sustainable biosensors [19]. Designing sustainable biosensors involves biodegradable and environmentally friendly materials such as paper, cellulose, bioplastics and hydrogels [27]. The use of such materials helps not only decrease the waste but also recycle biosensors [28]. Life cycle assessment can be applied to evaluate the sustainability that consists of the use of raw materials, energy, and other parameters of biosensor life cycles, including their durability and possibility of recycling [22],[29]. There are many examples of biosensors based on using recent enzymes in order to improve performance and detection characteristics [30]. Examples of enzymes used in biosensors include horseradish peroxidase, lactate dehydrogenase, phenylalanine hydroxylase, and enzymes for determining uric acid and genetic diseases [7]. Biosensors with such enzymes are characterised by higher sensitivity, specificity, and detection limit in clinical, environmental applications and others [22],[31]. Nowadays, biosensors attract scientists representing various scientific areas because of multiple applications in medicine, environmental monitoring, the food industry, and public health

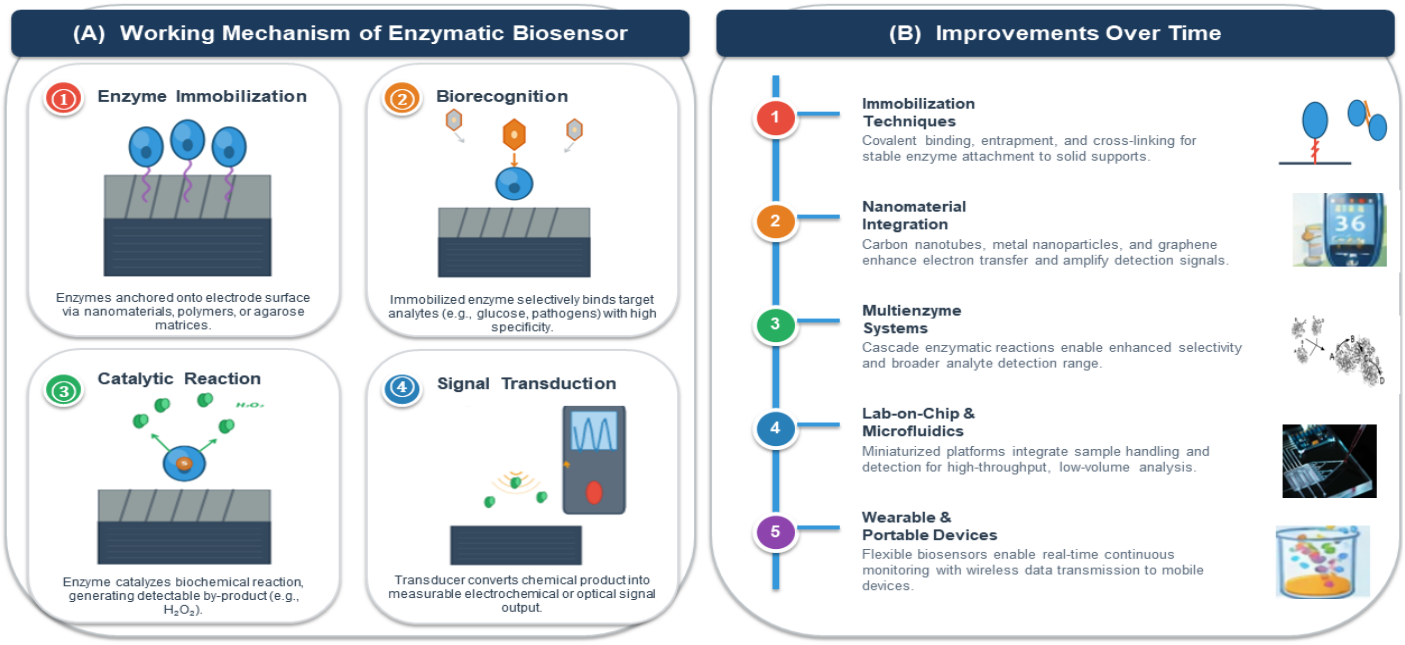


Figure 1: *Working mechanism and technological evolution of enzymatic biosensors. (a) The general working principles of an enzymatic biosensor: enzyme immobilization, analyte biorecognition, catalytic reaction, and signal transduction and (b) Development of new technologies in enzymatic biosensors, such as more sensitive and selective immobilization techniques, new nanomaterials, multienzyme cascade systems, lab-on-chip microfluidics, and wearable and portable biosensing devices increasing the sensitivity, selectivity, stability, and real-time monitoring capabilities of the biosensors.*

issues [10],[32]. Scientists are interested in the development of affordable, portable, and highly specific biosensors which can detect various diseases at an early stage and monitor environmental pollution [33],[34]. There is also great interest in creating sustainable biosensors which would help to preserve the environment while providing advanced medical and industrial achievements [35]. Recent advances in enzymatic biosensors include nanoaterial-assisted immobilization, microfluidic integration, multienzyme systems, and wearable sensing platforms as shown in Figure 1.

2. Carbohydrates-Degrading Enzymes

An important type of hydrolase is carbohydrate-degrading enzymes, which catalyse the breakdown of complex polysaccharides and oligosaccharides into simpler sugars, and are key players in natural metabolism and industry. They are broadly categorised as the Carbohydrate-Active Enzymes (CAZymes) system and have a few functionally different families, which are pectinases, cellulases, amylases or hemicellulases [36].

Despite the several recent reviews discussing enzyme-based biosensors, carbohydrate active enzymes (CAZymes), nanomaterial assisted sensing platforms and applications of food or environment biosensors, a detailed study focused on carbohydrate degrading enzymes (CAZymes) such as pectinases as biosensors is missing [37]. Currently, conventional oxidoreductases, like glucose oxidase, horseradish peroxidase and lactate oxidase, are the most frequently used in the literature for their analytical applications, while the use of pectinases, cellulases, amylases and hemicellulases in this field is comparatively less used [38]. This review combines uniquely the classification, catalytic mechanisms and production strategies of carbohydrate-degrading enzymes, their use in electrochemical, optical, colourimetric, and piezoelectric biosensors, immobilisation methods from traditional to nanomaterial-based, as well as new ideas such as nanozyme–enzyme hybrid systems, AI-assisted biosensing, wearable sensors, and sustainable sensor design. Special focus is given to pectinase-based biosensing for food quality monitoring, plant health assessment and the environment, which was not so well discussed in previous reviews [17].

2.1. Classification of Carbohydrate-Degrading Enzymes

Carbohydrate-Active Enzymes (CAZymes) include glycoside hydrolases (GHs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs). In this system, individual GH families link to particular polysaccharide substrates. These pairings are the basis for selective biosensing tools [39].

Table 1
Comparison of Present Review with Recent Reviews on Enzymes-based Biosensors and Carbohydrate-active Enzymes

Review	Main focus	Pectinase/CAZyme biosensing coverage	AI/ Nanozyme coverage	Distributing Features Comparison	Ref.
Carbohydrate-active enzyme (CAZyme) discovery and engineering via (Ultra)high-throughput screening	Discovery, engineering, and screening technologies for CAZymes	No dedicated Biosensing discussion	Computational approaches discussed; no biosensor AI focus	Emphasized enzyme discovery and analytical sensing applications and transducer integration	[39]
A review on pectinase properties, application in juice clarification, and membranes as immobilization support	Pectinase properties, industrial application, and membrane immobilization	Pectinases only; no biosensing emphasis	No	Focuses on juice processing and immobilization supports, whereas the present review examines pectinases as biorecognition eleents in multiple biosensor platforms	[40]
Nanozymes towards Personalized Diagnostics: A Recent Progress in Biosensing	Nanozyme-enabled Biosensing using diagnostics	No	Extensive nanozyme coverage	Concentrates on artificial enzyme mimics, while the present review integrates natural enzymes with nanozyme-assisted sensing concepts	[41]
Advances in the understanding and exploitation of carbohydrate-active enzymes	Recent advances and applications of CAZymes in biotechnology	Minimal biosensing coverage	No	Review broad CAZyme applications in therapeutics, foods, and biofules, but not enzyme-based sensing mechanisms or immobilization strategies	[36]
Systematic review on lectin-based electrochemical biosensors for clinically relevant carbohydrates and glycoconjugates	Electrochemical Biosensing targeting carbohydrates and glycoconjugates	Uses lectins rather than carbohydrate-degrading enzymes	No	Focuses on carbohydrate recognition by lectins, whereas the present work emphasizes catalytic degradation by CAZymes as sensing elements	[42]
Present review	Pectinases and carbohydrate-degrading enzymes in Biosensing	Comprehensive coverage of pectinases, cellulases, aylases, hemicellulases, PME, PG, and PL	Comprehensive discussion of AI and nanozyme-assisted Biosensing	Integrates enzyme classification, catalytic mechanisms, production strategies, immobilization methods, applications	

Comparison highlighting the unique focus on pectinase-centered Biosensing, advanced immobilization strategies, nanozyme integration, artificial intelligence, and sustainable sensing technologies.

Pectinases (pectinolytic enzymes) break down pectin, a complex acidic polysaccharide of the plant cell wall composed of α-1,4-linked galacturonic acid units (usually methyl-esterified) with rhamnose and neutral side chains [43].

2.2. Structure and Catalytic Mechanism

Glycoside hydrolases contain active-site clefts or pockets that interact with polysaccharides and determine orientation of glycosidic bond cleavage sites [44].

The substrate specificity of glycoside hydrolases is determined by the configuration of active site clefts and their chemical properties. Therefore, enzymes that hydrolyse α-1,4 linkages in starch fail to hydrolyse β-1,4 bonds in cellulose. Polygalacturonase is an example of a glycoside hydrolase that specifically hydrolyses α-1,4-galacturonan chains, producing galacturonic acid and oligomers that can be analysed using electrochemical and optical biosensing methods [45]. Carbohydrate-degrading enzymes have been broadly studied for analytical applications due to their substrate specificity and ability to produce measurable products. Their major applications and characteristics are summarised in Table 2.

3. Pectinases: Types, Mechanism of Action and Production

3.1. Types of Pectinases

Pectinases are a class of enzymes involved in the degradation of pectic substances, complex polysaccharides rich in galacturonic acid and abundant in plant cell walls [43].

- **Pectin methylesterase (PME, E.C. 3.1.1.11):** PME causes demethylation of highly methylesterified pectin and yields methanol along with pectic acid. PME works around neutral pH (6-7) [43].
- **Polygalacturonase (PG, E.C. 3.2.1.15):** PG is involved in the hydrolysis of α-1, 4 glycosidic bonds present in the de-esterified pectin and forms galacturonic acid and its oligomers. Usually, fungal PGs found in Aspergillus niger and Trichoderma are active under pH 4-6 and temperature ranging from 30-50 °C, suitable for food processing and juice analysis purposes [51].
- **Pectin lyase (PL, E.C. 4.2.2.10):** Pectin lyase degrades highly methylesterified pectin by β-elimination reaction, forming unsaturated oligogalacturonates. This enzyme normally works best under slightly acidic and high temperature conditions (pH 6-8). It makes bacterial PLs appropriate for industrial applications and biosensors [52].
- **Pectate lyase (E.C. 4.2.2.2):** Pectate lyase acts on de-esterified pectin (pectate), but also acts through 8-elimination, producing unsaturated oligomers. It is typical in phytopathogenic and soil-dwelling bacteria like *Erwinia* and *Bacillus spp*., and tends to be higher in pH (7-9) than fungal PGs, which is suitable in wastewater or bioremediation-oriented biosensors [53].

3.2. Mode of Action

The structure of pectin involves mainly α-1,4 glycosidically bound D-galacturonic acids along with rhamnogalacturonans, methyl esters, and neutral sugars as side-chains [54]. PME cleaves methyl ester groups from the C6 carbon of galacturonic acid, resulting in the formation of methanol and an increase in the net charge density of the molecule. The latter can be used as an approach in biosensing by detection of methanol via alcohol oxidase activity or by observing changes in impedance and charges [55]. Pectin and pectate lyases act via β-elimination, providing the formation of unsaturated compounds absorbing light at 235 nm [56].

3.3. Sources and Production

The microorganisms represent the main source of pectinases used in industry due to their high productivity, amenability to genetic modification and characterisation of their catalytic properties [57]. Different fungal strains, such as *A. niger*, *A. oryzae*, *Trichoderma* and *Penicillium, secrete* acidic PGs and pectin lyases that are commonly used in food industries.

Large-scale production is carried out either by submerged fermentation (SmF) or solid-state fermentation (SSF). While SmF allows for good process parameter regulation and easy purification of the enzymes, SSF uses cheap agricultural residues such as citrus peels, wheat bran and sugarcane bagasse to increase enzyme yield [58].

4. Principles of Enzyme-Based Biosensing

4.1. Enzyme–Substrate Interaction in Detection

Table 2
Carbohydrate Degrading Enzymes and Their Use in Biosensing

Enzyme	Substrate	Reaction/ Product	Detection Method	Biosensing Application	Ref.
Pectinase	Pectin	Hydrolysis of pectin into galacturonic acid and oligomers	Electrochemical, colorimetric, pH-based sensors	Juice quality assessment, food analysis	[46],[47]
Polygalacturonase	Polygalacturonic acid	Cleavage of α-1,4-glycosidic bonds	pH-based and electrochemical methods	Detection of pectin, degradation in food samples	[48]
Pectin methylesterase	Methylated pectin	Removal of methyl groups	Electrochemical and colorimetric methods	Monitoring fruit maturation and pectin modification	[49]
Pectin lyase	Esterified pectin	Cleavage by β-elimination	Optical and electrochemical methods	Pectin content analysis in food processing	[50]

Overview of carbohydrate-degrading enzymes, their substrates, reaction products, detection methods, and biosensing applications.

Enzymes are used as biological recognition components in biosensors. They identify and catalyse reactions with specific target analytes. Pectinase breaks down pectin into galacturonic acid and related oligosaccharides for carbohydrate analysis [58].

4.2. Signal Generation Mechanisms

The main role of enzyme-based biosensor technology is to translate an enzyme reaction into a quantifiable analytic signal using one of several different transduction approaches such as electrochemical, optical or cholorimetric methods. Table 3 shows the comparative overview of major signal generation mechanisms.

4.3. Role of Reaction Products in Signal Output

The measurable products created by enzyme-catalysed reactions serve as the basis for how the enzyme-based biosensors produce output [66]. In the case of detecting pectin, pectinase catalyses the hydrolysis of pectin to yield galacturonic acid, resulting in a medium that is more acidic than before and monitoring the change is possible with many different sensors [67].

5. Role of Pectinase in Biosensing

5.1. Detection of Pectin and Plant-Based Substrates

Pectin is one of the larger components of plant primary cell walls and the middle lamella, providing adhesion and rigidity for plant cells. The softening of pectin during ripening, storage and infection is of agronomic significance and is largely controlled by the enzymatic degradation of pectin, so its monitoring has direct value in agriculture and food quality control [68].

Pectinases, namely pectin methylesterases (PME), polygalacturonases (PG) and pectin lyases (PL), which convert pectin to galacturonic acid and oligosaccharides (Figure 2), are the basis of several strategies of signal transduction: pH drop sensing (LOD ~0.15 U/mL pectinase) and chromogenic/absorbance-based detection at 540 nm [69]. The following two are examples of the trade-off in the field. Due to limitations in the buffering capacity and ionic strength of the sample matrix, pH-based detection is simple and label-free, but the quantitative accuracy in complex plant tissue extracts is limited. The colourimetric kits that utilise chromogens that are heated to react reach a similar sensitivity (0.15 U/mL) but require a reaction/heating step that increases reaction time and complexity that is difficult to implement in the field [70]. This enzyme-reactive nanofiber packaging technology is extended to provide a real-time, in-line or in-package spoilage indicator, not a discrete assay; an important benefit, but at the cost of less quantitative information and less sensitivity to false-positive and false-negative results under fluctuating humidity/temperature storage conditions in the product are less well characterised [47].

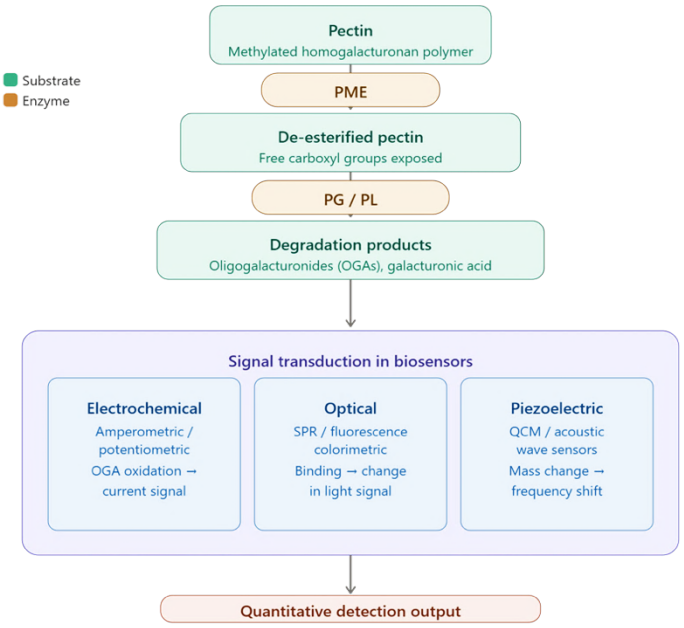


Figure 2: *Mechanism of pectin breakdown and biosensor signal transduction: Enzymatic degradation of pectin by PME and PG/PL results in oligogalacturonides that carry out signal transduction across electrochemical, piezoelectric, and optical biosensor platforms.*

5.2. Pectinase-Specific Biosensing Platforms

Apart from the mentioned pH drop and heated chromogenic systems, several biosensor designs have been developed based specifically on the recognition chemistry of pectinases, varying in the way the event of pectin hydrolysis is detected [7].

Assays utilising polygalacturonan substrates conjugated with dyes such as Remazol Brilliant Blue (RBB) offer a direct measurement of cleaved chromophore fragments produced without any heating, thus reducing the time of the analysis compared to the DNS assay requiring heating, yet maintaining the same sensitivity range. Analogous fluorogenic assays use substrates labelled with fluorescein or coumarin, providing increased sensitivity for low enzyme concentrations at the expense of using fluorescent labels [71]. Mass- and refractive index-based biosensors, Quartz Crystal Microbalance (QCM) biosensors coated with a thin layer of pectin, provide detection of enzymatic activity through real-time mass change, while Surface Plasmon Resonance (SPR) biosensors detect enzymatic activity through change of refractive index due to

Table 3
Signal Generation Mechanism Mostly Used in Enzyme-Based Biosensing Systems

Mechanism	Signal Type	Enzyme-Based Detection	Advantage	Reference
Electrochemical	Current, voltage, impedance, conductivity	Detection of electrical signals	High sensitivity, rapid response, easy miniaturization, suitable for real-time monitoring	[7],[59]
Optical	Absorbance, fluorescence, luminescence, refractive index	Detection of change in light intensity or fluorescence	Non-destructive analysis, high specificity, multiplex capability	[60],[61]
Colorimetric	Color intensity change	Detection of color change visually or spectrophotometrically	Simple operation, low cost, no sophisticated instrumentation required	[62]
Piezoelectric	Frequency or mass variation	Detection of change in crystal mass and resonance frequency	Label-free detection and high sensitivity	[63],[64]
Thermal (Calorimetric)	Change in temperature	Monitoring of heat change	Direct measurement without labels or dyes	[65]

Signal generation mechanisms used in enzyme-based biosensors and their applications.

hydrolysis of pectin. Both biosensors are label-free and provide continuous kinetic curves, which can be useful for studies of enzyme kinetics, yet QCM/SPR instrumentation is expensive and bulky [45].

Immunosensors and aptasensors are directed at the pectinase enzyme itself. Rather than measuring the hydrolysis products, immunosensors or aptasensors using antibody- or aptamer-functionalized electrode surfaces and SPR chips could measure the pectinase enzyme itself, which may be more suitable when the detection of a specific form of the enzyme is important [72], e.g., the polygalacturonase produced by phytopathogenic fungi like Botrytis cinerea or Verticillium spp. as an early sign of infection before the development of any visible symptoms. While the ability to detect individual isoforms is a real advantage over bulk activity assays, the cost and variability of antibodies/ aptamers production make it hard to use routinely [73].

Whole-cell and microbial biosensors. Genetically modified bacteria or yeast cells, which contain pectin-inducible promoter linked to genes encoding fluorescent protein (GFP) or luciferase reporter, serve as self-amplifying and cheap method for detection of pectinases in the environment or agriculture. As the output relies on the processes of transcription and translation, the reaction rate is lower compared to cell-free methods; moreover, viability issues reduce the portability of the technique [74]. Nevertheless, the

Table 4
Different Methods of Biosensing Systems

Method	immobilization	Sensitivity/LOD	Linear Range	Stability	Response time	Application	Ref
MBTH colorimetric assay for pectinase activity	Free pectinase using an optimized MBTH-based chromogenic reagent for reducing sugar detection under high alkaline and low temperature condition	Low LOD, High sensitivity (R^2= 0.9945)	2.5-15.8 mU/mL	Resolve background-interference and pectin precipitation problem of earlier DNS/MBTH protocol	Zero-order hydrolysis kinetics within 60 min; endpoint measurement	Enables routine; accurate quantification of pectin/pectinase depolymerase activity for industrial and research QC	[71]
Quartz crystal microbalance with dissipation (QCM-D) continuous assay	Insoluble polygalacturon-calcium immobilized on quartz sensor surface	Not a calibrated concentration LOD-continous kinetic-rate assay	Not applicable	Facilitate comparative analysis of enzyme preparation without requiring multiple sample	Continuous, dissipation trace and real time mass loss	Bench-top research tool for comparative kinetic analysis of endo-pectinase (e.g; GH28-family) mediated degradation activity on solid phase pectin substrate	[45]
Smartphone-read (paper based lateral flow strip	Smartphone based detection of Enzyme-mediated pectin viscosity changes measured by diffusion distance on pH indicator paper strip	0.025 U/mL	0.025-0.80 U/mL	-	Single endpoint diffusion readout	Field-deployable rapid screening of PG activity, Low cost	[56]
Pectinase immobilized on magnetic nanoparticles using PEG-trichlorotriazine chemistry	Pectinase immobilized covalently on PEG-grafted magnetic nanoparticles through trichlorotriazin coupling	-	Not applicable	94% retained after 125 days; 55% of activity retained after 10 cycles, store at 25°C	-	Immobilized enzyme mediated clarification of pineapple juice, reducing turbidity to 59% following 120 min of treatment	[69]
Fluorescent SWCNTs based biosensor for real time pectinase activity monitoring	Pectin-coated (6,5)-SWCNTs; enzyme pectin degradation promote fluorescence quenching and SWCNT aggregation and	-	Not reported	Not reported	Continous fluorescent readout, real time	NIR based real time monitoring of pectinase activity in process/biological specimen	[77]

whole-cell systems are convenient if in situ continuous monitoring is required. While the sensor signal relies on transcription/translation in cells, and not enzymatic activity, this results in a delay in response and portability issues due to the need to maintain the viability and culture status of cells. The method is promising when continuous in-situ detection becomes a priority, as opposed to fast single-point determination of an analyte concentration [75].

Molecularly imprinted polymers (MIPs) as synthetic receptors. MIPs obtained by imprinting galacturonic acid or pectinase itself are under investigation as cheaper and more robust chemical and thermal alternatives to antibodies for use in immobilisation formats. Low reproducibility of the imprinted cavities and relatively slow rebinding rate of MIP-based sensors make such pectinase sensors less sensitive than those employing antibodies and aptamers, yet the cost and stability of MIPs make the platform worth watching [76],[77],[78],[79],[80].

Overall, along with pH, chromogenic, electrochemical, nanoparticles-immobilised, and paper-based lateral flow platforms already described in this paper, there are at least eight different pectinase-specific platform classes known now, as mentioned in Table 4. None of the existing classes prevails: all of them occupy their own positions in the sensitivity, speed, cost and portability spectrum, and therefore the selection of the right platform should be based on its application [56].

5.3. Analytical Use of the Pectin Degradation Products

The principal product of the backbone of pectin being cleaved is galacturonic acid (GalA) and most pectinase biosensing chemistries are based on the detection of this product, but there are significant variations in sensitivity, speed, and infrastructure requirements. Well-established and economical, they are bulk end-point measurements that sacrifice spatial or kinetic resolution, as well as being potentiometric and optical detection of the associated pH drop (which is detected colourimetrically using DNS-reagent assays at 540 nm) [69]. Enzymatic sensors that monitor the current/impedance changes of the enzymes on the electrodes provide real-time kinetic readout, which is beneficial for the study of enzyme kinetics, but tend to be more complicated to fabricate and calibrate electrodes than colourimetric sensors. At the other end of the spectrum, however, are the paper-based lateral-flow strips that read PG activity by diffusion distance [56], which are low-cost and field-deployable, but less precise than electrochemical or nanomolar-sensitivity catalytic amplification approaches [98]. The net picture is not that of a single best way, but one of a sensitivity/throughput-vs-simplicity trade-off: nanomolar-sensitivity amplification assays work well for detailed kinetic or diagnostic work; lateral-flow strips work well in the field for rapid screening; and no single current platform combines both [81].

5.4. Recent Technological Advances

Nanomaterials other than graphene or gold nanoparticles, MXenes (Ti_3C_2Tx and other two-dimensional transition-metal carbide/nitride materials), have recently emerged as candidates in the wider domain of electrochemical biosensors that would combine conductivity with the hydrophilicity and surface functionalisability appropriate for enzymes [82]. Their potential use as a component in pectinase or pectin sensing has not yet been reported in the reviewed literature, but it constitutes a readily available possibility rather than an advance made by this subfield, as indicated here: production of wearable and flexible electrodes via printing technology [83]. Flexible substrate and screen-printing technologies, mainly developed for wearable electrochemical biosensors in healthcare applications, would enable inexpensive, disposable, in-line measurement of pectinase activity in the packaging line or in juices and wines. Similarly to MXenes, this represents an advance in biosensors that can be applied in pectinase detection but has not yet been achieved by pectinase biosensors themselves [84].

Signal processing using machine learning. In enzyme detection and food quality monitoring in general, machine learning approaches are being used more and more often to account for matrix effects and for the combination of signals from various sensor outputs. In the case of pectinase sensing, this can be translated to integrating pH, colourimetric, and impedance channels into a single ripeness/spoilage metric [85]. Chromogenic or fluorogenic pectinase detection assays coupled to smartphone camera-based colour analysis software get rid of the requirement for a benchtop spectrophotometer, bringing lab-validated assay chemistries closer to practical field applications. One of the more developed technologies among those described in this section, smartphone-readout colourimetric devices have already been proven for use with other food-quality chemistries and need only an assay adaptation, not device development, for pectinase detection [62].

5.5. Future in New Biosensing Systems

The two most prominent levers for advancing pectinase biosensors beyond some of the above-detailed limitations are nanomaterial integration (such as graphene, AuNPs, conducting polymers) and microfluidics. A major advantage of nanomaterials is that they have a significantly higher surface area (100-1000 times higher) and electron-transfer rate (10-50 times) [86] than in the other systems described above, in addition to immobilization stability (80-90% activity retained after 10-20 cycles) [87], which directly overcomes the reusability and the sensitivity restrictions of the electrochemical and immobilized-enzyme systems. Microfluidic/lab-on-chip formats solve the throughput and matrix-interference issues mentioned above by using small volumes (<100 nL) and can provide high-throughput (100-sample) screening, fast impedance-based readout (<5 min) and improved signal quality (SNR>20 dB), but require less sample (10-100×) than the microplate format [88]. Real-world gains are achievable, but most of them have been demonstrated individually and/or under controlled laboratory conditions, and the challenge now is to make these novel functions available as a single validated platform for real, variable food or environmental matrices, which are not ideal buffers. While applications like anthocyanin-based indicators of food spoilage in smart packaging, pathogen detection in precision agriculture or diagnostic applications are promising, they are still largely in concept and reproducibility, cost-per-test and shelf stability under real storage/transport conditions are the greatest barriers to translation from bench to field [89]. There are four such opportunities that come out of the platform review listed above and should be explicitly stated rather than left implied. First, multiplexed assays: using probes selective for PME, PG, and PL enzymes together on a single chip or strip will result in a compound ripeness or spoilage indicator, thus tackling the sensitivity-vs-simplicity dilemma [88]. Second, logistics-integrated sensing: integrating in-package or in-line pectinase detection with wireless sensing (RFID, NFC, or IoT) will enable continuous ripeness/spoilage monitoring along the entire cold chain from storage through distribution to retail, as opposed to the one-shot readings that predominate among the platforms reviewed [90]. Third, sustainable biodegradable sensor materials: paper-, cellulose-, or biopolymer-based sensing platforms will make pectinase sensors conform to the emerging trend of biodegradable smart packaging, without adding to the electronic waste generated by what is often compostable packaging [70].

Fourth, validation and standardisation: practically all the developments covered in this chapter, from nanomaterials immobilisation to microfluidic designs and thermostable enzyme variants, have been tested individually in controlled lab conditions [80]. What now stands between us and the real-world application of these devices is not the discovery of new chemistries, but rather the validation of such a system as a whole against real food and environmental matrices and setting the reproducible cost per test figures; not to mention inter-laboratory experience needed before any standards acceptance at the industry level. Prioritising these practical considerations will contribute more significantly to advancing next-generation pectinase biosensors than continued development of standalone proof of concept studies.

6. Applications of Enzyme-Based Biosensors

The capability to detect biochemical substances in real time, rapidly, and sensitively has seen biosensors widely used in various fields. These devices combine both biological recognition components and transducers to detect physical, chemical and biological processes. Their versatility allows their use in food safety, environmental surveillance, in controlling industrial processes, and in biomedical diagnostics and makes them an essential part of a modern analytical system [10] as shown in Figure 3.

6.1. Food Quality and Safety Monitoring

One of the most important fields in biosensor applications is food quality and safety monitoring. Conventional methods of analysis that are being used in food industries tend to be time-consuming and involve the use of skilled personnel; biosensors are fast, cost-effective, and highly selective in detecting contaminants and quality indicators [91]. Biosensors have been extensively applied in the processing of fruit juice, to monitor glucose level and sugar composition variation in storage and preparation of fruit juice. The glucose biosensors are of great significance since the food products might alter in their composition during storage, thus influencing the quality and nutritional value [92]. The application of glucose oxidase immobilised on electrodes that have been modified with gold nanoparticles has been reported to enhance the level of sensitivity in the detection of glucose in food products like juices and beverages [93].

The microbial contamination and spoilage detection in food systems are also critical in the use of biosensors. As an illustration, Escherichia coli can be detected in vegetables and this is an indicator of fecal contamination. This is done by measuring changes in pH or impedance changes brought about by the metabolites or ammonia using potentiometric or impedance biosensors [94]. These systems can quickly identify contamination and are useful in ensuring food safety standards. Biosensors are also used in the dairy industry to identify pesticide and herbicide residues and other contaminants. Recently, polymer-based and nanomaterial-based biosensors have been developed to be used on-site to detect heavy metals, antibiotics, allergens, and pesticide residues in milk and other food samples [95]. Biosensors based on automated flow that combine enzyme systems and automation have also been created to analyze the quantity of the organophosphate

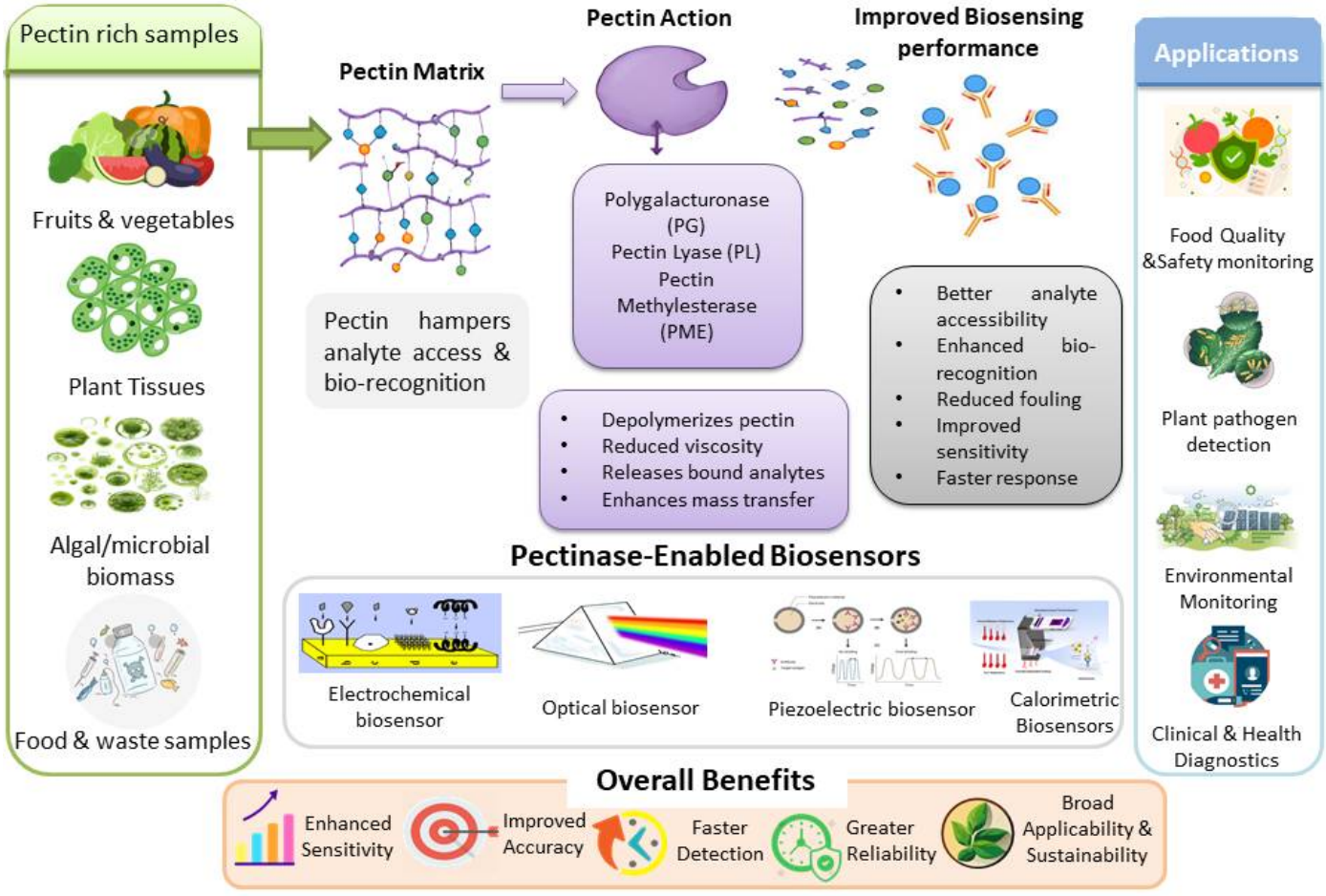


Figure 3: *Applications of pectinase in biosensors. Enzymatic degradation of pectin by PG, PL, and PME enhances analyte accessibility and signal generation improving the performance of biosensor across environmental, clinical and food applications..*

and carbamate pesticides in the milk sample, guaranteeing food safety and adherence to regulations [96]. Recent developments highlight the use of immunosensors and CRISPR- or nanozyme-based biosensors for pathogen detection (e.g., E. coli, Salmonella, Listeria) with improved sensitivity, specificity, and field-ready operation. All in all, biosensors play a vital role in food quality assurance, spoilage prevention, and consumer safety due to continuous and on-site monitoring and rapid detection systems [97].

6.2. Environmental Monitoring

Another important field of biosensor use is in environmental monitoring. These instruments make it possible to detect pollutants, poisonous substances, and biological pollutants in water, soil, and air in a short time. Their selectivity and sensitivity enable them to be detected at very low concentrations [35]. A significant application is in biomass degradation and waste analysis, where biosensors are employed to measure biochemical oxygen demand (BOD), variations in organic load, and metabolic activity. Whole-cell biosensors have also been invented to sense heavy metal ions via metallothionein or metal-responsive promoters of microorganisms [98]. These biosensors are specific to toxic metals and thus can be used as tools in monitoring pollution in the environment as well as ecological risks. Pesticide residues are also detected using biosensors and these are major hazards to the environment. Biosensors based on enzymes have been developed to identify the presence of organophosphate and carbamate pesticides in water samples by using the enzyme acetylcholinesterase (AChE) and butyrylcholinesterase [99]. These biosensors are fast in response and sensitive, and thus they can be used in real-time monitoring of the environment. Moreover, biosensors based on nanomaterials, as well as bacterial bioassays, have been utilised to detect arsenic, cyanide, and other toxic metals in natural water sources [35]. These systems offer dependable identification of harmful materials and help in protecting the environment. In general, biosensors are more environmentally friendly because they allow quick and sensitive detection of pollutants and efficient waste- and water-management mechanisms.

6.3. Industrial Process Monitoring

Biosensors are also important in industrial biotechnology to monitor the production processes and improve the efficiency of the operations. Fermentation and bioconversion processes are among these and they are major areas of application. The process of fermentation is complicated and entails various biochemical reactions, during which strict regulation of the metabolites, including glucose, lactate, ethanol and amino acids, is critical [100]. To monitor these compounds in real time, biosensors have become common to enable better product yield and better control over the processes. The glucose biosensors used in modern fermentation industries are more accurate in controlling the saccharification and fermentation processes as opposed to the traditional titration methods.

Fermentation systems have been enhanced greatly with the application of biosensors to help with better productivity. As an illustration, biochemical changes in an ion-exchange process of fermentation systems have been monitored using glutamate biosensors [101]. Critical metabolites are detected in real time, and process conditions can thus be optimised more quickly and the product quality is maintained. Biosensors also contribute to the conversion processes of bioconversion processes where biological materials are changed into valuable products such as enzymes, organic acids and biofuels [98]. The ability to monitor metabolic activity using biosensors online can help maintain better control of reaction conditions and improve efficiency. Recent developments have incorporated microfluidic and lab-on-chip biosensors in bioprocess control systems, which have enabled high-throughput, automated and miniaturised monitoring of bioreactors in industries. All in all, biosensors are useful tools in current biotechnological industries as they offer automated, fast and reliable monitoring of the industrial process [88].

6.4. Emerging Biomedical Applications

One of the most rapidly developing areas of biosensor technology is biomedical applications. These applications aim at diagnosing diseases, monitoring health and early detection of biological markers of several medical conditions. The glucose biosensor is one of the most popular biosensors that are currently utilised in healthcare and is widely used to monitor the levels of blood glucose in patients with diabetes mellitus [102]. These devices can be monitored around the clock or nearly 24 hours and they give precise readings, which are required in the management of a disease. A high percentage of the biosensor market worldwide is taken by blood glucose monitoring systems because of their extensive clinical applications.

Detecting infectious diseases and pathogens is also done by using biosensors. Immunosensors, DNA biosensors, and CRISPR-based biosensors are rapidly developing biosensor technologies which can be used to identify microbial infections, including urinary tract infections, bacteria that cause sepsis, and viral infections (e.g., SARS-CoV-2, influenza) [103]. These devices minimise the time of diagnosis, aid in point-of-care testing, and enhance treatment outcomes. The other biomedical application that is emerging is the detection of cytokines and biomarkers related to inflammation, such as interleukin-6 (IL-6), interleukin-10 (IL-10), and tumour necrosis factor-alpha (TNF-alpha), which are involved in inflammatory reactions as well as cardiovascular or autoimmune disorders [104]. Advanced materials like hafnium oxide, graphene or antibody-functionalized electrodes have been developed as biosensors to enable detection of disease-related biomarkers in their early stages to allow timely medical intervention [105]. The latest developments in antibody- and aptamer-based biosensors have broadened the scope of detection of cancer biomarkers, cardiac markers and neurochemical species in blood, serum and even in vivo. Moreover, the biosensor based on carbohydrate-protein interaction has been investigated to detect pathogens [106]. Lectin carbs the biosensors read lectin-carbohydrate recognition to detect microbial targets like E. coli and other bacteria that have particular surface glycans. As an example, mannose or other sugar-functionalized surfaces have the capacity to bind bacterial pili, resulting in detectable impedance or piezoelectric responses, enabling label-free and specific detection of pathogens. In general, the new biomedical biosensor technologies have great potential in the field of enhancing diagnostic accuracy, personalised medicine and point of care medicine, and in improving healthcare delivery system [5].

7. Challenges and Limitations

Despite the widespread use of enzymes in biosensors and industrial biotechnological processes, some issues affect the efficiency and applicability of enzymes and become especially important regarding the commercialisation of pectinase biosensors [80].

- **Enzyme stability:** One of the major limitations of using enzymes is related to their instability and loss of activity during storage and operation. Enzymes are highly labile biological molecules whose catalytic properties depend upon several external factors, including temperature, pH and ionic

strength [86]. Nevertheless, inappropriate use or lack of control of the operating conditions may lead to denaturation or conformational changes in the enzyme, which results in the decrease of catalytic properties and reduction of the lifetime of the enzyme [107]. In the case of pectinases, this problem is particularly significant because of their highly selective catalytic properties that work in rather narrow pH and temperature windows. Exposure to various matrices containing acids, sugars and different temperatures that accompany analysis of fruit juices and other food samples may result in the rapid decay of pectinases' activity and reduced shelf life of the sensor [69].

- **Sensitivity:** The lack of sensitivity, which can be observed in some systems, is another limitation that affects detection of very low concentrations of analytes. In some cases, when it is important to detect very low concentrations of something in diagnostics, environmental monitoring, or analysis of food quality, insufficiency of the sensitivity of enzymatic systems could become a problem [108].
- **Structural heterogeneity of the substrate:** One more often underestimated limitation of pectinase-based systems is that of the structural heterogeneity of the substrate – pectin. The structure of the pectin differs from sample to sample and depends on the plant, its stage of ripeness, etc. Moreover, there exist several types of enzymes that belong to pectinases (polygalacturonases, pectin lyases, pectin methylesterases, etc.). Therefore, the biosensor that was calibrated on some particular pectin source will not necessarily work well with others, which makes assay development quite complicated [109].
- **Matrix interference:** Moreover, sample matrix interference might impact the efficiency of the enzyme and the analysis itself. There are many components such as inhibitors, organic matter, salts, and proteins contained in biological fluids, food, and environmental samples that may interact with the enzyme-substrate reaction and give misleading results [110]. Interference in the matrix will result in a lack of specificity of the response and false positives and negatives. This is especially important for pectinase biosensors since food and juices contain sugar, have variable pH, and have other polysaccharides that can interfere with the reactions [69].
- **Issues in high-volume manufacture:** Besides the issues with performance in the lab, scaling up of the pectinase biosensor manufacture process is also associated with its specific problems. Obtaining and purifying enzymes at a high volume is a challenge in itself due to batch to batch variations of activity and purity, immobilization techniques that worked well on hand-made electrodes sometimes don't scale up easily, and especially nanomaterial-based enzyme immobilization [111]. In addition, the cold-chain requirement and short shelf-life further increase complications associated with distribution and cost, which can act as practical limitations towards making a pectinase biosensor transition from prototype into a commercially available device [112].
- **Standardisation and reproducibility:** Additionally, standardisation becomes another issue when developing and commercialising technologies based on enzymes. Differences may occur in terms of enzyme activity within different studies and laboratories due to differences in the enzyme source, enzyme purification, enzyme immobilisation, conditions of assay and used analytical techniques [113]. There are no standard protocols; therefore, it becomes difficult to reproduce results and compare the outcomes of experiments. In the case of pectinase biosensors, the problem of non-standardised reference substrates and assays increases the problem of results comparison and the creation of a standard protocol necessary for validation and commercialisation [114]. When put together, enzyme instability, substrate variability, matrix interference, scale-up manufacture, and lack of standardised methodology for analyses make up an intricate set of obstacles that prevent the transfer of pectinase biosensors from laboratory prototypes to commercial applications. Therefore, stabilisation of enzyme, enhanced sensitivity, consideration of variability of pectin substrates, elimination of matrix interference, creation of reproducible manufacturing technology, and development of standardised methodology become important tasks for the future progress of the reliability of enzyme-based systems [115].

8. Future Perspectives

It is expected that future development of enzyme-based biosensing techniques will concern the improvement of performance, wider implementation of the technology, and sustainability. An approach to the engineering of enzymes by protein engineering, directed evolution and genetic manipulations is a relatively new method for the optimisation of enzymatic catalysis, specificity of reactions, thermal stability, and environmental stress resistance of the enzymes. As for pectinases, directed evolution or rational engineering aimed at increasing pH and thermal stability or reducing the influence of substrate heterogeneity may be applied for overcoming two of the main challenges of pectinase biosensors. Enzyme degradation and reduced activity limitation issues can be overcome by these means.

- **AI-assisted Biosensing:** More advanced integration of artificial intelligence and machine learning techniques with enzyme-based sensors is expected. In the case of pectinase biosensors, this approach may be useful for the differentiation of a complex response generated by the heterogeneity of pectin structure and interference from sugars, salts, and other polysaccharides in the juice or food matrix, thus providing adaptive calibration. Machine learning techniques can be employed for compensating for baseline drift due to reduced enzymatic activity of the sensor throughout its operational lifetime, thus prolonging the usable life of sensors without the need for recalibration after each production batch, as well as detecting any unusual measurements that can be attributed to the presence of interferents rather than actual changes in concentration of the analyte.
- **Hybrid pectinase-nanozyme biosensors:** Nanozymes are nanomaterials possessing enzyme-mimicking catalytic properties and have been investigated as partners and even potential substitutes for natural enzymes in designing biosensors. The use of a hybrid of natural pectinase and a nanozyme may provide an opportunity for decoupling the performance of the biosensor from the instability of the enzyme by maintaining the catalytic reaction and signal transduction despite the gradual decrease in activity of the natural pectinase, thus making it possible to achieve greater stability and longevity beyond the possibilities of just the immobilisation of the enzyme. This type of approach is quite consistent with the previously mentioned approaches of using nanomaterials in biosensors due to the identical nature of materials.
- **Flexible and wearable biosensors:** Although most of the current biosensor applications involving pectinase focus on food quality or environmental monitoring, not wearable healthcare, the flexible substrate technologies used for wearable sensors are gaining importance for pectinase tests

carried out using decentralized, mobile, field-based equipment such as flexible sensors in strips or patches that are used to assess the ripeness of fruits or quality of their juice in harvesting, processing, or transportation stages, not only in stationary laboratories. The use of flexible electrode material and printing techniques to immobilise pectinase would help here.

- **Microfluidics integration:** Combining pectinase biosensors with microfluidic devices provides a direct solution to overcome many of the barriers to commercialisation. The integration of microfluidics in the process of biosensing allows to standardize the sample volume, dilution, and pretreatment of heterogeneous samples containing pectin. In addition, microfluidic integration combined with small-scale reagents and enzyme storage helps to achieve the kind of small-volume, automated, and reproducible preparation needed for scaling up from manually assembled research electrodes.
- **Multiplex sensing platforms:** Since pectin breakdown is only one parameter among several that indicate the quality of food/juice (along with other parameters such as sugar content, acidity, or spoilage markers, for instance), the development of multiplexed sensing platforms which integrate detection based on pectinase with other enzymatic or non-enzymatic sensing channels into a single chip will allow obtaining a comprehensive profile of food quality with a single sampling/measurements process. Besides being able to compensate for the lack of sensitivity and selectivity of any one enzymatic channel, multiplexing will allow validating results through multiple parallel measurements. Moreover, increasing demand for fast, accurate and non-invasive disease detection methods will definitely contribute to the increased application of enzyme-based biosensors in medical diagnostics in the future. Biosensors can be used for early disease detection of metabolic disorders, tumour markers, infectious diseases and personalised medicine approaches. Health monitoring in real time is sure to be enhanced due to the use of modern technologies, including artificial intelligence and wearable sensors. In addition, the creation of environmentally sustainable biosensors has become an important field of research with an emphasis on environmentally friendly materials, biodegradable materials, environmentally sustainable synthesis, and energy-saving production. This is a type of development that corresponds to the global aims of sustainability and can assist in reducing environmental impact without compromising the analytical capabilities of these sensors.

All of the above-mentioned trends in combination constitute the way to implement the concept of pectinase biosensor into practice through creating a robust, deployable, and economically efficient sensor. Biosensors based on enzymes are expected to find widespread applications and make a breakthrough due to the continuous improvements in the areas of enzyme engineering, hybrid systems design, and clinical applications.

9. Conclusion

Carbohydrate-degrading enzyme-based biosensors represent an emerging frontier in intelligent sensing technologies, offering exceptional molecular recognition, catalytic specificity, and versatile analytical capabilities. Among these systems, pectinase-based biosensors provide unique opportunities for monitoring food quality, plant health, environmental changes, and industrial processes through selective detection of pectin-related biochemical transformations. Recent advances in nanomaterial-assisted immobilisation, microfluidic integration, flexible sensing platforms, and sustainable biomaterials have significantly improved sensor performance, stability, and practical applicability. However, challenges associated with enzyme stability, substrate complexity, matrix interference, reproducibility, and large-scale manufacturing continue to limit real-world deployment. Future progress will depend on the synergistic integration of enzyme engineering, advanced functional materials, and intelligent data-driven sensing architectures. By addressing these limitations, carbohydrate-active enzyme-based biosensors are expected to evolve into robust, portable, and sustainable analytical platforms, contributing significantly to next-generation healthcare, food safety, environmental monitoring, and industrial biotechnology.

Declaration

AI Disclosure: N/A

Author Contribution Statement: R. U. wrote this paper.

Availability of Data and Materials: Data will be made available by the corresponding author on reasonable request.

Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Issues: There are no ethical issues.

References

1. Gao, W., et al., Recent advances in biosensors: Structure, principles, classification, and application in bio-manufacturing. Methods, 2025. 242: p. 123-142.
2. Sharma, S., R. Butola, and J. Kumar, Transforming Industry: The Role of Biosensors in Enhancing Efficiency, Safety, and Sustainability. ECS Journal of Solid State Science and Technology, 2025. 14(5): p. 057001.
3. Guo, J., et al., Effect of deep eutectic solvents on the activity and stability of cellulases and pectinases. ACS omega, 2023. 8(48): p. 45678-45686.
4. Khor, S.M., et al., Challenges and strategies in developing an enzymatic wearable sweat glucose biosensor as a practical point-of-care monitoring tool for type II diabetes. Nanomaterials, 2022. 12(2): p. 221.
5. Elgiddawy, N. and H.M.E.-S. Azzazy, Carbohydrate-based biosensors for enhanced pathogen detection. Analytical and Bioanalytical Chemistry, 2025: p. 1-22.
6. Eivazzadeh-Keihan, R., et al., Recent advances on biomedical applications of pectin-containing biomaterials. International journal of biological macromolecules, 2022. 217: p. 1-18.
7. Wijayanti, S.D., L. Tsvik, and D. Haltrich, Recent advances in electrochemical enzyme-based biosensors for food and beverage analysis. Foods, 2023. 12(18): p. 3355.
8. Wang, X., J. Zhou, and H. Wang, Bioreceptors as the key components for electrochemical biosensing in medicine. Cell Reports Physical Science, 2024. 5(2).
9. Khaleque, M.A., et al., Bioreceptor modified electrochemical biosensors for the detection of life threating pathogenic bacteria: a review. RSC advances, 2024. 14(39): p. 28487-28515.
10. Assudani, P.J., et al., Biosensing technologies for foodborne pathogen detection and healthcare: principles, emerging materials, and intelligent platforms. Microchimica Acta, 2026. 193(4): p. 231.
11. Singh, R., et al., Advancements in glucose biosensors: Innovations for wearable and real-time monitoring. Talanta, 2025: p. 129045.
12. Chen, K., et al., Exploring nucleic acid nanozymes: a new frontier in biosensor development. Biosensors, 2025. 15(3): p. 142.
13. Zhao, J., et al., The advancement of biosensor design and construction utilizing biomolecular motors. Synthetic and Systems Biotechnology, 2025. 10(2): p. 543-554.
14. Hussain, A., et al., Recent Advances in Integrating Graphene into Polymeric Nanocomposite Hydrogels for Biomedical Applications. Macromolecular Bioscience, 2026. 26(2): p. e00553.

15. Malode, S.J., et al., Bio-based nanomaterials for smart and sustainable nano-biosensing and therapeutics. Industrial Crops and Products, 2025. 234: p. 121603.
16. Khumngern, S. and I. Jeerapan, Synergistic convergence of materials and enzymes for biosensing and self-sustaining energy devices towards on-body health monitoring. Communications Materials, 2024. 5(1): p. 135.
17. Melo, R.L.F., et al., A comprehensive review on enzyme-based biosensors: advanced analysis and emerging applications in nanomaterial-enzyme linkage. International Journal of Biological Macromolecules, 2024. 264: p. 130817.
18. Kausaite-Minkstimiene, A., et al., An amperometric enzyme–nanozyme biosensor for glucose detection. Biosensors, 2025. 15(8): p. 545.
19. Kumar, A. and P. Maiti, based sustainable biosensors. Materials Advances, 2024. 5(9): p. 3563-3586.
20. Nazreen, A., et al. Biopolymers-based Electrochemical Sensors: An Overview of Synthesis and Applications. in Acta Biol. Forum. 2024.
21. Guoqiang, G., et al., Recent advances in glucose monitoring utilizing oxidase electrochemical biosensors integrating carbon-based nanomaterials and smart enzyme design. Frontiers in Chemistry, 2025. 13: p. 1591302.
22. Williams, A., et al., Biosensors for public health and environmental monitoring: the case for sustainable biosensing. ACS sustainable chemistry & engineering, 2024. 12(28): p. 10296-10312.
23. Sun, G., et al., Immobilization of enzyme electrochemical biosensors and their application to food bioprocess monitoring. Biosensors, 2023. 13(9): p. 886.
24. Han, Y., J. Wei, and W. Feng, Hybrid of glucose oxidase and enzyme-like Cu&Ce: Efficient glucose scavenging and generation of hydroxyl, hydroperoxyl, and superoxide radicals for wound healing. Materials Today Bio, 2025. 32: p. 101888.
25. Jung, H.H., et al., Wearable electrochemical sensors for real-time monitoring in diabetes mellitus and associated complications. 2024.
26. Harun-Or-Rashid, M., et al., Advances in electrochemical sensors for real-time glucose monitoring. Sensors & Diagnostics, 2024. 3(6): p. 893-913.
27. Yan, Y., et al., Bacterial cellulose as a promising biodegradable bioplastic for sustainability. Nature Communications, 2026.
28. Zahedi, A., R. Liyanapathirana, and K. Thiyagarajan, Biodegradable and renewable antennas for green iot sensors: a review. IEEE Access, 2024. 12: p. 189749-189775.
29. Gokhale, N.A., S.N. Joglekar, and S. Panda, Life cycle assessment of enzymatic and non-enzymatic electrochemical sensors: A comparative study using glucose oxidase and metal nano-catalysts. Journal of Cleaner Production, 2025. 522: p. 146327.
30. Ayoib, A., Hybrid nanomaterials in biosensors for advanced point-of-care diagnostics: DNA-, enzyme-, or immunosensor-based, for healthcare, in Hybrid-nanomaterials: Fabrication, characterization and applications. 2024, Springer. p. 123-153.
31. Fan, Y.-F., Z.-B. Guo, and G.-B. Ge, Enzyme-based biosensors and their applications. 2023, MDPI. p. 476.
32. Jiao, B., Applications of Electrochemical Biosensors across Medical, Environmental and Food Safety Fields. MedScien, 2025. 1(5).
33. Mousavian, Z., et al., Recent Advances in Development of Biosensors for Monitoring of Airborne Microorganisms. Iranian Journal of Biotechnology, 2024. 22(2): p. e3722.
34. Upadhaya, A., et al., Nanoparticle-Enabled Portable Biosensors for Early Detection and Monitoring of Non-Communicable Diseases: A Focus on Diabetes, Cardiovascular, and Cancer Diagnostics. Biosensors and Bioelectronics: X, 2025: p. 100675.
35. Huang, C.-W., et al., A review of biosensor for environmental monitoring: principle, application, and corresponding achievement of sustainable development goals. Bioengineered, 2023. 14(1): p. 58-80.
36. Bains, R.K., et al., Advances in the understanding and exploitation of carbohydrate-active enzymes. Current Opinion in Chemical Biology, 2024. 80: p. 102457.
37. Kaur, D., et al., Pectinases as promising green biocatalysts having broad-spectrum applications: Recent trends, scope, and relevance. Biotechnology and Applied Biochemistry, 2023. 70(5): p. 1663-1678.
38. Lzaod, S. and T. Dutta, Recent advances in the development of oxidoreductase-based biosensors for detection of phenolic antioxidants in food and beverages. ACS omega, 2022. 7(51): p. 47434-47448.
39. Wardman, J.F. and S.G. Withers, Carbohydrate-active enzyme (CAZyme) discovery and engineering via (Ultra) high-throughput screening. RSC Chemical Biology, 2024. 5(7): p. 595-616.
40. Patel, V.B., S. Chatterjee, and A.S. Dhoble, A review on pectinase properties, application in juice clarification, and membranes as immobilization support. Journal of Food Science, 2022. 87(8): p. 3338-3354.
41. Kurup, C.P. and M.U. Ahmed, Nanozymes towards personalized diagnostics: a recent progress in biosensing. Biosensors, 2023. 13(4): p. 461.
42. Abrantes-Coutinho, V.E., et al., Systematic review on lectin-based electrochemical biosensors for clinically relevant carbohydrates and glycoconjugates. Colloids and Surfaces B: Biointerfaces, 2021. 208: p. 112148.
43. Roman-Benn, A., et al., Pectin: An overview of sources, extraction and applications in food products, biomedical, pharmaceutical and environmental issues. Food Chemistry Advances, 2023. 2: p. 100192.
44. Hrmova, M. and J.G. Schwerdt, Molecular mechanisms of processive glycoside hydrolases underline catalytic pragmatism. Biochemical Society Transactions, 2023. 51(3): p. 1387-1403.
45. Cannac, N., et al., A QCM-D-based continuous assay for the study of the enzymatic degradation of insoluble calcium-polygalacturonate. Carbohydrate Polymers, 2025. 364: p. 123759.
46. Bykhovsky, D., Coherence time evaluation in indoor optical wireless communication channels. Sensors, 2020. 20(18): p. 5067.
47. Dong, Y., et al., Enzyme-responsive controlled-release materials for food preservation and crop protection-A review. International Journal of Biological Macromolecules, 2024. 254: p. 128051.
48. Abd El-Aziz, N.M., et al., RETRACTED ARTICLE: Enhancement of novel Endo-polygalacturonase expression in Rhodotorula mucilaginosa PY18: insights from mutagenesis and molecular docking. Microbial Cell Factories, 2023. 22(1): p. 252.
49. Gallemí, M., et al., Dual role of pectin methyl esterase activity in the regulation of plant cell wall biophysical properties. Frontiers in Plant Science, 2025. 16: p. 1612366.
50. Yadav, K., et al., Recent insights into microbial pectin lyases: a review. Process Biochemistry, 2023. 134: p. 199-217.
51. Mallick, P., et al., Revisiting interactions between polygalacturonases and polygalacturonase-inhibiting proteins and their effects on plant health: a review. Frontiers in Plant Science, 2025. 16: p. 1691420.
52. Stanek-Wandzel, N., et al., Evaluation of cellulase, pectinase, and hemicellulase effectiveness in extraction of phenolic compounds from grape pomace. International journal of molecular sciences, 2024. 25(24): p. 13538.
53. Li, P., et al., Improvement of optimum pH and specific activity of pectate lyase from Bacillus RN. 1 using loop replacement. Frontiers in Bioengineering and Biotechnology, 2023. 11: p. 1242123.
54. Chandel, V., et al., Current advancements in pectin: Extraction, properties and multifunctional applications. Foods, 2022. 11(17): p. 2683.
55. Rößling, A.-K., et al., Pectin methylesterase activity is required for RALF1 peptide signalling output. Elife, 2024. 13: p. RP96943.
56. Zhang, H. and F.-Q. Yang, A paper-based lateral flow strip assay based on enzyme-mediated pectin viscosity change for the determination of polygalacturonase activity. Microchemical Journal, 2023. 186: p. 108322.
57. Alqahtani, Y.S., et al., Production and purification of pectinase from Bacillus subtilis 15A-B92 and its biotechnological applications. Molecules, 2022. 27(13): p. 4195.
58. Pathan, S.U., et al., Enzymes as indispensable markers in disease diagnosis. Bioanalysis, 2024. 16(10): p. 485-497.
59. Singh, R., et al., A review on recent trends and future developments in electrochemical sensing. ACS omega, 2024. 9(7): p. 7336-7356.
60. Naresh, V. and N. Lee, A review on biosensors and recent development of nanostructured materials-enabled biosensors. Sensors, 2021. 21(4): p. 1109.
61. Chen, C. and J. Wang, Optical biosensors: An exhaustive and comprehensive review. Analyst, 2020. 145(5): p. 1605-1628.
62. Zhou, J., et al., Recent advances in design and application of nanomaterials-based colorimetric biosensors for agri-food safety analysis. ACS omega, 2023. 8(49): p. 46346-46361.
63. Pohanka, M., Piezoelectric chemosensors and biosensors in medical diagnostics. Biosensors, 2025. 15(3): p. 197.

64. Skládal, P., Piezoelectric biosensors: shedding light on principles and applications. Microchimica Acta, 2024. 191(4): p. 184.
65. Sonowal, K., P.P. Borthakur, and K. Pathak, Advances in Enzyme-Based Biosensors: Emerging Trends and Applications. Engineering Proceedings, 2025. 106(1): p. 5.
66. Sadani, K., et al., Enzymatic optical biosensors for healthcare applications. Biosensors and Bioelectronics: X, 2022. 12: p. 100278.
67. Majer-Baranyi, K., A. Székács, and N. Adányi, Application of electrochemical biosensors for determination of food spoilage. Biosensors, 2023. 13(4): p. 456.
68. Li, D., et al., Ripening induced degradation of pectin and cellulose affects the far infrared drying kinetics of mangoes. Carbohydrate Polymers, 2022. 291: p. 119582.
69. Navarro-López, D.E., et al., Nanocatalytic performance of pectinase immobilized over in situ prepared magnetic nanoparticles. Heliyon, 2023. 9(8).
70. Said, N.S. and W.Y. Lee, Pectin-based active and smart film packaging: a comprehensive review of recent advancements in antimicrobial, antioxidant, and smart colorimetric systems for enhanced food preservation. Molecules, 2025. 30(5): p. 1144.
71. Liu, Y., et al., A highly sensitive, accurate, and stable method for measuring pectin depolymerase activity. Food Chemistry, 2025. 463: p. 141229.
72. Nazari-Vanani, R. and M. Negahdary, Recent advances in electrochemical aptasensors and genosensors for the detection of pathogens. Environmental Research, 2024. 243: p. 117850.
73. Gao, S., et al., Magnetic nanoparticle-based immunosensors and aptasensors for mycotoxin detection in foodstuffs: An update. Comprehensive Reviews in Food Science and Food Safety, 2024. 23(1): p. e13266.
74. Guan, Y., et al., Recent advances in microbial whole-cell biosensors for detecting food contaminants: a review. Food Control, 2025: p. 111674.
75. Bergman, G.Ö., et al., Engineering chimeric signaling proteins for microbial whole-cell biosensors: from design to deployment. Trends in Biotechnology, 2025.
76. Feng, Y., et al., Advancing food safety surveillance: rapid and sensitive biosensing technologies for foodborne pathogenic bacteria. Foods, 2025. 14(15): p. 2654.
77. Basu, S. and G. Bisker, Near-Infrared Fluorescent Single-Walled Carbon Nanotubes for Biosensing. Small, 2025. 21(26): p. 2502542.
78. Wang, F., et al., Application of immobilized enzymes in juice clarification. Foods, 2023. 12(23): p. 4258.
79. Hasanah, U., et al., Construction of a hydrogel pectin-based triglyceride optical biosensor with immobilized lipase enzymes. Biosensors, 2019. 9(4): p. 135.
80. Zhao, M., et al., Advances in Pectinase Engineering for Food Bioprocessing: Novel Sources, Mechanisms, and Optimization Strategies. Journal of Agricultural and Food Chemistry, 2025. 73(37): p. 23078-23097.
81. Mustafa, F., S. Liebich, and S. Andreescu, Nanoparticle-based amplification for sensitive detection of β-galactosidase activity in fruits. Analytica Chimica Acta, 2021. 1186: p. 339129.
82. Zhao, Z., et al., Recent advances in MXene-based electrochemical sensors. Biosensors, 2025. 15(2): p. 107.
83. Ali, M., et al., Unlocking the Potential of MXene-Based Electrochemical Biosensors: A Review of Biofunctionalization Strategies and Biosensing Principles. Advanced Materials Technologies, 2026. 11(12): p. e02120.
84. Fakhr, M.H., et al., Recent advances in wearable electrochemical biosensors towards technological and material aspects. Biosensors and Bioelectronics: X, 2024. 19: p. 100503.
85. Zhu, S., et al., AI-assisted molecular biosensors: design strategies for wearable and real-time monitoring. International Journal of Molecular Sciences, 2026. 27(7): p. 3305.
86. Abdel-Mageed, H.M., Frontiers in nanoparticles redefining enzyme immobilization: a review addressing challenges, innovations, and unlocking sustainable future potentials. Micro and Nano Systems Letters, 2025. 13(1): p. 7.
87. Baruah, A., et al., Biomedical applications of graphene-based nanomaterials: recent progress, challenges, and prospects in highly sensitive biosensors. Discover Nano, 2024. 19(1): p. 103.
88. Zhang, J., et al., Emerging biosensors integrated with microfluidic devices: a promising analytical tool for on-site detection of mycotoxins. npj Science of Food, 2025. 9(1): p. 84.
89. Weston, M., et al., Anthocyanin-based sensors derived from food waste as an active use-by date indicator for milk. Food Chemistry, 2020. 326: p. 127017.
90. Bhatlawande, A.R., et al., Unlocking the future of smart food packaging: biosensors, IoT, and nano materials: AR Bhatlawande et al. Food Science and Biotechnology, 2024. 33(5): p. 1075-1091.
91. Meliana, C., et al., Biosensor in smart food traceability system for food safety and security. Bioengineered, 2024. 15(1): p. 2310908.
92. Ayenimo, J.G. and S.B. Adeloju, Amperometric detection of glucose in fruit juices with polypyrrole-based biosensor with an integrated permselective layer for exclusion of interferences. Food chemistry, 2017. 229: p. 127-135.
93. Lipińska, W., K. Grochowska, and K. Siuzdak, Enzyme immobilization on gold nanoparticles for electrochemical glucose biosensors. Nanomaterials, 2021. 11(5): p. 1156.
94. Abdullah, A., et al., Microfluidic based impedance biosensor for pathogens detection in food products. Electrophoresis, 2019. 40(4): p. 508-520.
95. Wang, B., D. Huang, and Z. Weng, Recent advances in polymer-based biosensors for food safety detection. Polymers, 2023. 15(15): p. 3253.
96. Bucur, B., et al., Advances in enzyme-based biosensors for pesticide detection. Biosensors, 2018. 8(2): p. 27.
97. Valenzuela-Amaro, H.M., et al., Emerging applications of nanobiosensors in pathogen detection in water and food. Biosensors, 2023. 13(10): p. 922.
98. Gan, Q., et al., Advancing lignocellulosic conversion though biosensor-enabled metabolic engineering. Green Chemistry, 2025. 27(33): p. 9862-9873.
99. Salek Maghsoudi, A., et al., Recent advances in nanotechnology-based biosensors development for detection of arsenic, lead, mercury, and cadmium. International Journal of Nanomedicine, 2021: p. 803-832.
100. Pontius, K., et al., Automated electrochemical glucose biosensor platform as an efficient tool toward on-line fermentation monitoring: novel application approaches and insights. Frontiers in Bioengineering and Biotechnology, 2020. 8: p. 436.
101. Yang, L., et al., A biosensor based on oriented immobilization of an engineered l-glutamate oxidase on a screen-printed microchip for detection of l-glutamate in fermentation processes. Food Chemistry, 2023. 405: p. 134792.
102. Waly, Y., et al., Evolution of Biosensors and Current State-of-the-Art Applications in Diabetes Control. Biosensors, 2026. 16(1): p. 39.
103. Parihar, M., et al., Point-of-care biosensors for infectious disease diagnosis: recent updates and prospects. RSC advances, 2025. 15(36): p. 29267-29283.
104. Ondevilla, N.A.P., et al., A point-of-care electrochemical biosensor for the rapid and sensitive detection of biomarkers in murine models with LPS-induced sepsis. Biosensors and Bioelectronics, 2024. 254: p. 116202.
105. Han, Q., et al., Graphene biodevices for early disease diagnosis based on biomarker detection. ACS sensors, 2021. 6(11): p. 3841-3881.
106. GeGen, S., et al., Advances in aptamer-based electrochemical biosensors for disease diagnosis: integration of DNA and nanomaterials. Nanoscale Horizons, 2025. 10(11): p. 2668-2687.
107. Ansari, S.A., et al., Recent updates on stabilization of recombinant enzymes via nanotechnology-based approaches. Discover Food, 2024. 4(1): p. 159.
108. Wang, J., Y. Zeng, and W. Wang, Recent Advances in Enzyme Activity Assay Methods: A Systematic Review of Emerging Technologies and Applications. Analytical Chemistry, 2026.
109. Abdullahi, H.Y., et al., Spotlight on pectinase: a comprehensive review of large-scale production strategies. Critical Reviews in Biotechnology, 2026: p. 1-21.
110. Rahimnejad, M., R. Abd Alsaheb, and M. Atyia, Current developments in biosensors for monitoring environmental quality: A critical review. 2025.
111. Sivakumar, S., et al., Bioengineered Nano-sorbents: Microbial Allies in Pollutant Sequestration, in Nano-microbiology for Sustainable Development. 2025, Springer. p. 331-372.
112. Yusuf, M. and M. Shahid, Biotechnology Approaches in Textile Technology: Progress and Trends. 2025: CRC Press.

113. Malzacher, S., et al., The STRENDA Biocatalysis Guidelines for cataloguing metadata. Nature Catalysis, 2024. 7(12): p. 1245-1249.
114. Zahra, H., et al., From nature to industry: harnessing the power of pectinase towards potential biotechnological applications. Int. J. Biol. Biotech., 2024. 21(1): p. 17-35.
115. Mor, R., A review on development of enzymatic biosensors for industrial applications. World journal of advanced Research and Reviews, 2024. 23(1): p. 2582-2590.