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Review

A Review of Electrochemical Sensors and Biosensors for Ethanol Detection in Beverages

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***Abstract*— Ethanol detection is critical in the beverage industry, where it is essential to monitor alcohol concentrations for quality control and compliance with regulatory standards. Traditional analytical methods, such as gas chromatography and distillation, offer accuracy but are often labor-intensive, time-consuming, and require sophisticated equipment. In contrast, electrochemical sensors and biosensors have emerged as promising alternatives due to their rapid response, portability, cost-effectiveness, and potential for real-time monitoring. Electrochemical sensors, particularly those enhanced with metal nanoparticles like platinum, palladium, or gold, have shown significant improvements in sensitivity, selectivity, and response time. These sensors offer the advantage of miniaturization, making them ideal for on-site analysis, although issues such as electrode stability, susceptibility to interference, and long-term reliability remain. On the other hand, biosensors, which leverage biorecognition elements like alcohol dehydrogenase (ADH) or alcohol oxidase, provide high specificity for ethanol, reducing interference from other compounds commonly found in beverage samples. Recent advancements in biosensor technology have focused on improving sensor stability, enzyme immobilization techniques, and reducing production costs. While biosensors offer high selectivity and sensitivity, they may still face challenges related to enzyme denaturation and environmental factors such as temperature and pH fluctuations. Both electrochemical sensors and biosensors are continuously evolving, with recent developments including the use of nanomaterials and novel biorecognition elements to enhance performance. This review will explore recent advances in electrochemical sensors and biosensors for ethanol detection in beverage samples, highlighting their potential, challenges, and future directions in this field.**

***Keywords*— Electrochemistry, ethanol, sensor, biosensor, beverage analysis.**

I. INTRODUCTION

Ethanol, the primary psychoactive component in alcoholic beverages has been produced and consumed since ancient times through fermentation processes dominated by *S. cerevisiae* due to its high ethanol tolerance and metabolic efficiency [1]–[3]. Today, ethanol quantification remains essential for ensuring product quality, regulatory compliance, and taxation [4].

Conventional analytical techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), refractometry, and spectrophotometry are widely reagrded as standard methods for ethanol detection [5]–[8]. However, their reliance on expensive instrumentation, complex simple preparation, and centralized laboratory operation limits their applicability for real-time monitoring or portbale solutions.

Despite advances in analytical chemistry, a significant unmet need exists for ethanol detection systems that combine the accuray of traditional methods with the portability, speed, and cost-efficiency required for modern applications. Current electrochemical sensors, while promising, still face challenges in selectivity such as interference from sugars or other alcohols, long-term stability like enzyme degradation in biosensors, and standardization across diverse beverage matrices [9], [10].

Furthermore, most studies focus on aqueous ethanol solutions rather than complex real-world samples like fermented beverages or biological fluids, creating a translational gap between laboratory research and industrial or clinical implementation [11], [12]. Electrochemical sensors address many of these limitations by leveraging redox reactions to generate measurable electrical signals from ethanol oxidation. Their miniaturization potential, rapid response (<30 s), and adaptability to portable formats make them ideal for on-site testing. Recent innovations in nanomaterials, enzyme immobilization techniques, and machine learning-assisted signal processing have begun to overcome historical barriers in selectivity and reproducibility [13].

This review provides a comprehensive and critical analysis of electrochemical approaches for ethanol detection in beverages, spanning both non-enzymatic sensors and enzymatic biosensors. We examine how nanostructured metals and metal oxides (Pt, Pd, Au, ZnO, CuO) enhance non-enzymatic sensors through improved electron transfer and catalytic activity, while also evaluating recent advances in enzymatic systems using alcohol oxidase (AOx) and alcohol dehydrogenase (ADH), with particular attention to enzyme stabilization techniques and novel immobilization strategies. The discussion systematically

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compares detection principles (amperometry, voltammetry, impedimetry), fabrication methods (screen printing, nanomaterial modification), and performance metrics (sensitivity, selectivity, response time) across these platforms, while critically assessing their real-world applicability in terms of cost, portability, and compliance with industry standards. By highlighting current limitations, including electrode fouling, interference effects, and enzyme instability, and emerging solutions like hybrid designs and AI-assisted calibration, this review not only synthesizes the state-of-the-art but also charts a clear pathway toward next-generation ethanol detection systems that balance analytical performance with practical implementation in beverage quality control.

II. ELECTROCHEMICAL SENSOR

Electrochemistry is a specialized field within chemistry that investigates the relationship between electrical energy and chemical changes, particularly those involving electron transfer in conductive media. At the heart of electrochemical systems are electrodes, typically composed of a working electrode, a reference electrode, and a counter electrode, which serve as interfaces between an electrical circuit and an ionic conductor such as an electrolyte. The foundation of electrochemical processes lies in redox (reduction-oxidation) reactions, where oxidation and reduction occur simultaneously at separate electrodes. By applying a controlled electrical potential, these redox reactions can be initiated, producing a measurable electric current that is directly related to the presence and concentration of specific analytes [14]. Various electrochemical techniques, including potentiometry, amperometry, voltammetry, impedance spectroscopy, and conductometry, have been developed to exploit this principle for analytical applications [15], [16]. Electrochemical sensors, a crucial application of these techniques, function by converting chemical information, such as analyte concentration or sample composition into an electrical signal using a transducer. The illustration can be seen in Figure 1. As defined by the IUPAC, these sensors are capable of detecting, identifying, and quantifying chemical substances by responding to interactions between analytes and recognition elements, typically producing an electrical output that is analytically meaningful [17], [18]. Due to their inherent advantages including high sensitivity and selectivity, miniaturized design, low cost, and suitability for real-time and automated analysis electrochemical sensors have become essential tools in modern analytical chemistry, with wide-ranging applications in environmental monitoring, clinical diagnostics, industrial process control, and food safety [19], [20].

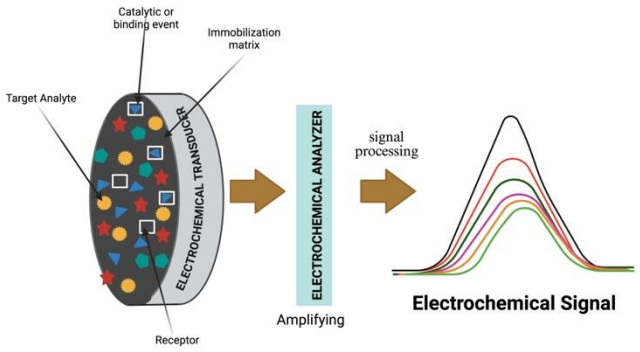

Figure. 1 Electrochemical biosensor workflow: The target analyte binds to an immobilized receptor, triggering a catalytic/binding event. This generates an electrochemical signal, which is amplified and processed for detection.

The advantages of electrochemical methods over conventional techniques include not only operational simplicity and cost-efficiency but also enhanced adaptability to modern analytical needs. These systems allow for quick analysis with high sensitivity, excellent redox reversibility, and robust performance even under varying environmental conditions. A typical electrochemical sensor comprises a transducer system, where a measurand (the analyte of interest) interacts with a chemically selective recognition element—commonly an electrode. Upon this interaction, a chemical change occurs that generates an electrical signal. This signal is then amplified and processed by an integrated electronic circuit to yield a readable output. These characteristics make electrochemical systems highly versatile for monitoring ethanol in diverse beverage matrices and point-of-care settings.

Among the most widely used electrochemical methods is voltammetry, which involves the application of a time-varying potential to an electrochemical cell and measurement of the resulting current. The data are typically presented in the form of a voltammogram, a plot of current versus potential, which reveals valuable information about redox-active species [21]. One variant, differential pulse voltammetry (DPV) enhances analytical sensitivity and resolution by superimposing potential pulses on a linearly increasing base potential and recording the difference in current before and after each pulse. This method produces sharp, well-defined peaks with high signal-to-noise ratios, making it ideal for detecting low concentrations of analytes [22]. DPV response can be fine-tuned by adjusting parameters such as scan rate, pulse amplitude, and electrode surface properties, further improving analytical performance [23]. Another prominent technique, cyclic voltammetry (CV), involves sweeping the potential linearly between two set values and then reversing the scan, allowing for the observation of both oxidation and reduction reactions within a single cycle [24], [25]. CV is versatile, simple, and informative, capable of revealing key characteristics such as reaction reversibility, redox potential, and diffusion kinetics. The peak currents in CV typically increase with scan rate, indicating diffusion-controlled processes and enabling kinetic analysis. These techniques are

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underpinned by mathematical models such as the Randles–Sevcik equation, which relates peak current to variables like analyte concentration, scan rate, and diffusion coefficient [26].

Electrochemical sensors are particularly valuable in the detection of ethanol, a compound of importance in clinical, environmental, and industrial contexts. Ethanol detection can be achieved either directly, through its oxidation at modified electrodes using metal or metal oxide catalysts, or indirectly via enzymatic methods employing enzymes such as alcohol dehydrogenase. In both cases, the redox reaction produces a measurable electrical signal—either current or potential—that is proportional to ethanol concentration. The choice between direct and enzymatic approaches depends on the required sensitivity, stability, and selectivity of the sensor. While enzymatic sensors offer superior selectivity, non-enzymatic sensors often provide greater stability and ease of fabrication. Ultimately, the continued advancement of electrode materials, nanostructuring techniques, and surface modifications is expanding the capabilities and applications of electrochemical ethanol sensors, making them indispensable in a wide array of analytical domains [27].

Electrochemical sensors and biosensors have emerged as compelling alternatives due to their rapid response, miniaturization potential, high stability, and low operating costs. These devices offer the flexibility to be incorporated into portable or even wearable formats, making them ideal for real-time, in-field ethanol analysis. The electrochemical process typically relies on the application of direct current (DC) to drive oxidation-reduction reactions. In such systems, positively charged ions (cations) migrate towards the cathode (negatively charged electrode), and negatively charged ions (anions) move toward the anode (positively charged electrode), resulting in metal deposition at the cathode and oxidation at the anode [28]. This redox activity underpins the sensor's ability to transduce a chemical signal into a measurable electrical output, which forms the basis of analyte detection. Advantages, disadvantages, and strategies of further challenges of two methods can be seen in Table 1.

Table 1. Comparison of Electrochemical Sensors vs. Biosensors for Ethanol Detection

Aspect	Electrochemical Sensors	Biosensors
Advantages	- Fast response time - High stability - Works in harsh conditions (pH/temperature) - Lower cost (no enzymes) - Ease of miniaturization	- High specificity (enzyme-based recognition) - Excellent selectivity (minimal interference) - Suitable for complex matrices (e.g., beverages) - Tunable sensitivity (via enzyme loading)
Disadvantages	-Susceptible to electrode fouling - Interference from other oxidizable compounds (e.g., sugars, acids) - Requires surface regeneration - Limited selectivity in multicomponent samples	- Enzyme denaturation over time - Sensitive to pH/temperature changes - Higher cost (enzyme purification/immobilization) - Shorter operational lifetime
Strategies to Overcome Challenges	- Nanomaterial modification (e.g., Pt/Au NPs for anti-fouling) - Hybrid sensing layers (e.g., molecularly imprinted polymers for selectivity) - Advanced signal processing (machine learning for interference correction)	- Enzyme engineering (thermostable ADH/AOx mutants) - Improved immobilization (nanocarriers, cross-linking) - Protective membranes (e.g., Nafion for pH stability) - Disposable/low-cost substrates (paper-based electrodes)

III. ALCOHOL PRODUCTION THROUGH BEVERAGE FERMENTATIONAIN

As previously mentioned in the introduction, alcoholic fermentation is commonly carried out using yeast. There are two principal brewing methods: top-fermenting and bottom-fermenting. These methods yield two distinct types of beer, ale and lager, respectively. The primary differences between these techniques lie in the strain of yeast employed and the temperature at which fermentation occurs. Additionally, the terms "top" and "bottom" refer to the position of yeast cells at the conclusion of the fermentation process. The major yeast strains commonly used in the fermentation of alcoholic beverages are presented in Table 2 [29].

Table 2. Fermentation Methods, Beverages, Yeast Strains, and Conditions

Beverage Type	Fermentation Type	Yeast Species/Strains	Ferment. Temp.
Ale	Top-fermenting	*Saccharomyces cerevisiae*	60–70 °F
Lager	Bottom-fermenting	*Saccharomyces pastorianus*	35–50 °F
Beer (general)	Mixed	*S. cerevisiae*, *S. pastorianus*, *Brettanomyces bruxellensis*	Varies
Wine	--	*S. cerevisiae*, *S. bayanus*	Varies
Whiskey	--	*S. cerevisiae*	Varies
Rum	--	*S. cerevisiae*, *Schizosaccharomyces pombe*	Varies
Brandy, Gin, Vodka	--	*S. cerevisiae*	Varies

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Beverages from Cheese Whey	--	*Kluyveromyces marxianus*	Varies

Note: Dashes (--) indicate that specific fermentation or temperature is not typically categorized in those beverages or varies based on the production process.

This discussion focuses particularly on *S. cerevisiae*. There are two fundamental requirements for successful fermentation using *S. cerevisiae*: physical conditions and nutrient availability. One critical physical factor is the presence of water, essential for maintaining yeast cell physiology. High sugar concentrations in the fermentation medium can exert osmotic pressure on yeast cells, leading to stress responses. A common reaction by yeast cells under water stress is the excessive production of glycerol, which subsequently reduces ethanol yield [29].

Temperature and pH also significantly influence yeast activity. *S. cerevisiae* grows optimally within a temperature range of 20–30 °C and a pH of 4.5–6.5. Oxygen availability is another consideration. Although *S. cerevisiae* is classified as a facultative anaerobe, it still requires oxygen for the biosynthesis of essential fatty acids such as oleic acid and sterols like ergosterol. An alternative to oxygen supplementation is to provide these compounds—fatty acids and sterol growth factors—directly in the fermentation medium [29]. The nutrients required during fermentation, categorized by the type of fermentation media, are summarized in Table 3.

Table 3. Key Nutrients Available from Fermentation Media in Alcoholic Beverage Production

Component	Molasses	Grains	Grapes	Cheese Whey
Carbon Source	Sucrose, glucose, fructose, raffinose	Glucose, maltose, maltotriose	Glucose, fructose	Lactose
Nitrogen Source	–	Amino acids	Amino acids	Amino acids, albumin, globulin
Minerals	P, S available, small amounts of K and Ca	Most minerals available except Zn	Most minerals available, except Zn and Mg	Most minerals available
Vitamins	Most vitamins available, except biotin	Most vitamins available, except biotin	Most vitamins available	Biotin, pyridoxine, thiamine available
Minor Components	Betaine, organic acids, waxes, silica	Maltodextrin	Pentoses, fatty acids	High in lactic acid, fats, and dietary fiber

Fermentation in these media begins with glycolysis, the metabolic process that converts glucose into pyruvate, as represented in the following reaction:

Glycolysis Reaction:

$Glucose + 2\ ADP + 2\ Pi + 2\ NAD^+ \rightarrow 2\ Pyruvate + 2\ ATP + 2\ NADH + 2\ H^+$

Through this process, yeast cells acquire both energy (in the form of ATP) and reduce power via the production of NADH. During anaerobic alcoholic fermentation, *S. cerevisiae* regenerates NAD^+ to maintain redox balance and sustain glycolysis. This regeneration occurs through the following biochemical reaction:

Alcoholic Fermentation Reaction:

$2\ Pyruvate + 2\ NADH + 2\ H^+ \rightarrow 2\ NAD^+ + 2\ Ethanol + 2\ CO_2$

In this reaction, acetaldehyde serves as an intermediate electron acceptor and is produced through the decarboxylation of pyruvate. The complete transformation can be described as:

Pyruvate Decarboxylation and Ethanol Formation:

$CH_3COCOOH \rightarrow CH_3CHO + CO_2 \rightarrow CH_3CH_2OH$
[29].

Here is the revised and cleaned-up version of the section, with all entries unrelated to beverages removed. This applies both to the text and the table, keeping only sensors and biosensors used in beverage matrices like wine, beer, spirits, etc.

Electrochemical principles such as electron transfer kinetics, mass transport, and interfacial reactions can be applied to ethanol detection, but they require careful adaptation. The matrix in alcoholic beverages presents unique challenges, including interference from sugars, acids, and polyphenols, as well as variations in pH and viscosity that can disrupt signals and reduce accuracy. Therefore, effective ethanol detection requires optimized design, including the electrode materials used, surface modifications, and measurement methods that are fine-tuned not only for pure ethanol but also for alcoholic beverages such as wine, beer, and long-stored fruit juices. This critical balance between fundamental electrochemistry and real-world applicability drives innovation in sensor development.

IV. Electrochemical Sensors and Biosensors for Monitoring Alcohol Fermentation

A. Monitoring Alcohol Fermentation Using Electrochemical Sensors

The development of electrochemical sensors and biosensors for ethanol detection has seen significant advancements, particularly in their application to real beverage samples. Several studies have demonstrated the effectiveness of these

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biosensors in accurately measuring ethanol levels in various alcoholic beverages, showcasing their potential for quality control and regulatory compliance. Electrochemical sensors for monitoring alcohol fermentation are commonly enhanced with metallic nanoparticles to improve their efficiency, such as Nickel, Palladium, and Platinum. For example, an ethanol sensor utilizes a nickel electrode modified with a ruthenium oxide (RuO_2) thin film, which facilitates the electrocatalytic oxidation of ethanol in alkaline media through a synergistic redox mechanism involving surface-bound Ru species. This sensor is unique in its non-enzymatic design, providing enhanced stability, reproducibility, and sensitivity compared to conventional enzyme-based sensors, due to the high catalytic activity and electronic conductivity of the RuO_2 layer. With a detection limit of 0.1 mM, the sensor demonstrated accurate and reliable performance in real sample matrices such as commercial alcoholic beverages, confirming its practical applicability for ethanol quantification in complex environments [30]. On the other hand, Tao et. al. also use Ni based electrode, Pd–Ni/SiNWs as ethanol sensor employs a nanostructured silicon nanowire (SiNW) substrate co-deposited with palladium and nickel, where each component plays a critical role in enhancing ethanol detection performance. The SiNWs provide a high surface-to-volume ratio and nanoscale curvature that significantly increase the active electrochemical area and facilitate rapid electron transfer, while the co-plated Ni layer contributes to the electrocatalytic oxidation of ethanol through the formation of $Ni(OH)_2/NiOOH$ redox couples. The trace Pd component enhances catalytic efficiency at lower potentials, promotes oxidative intermediates like CH_3COO^-, and ensures stable redox cycling, resulting in excellent stability and sensitivity—with detection limits as low as 6 μM (via cyclic voltammetry) and 10 μM (via fixed potential amperometry) in 1 M KOH, demonstrating the sensor's applicability for precise ethanol quantification in aqueous [31].

Liu et al. (2010) utilized NiCFP ethanol sensor, constructed using a novel composite of nickel nanoparticles embedded in electrospun carbon fibers, enabling efficient electrocatalytic oxidation of ethanol through the formation of Ni(III) oxyhydroxide species in alkaline media. The carbon fiber matrix not only provides a high surface area and excellent conductivity but also ensures mechanical stability and minimizes fouling, while the embedded Ni nanoparticles serve as active sites for ethanol oxidation, leading to a high current response, reproducibility, and long-term operational stability. The sensor exhibits a detection limit of 0.25 mM with a wide linear range up to 87.5 mM, and it successfully quantified ethanol in real liquor samples with results consistent with labeled concentrations, demonstrating its practical applicability for commercial and industrial use [32].

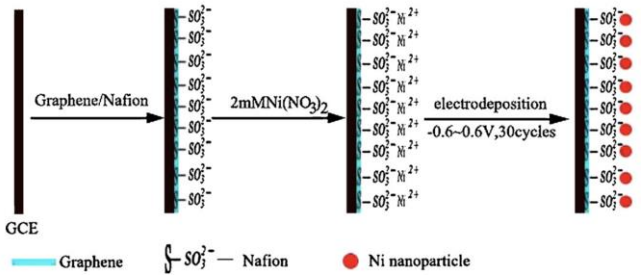

Figure 2. The GCE is modified with a graphene/Nafion layer, followed by immersion in 2 mM $Ni(NO_3)_2$ to load Ni^{2+} ions. Electrodeposition (−0.6 to 0.6 V, 30 cycles) forms Ni nanoparticles on the surface, yielding a Ni-decorated graphene/Nafion-modified electrode [33].

Jia et al. developed a Ni/Nafion/graphene ethanol sensor combines the catalytic properties of nickel nanoparticles enhancing sensitivity, the ionic conductivity of Nafion improving signal response and stability, and the high surface area and excellent electron mobility of graphene to facilitate efficient ethanol oxidation via the Ni(II)/Ni(III) redox cycle in alkaline media facilitating faster electron transfer and better adsoprtion of ethanol molecules. The illustration can be seen in Figure 2. Graphene enhances charge transfer and provides a robust support for homogeneous Ni nanoparticle deposition, while Nafion assists in selectively incorporating Ni^{2+} ions and stabilizing the film, together resulting in a highly dispersed catalytic network with rapid electron/proton transport. The sensor exhibits a low detection limit of 0.12 mM, a broad linear range (0.43–88.15 mM), and accurate ethanol quantification in real liquor samples, validating its practical utility for complex matrices [33].

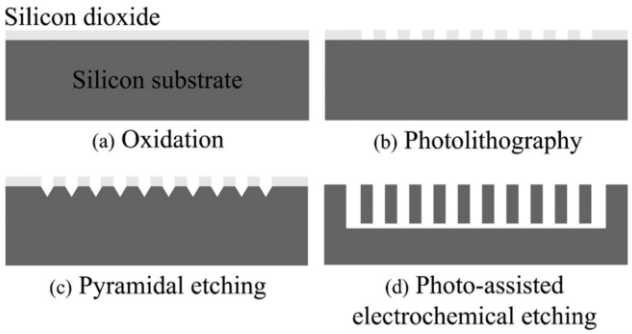

Figure 3. The silicon substrate is first thermally oxidized to form a silicon dioxide layer (a), followed by photolithography to pattern the oxide surface (b). Pyramidal etching creates textured features on the silicon surface (c), which are then further processed using photo-assisted electrochemical etching to form well-defined microstructures (d) [35].

Another catalyst that is also often used for ethanol oxidation is Pd. However, Pd nanoparticles (PdNPs) alone, without co-catalysts, demonstrated lower catalytic activity [34]. Shi et. al. combined Ni and Pd and develop Pd/Ni/Si-MCP ethanol sensor features a 3D silicon microchannel plate (MCP) structure coated with nickel and decorated with palladium nanoparticles,

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each component synergistically enhancing ethanol detection. The illustration can be seen in Figure 3. MCP architecture offers a high surface-to-volume ratio and efficient mass transport, while the Ni layer restores conductivity and supports uniform Pd deposition; Pd nanoparticles serve as the key electrocatalyst, facilitating ethanol oxidation via adsorbed acetaldehyde intermediates in alkaline media. The sensor demonstrates high sensitivity ($0.992\ mA\ mM^{-1}\ cm^{-2}$), a low detection limit (16.8 µM), and a wide linear range up to 60 mM, with high stability and reproducibility, making it well-suited for ethanol quantification in real-world applications [35]. This was also confirmed by Tavakolian et al., whose findings showed that ZnO-modified Pd electrodes yielded better ethanol oxidation currents than bare Pd [36].

Pt was one of the most used electrocatalyst for oxidation of organic compound, due to its high catalytic activity towards hydrogen. This was also applied to ethanol. Neves et al. developed a disposable ethanol sensor in the form of a nanostructured catalytic surface consisting of platinum nanoparticles (PtNPs) immobilized on screen-printed carbon electrodes (SPCEs), exploiting the high catalytic activity and large surface area of PtNPs to enhance ethanol electro-oxidation in alkaline media. The PtNPs facilitate electron transfer and selectively catalyze ethanol oxidation via a dual-path mechanism, while the SPCE platform offers portability, disposability, and compatibility with miniaturized instrumentation, making it highly suited for rapid in situ analysis. The sensor demonstrates a detection range of 700–4700 ppm, accurate quantification of ethanol in real alcoholic beverages (beer and wine), and excellent specificity against common interfering substances, aligning with EU tolerance limits for alcoholic labeling. developed a sensor modified with Pt nanoparticles (PtNPs), capable of detecting ethanol at –0.8 V through interactions between PtNPs and the hydroxyl group of ethanol [37].

Electrochemical sensors for ethanol detection have advanced significantly, especially for real beverage analysis, offering high sensitivity, stability, and practicality. Key developments include non-enzymatic designs using metal nanoparticles like Ni, Pd, and Pt to enhance catalytic performance. Nickel-based sensors, such as those incorporating RuO_2 films or nanostructured SiNWs co-deposited with Pd, show excellent redox activity and detection limits as low as 6 µM. Carbon fiber-based Ni sensors and graphene/Nafion-modified electrodes further improve sensitivity, conductivity, and operational stability. Pd-based sensors benefit from structural supports like Si microchannel plates or ZnO modifiers, while PtNP-modified screen-printed electrodes offer portable, disposable solutions with strong ethanol oxidation capabilities. These innovations demonstrate the practical utility of electrochemical sensors for accurate, real-time ethanol monitoring in complex media.

B. Monitoring Alcohol Fermentation Using Electrochemical Biosensors

A biosensor is a detection device that utilizes a biorecognition element as its receptor. Enzymes serve as excellent biorecognition elements due to their high selectivity. The most used enzymes for alcohol detection are AOx and ADH [38].

The developed ethanol biosensor integrates a bienzymatic system—carboxyl esterase and alcohol oxidase—immobilized within a gelatin matrix on a graphite epoxy composite electrode (GECE), enabling selective detection of ethanol via oxygen consumption monitoring. In this configuration, alcohol oxidase catalyzes the oxidation of ethanol to acetaldehyde with simultaneous oxygen reduction, and the GECE provides a stable, conductive, and renewable surface ideal for enzyme immobilization and signal transduction. The biosensor exhibited a low detection limit with a linear response range of 2.5–25 µM for ethanol and demonstrated accurate quantification in real wine and beer samples with recoveries between 97.6–105.3%, highlighting its practicality for low-concentration ethanol detection in complex beverage matrices [39].

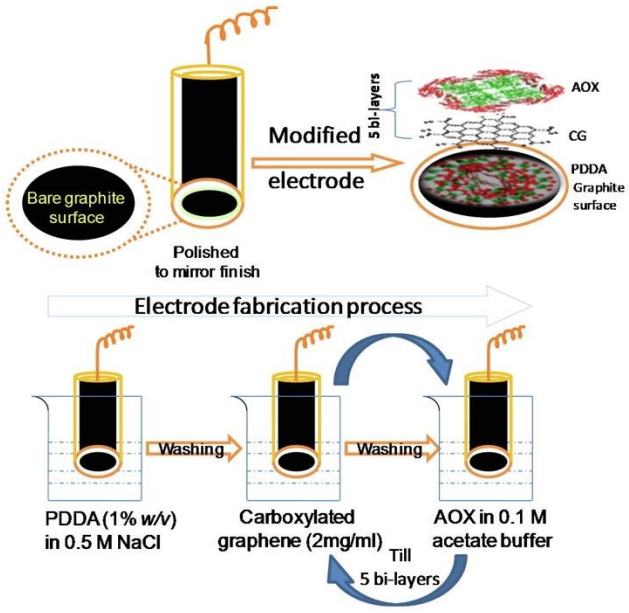

Figure 4. A bare graphite electrode is polished to a mirror finish and modified through a layer-by-layer assembly process. The electrode is sequentially immersed in PDDA (1% w/v in 0.5 M NaCl), carboxylated graphene (2 mg/mL), and AOX in 0.1 M acetate buffer, with washing steps in between. This cycle is repeated until 5 bilayers are formed, resulting in a composite-modified electrode with enhanced surface functionality [41].

A copolymer-based ethanol biosensor was developed by electropolymerizing TBeSe-co-P3CA onto a graphite electrode, creating a conductive, enzyme-friendly surface where alcohol oxidase was immobilized via carboxyl groups, enabling efficient electron transfer and high sensitivity. Another approach used a layer-by-layer assembly of carboxylated graphene and alcohol oxidase on a PDDA-modified graphite electrode, where graphene provided a large surface area and enhanced conductivity, supporting direct electron transfer from the enzyme. The illustration can be seen in Figure 4. Both sensors achieved low detection limits—0.052 mM and

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0.050 mM respectively—and demonstrated reliable ethanol quantification in real alcoholic beverages with recoveries close to 100% [40], [41]. The paper-based ethanol biosensor combines a nanocomposite of carbon black and Prussian Blue nanoparticles (CB/PBNPs) with alcohol oxidase immobilized on a screen-printed electrode fabricated on common office paper. The CB/PBNPs enable low-potential and highly sensitive detection of hydrogen peroxide, the enzymatic byproduct of ethanol oxidation, while the paper substrate offers flexibility, low cost, and eco-friendly disposal. With a detection limit of 0.52 mM and successful quantification of ethanol in various beer samples—including alcohol-free beer—the sensor proves highly accurate, selective, and practical for point-of-care and food quality control applications [42].

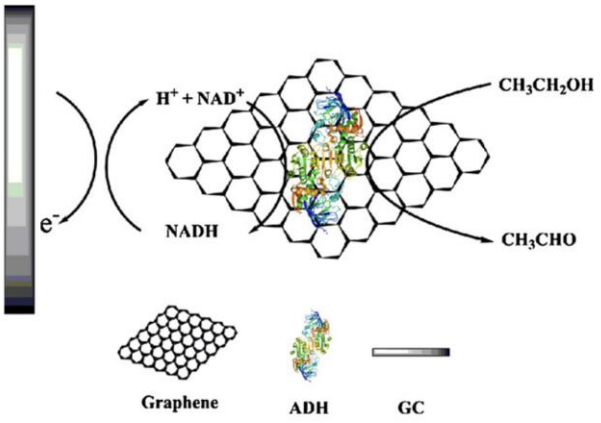

Figure 5. A bare graphite electrode is polished and modified via a layer-by-layer assembly by alternating immersion in PDDA, carboxylated graphene, and AOX solutions, with washing steps in between. This process is repeated to form 5 bilayers, resulting in a PDDA/CG/AOX-modified electrode surface [46]. (Q2R3)

The ethanol biosensor developed with alcohol dehydrogenase (ADH) covalently immobilized on a cellulose acetate membrane linked to Toluidine Blue O (TBO) presents an elegant strategy for lowering the overpotential required for NADH oxidation, enhancing selectivity and minimizing electrode fouling. The cellulose acetate-TBO matrix ensures stable mediator confinement and efficient electron transfer, enabling sensitive voltammetric detection of ethanol down to 5 µM with reliable performance in real beer samples [43]. Complementing this, the review by Hooda et al. contextualizes the significance of such ADH-based biosensors, highlighting their superior selectivity for primary alcohols, the critical role of mediator-assisted electron transfer to overcome high overpotentials, and the integration of nanomaterials to further boost sensitivity, stability, and operational lifetime in real-world ethanol sensing applications [44].

A voltammetric ethanol biosensor was fabricated by adsorbing toluidine blue (TBO) onto an alcohol dehydrogenase (ADH)-modified glassy carbon electrode (GCE), followed by Nafion coating, which stabilized the biofilm and enabled sensitive detection of NADH produced during enzymatic ethanol oxidation. The TBO served as a redox mediator, significantly lowering the overpotential for NADH oxidation (−0.14 V) and enhancing electron transfer kinetics sixfold compared to non-enzymatic films, resulting in high sensitivity (7.91 µA mM^{-1} cm^{-2}) and stable performance across a wide pH range [45]. A graphene-based counterpart further improved the catalytic oxidation of NADH and ethanol, leveraging the high surface area and conductive properties of graphene to achieve a low detection limit of 25 µM and accurate ethanol quantification in real alcoholic beverages like beer and wine, with recovery rates between 93% and 108% [46]. The illustration can be seen in Figure 5. Bilgi (2016) detected ethanol using an ADH-based biosensor with MWCNT/AuNPs/PNR/ADH/GA/SPCE electrodes. The incorporation of MWCNTs, AuNPs, and PNR reduced the NADH oxidation potential [47].

Enzyme-based ethanol biosensors utilize highly selective biorecognition elements like AOX and ADH to detect ethanol with high precision. Various sensor designs enhance performance through innovative electrode modifications. Examples include a bienzymatic AOX/carboxyl esterase system on a graphite epoxy electrode for low-concentration ethanol detection in beverages, and PDDA/carboxylated graphene/AOX-modified graphite electrodes (Figure 4 & 5), which offer high sensitivity and efficient electron transfer. Copolymer- and nanocomposite-based platforms further improve conductivity and stability. ADH-based biosensors incorporate mediators like Toluidine Blue O to lower NADH oxidation potential, boosting selectivity and minimizing fouling, while nanomaterials such as graphene and MWCNTs enhance surface area and electron mobility. These biosensors consistently demonstrate low detection limits, high accuracy, and effective performance in complex matrices, confirming their suitability for real-world ethanol monitoring applications.

Table 4. Alcohol Fermentation Monitoring using Electrochemical Sensor/Biosensor Platforms

Target Analyte	Matrix	Parameters	Sensor/Biosensor Configuration	Ref
Sensor				
Ethanol	Ethanol	LR: 100–1000 ppm, LOD: 4.92 µM	RuO_2/Ni vs. Ag/AgCl	[30]
Ethanol	Ethanol	LR: 0–20.4 mM, LOD: 6 µM (Pd), 10 µM (Ni)	Pd/Ni/SiNWs vs. Ag/AgCl	[31]
Ethanol	Liquor	LR: 0.34–1.71 M, LOD: 0.25 mM	NiNPs/CFP vs. Ag/AgCl	[32]
Ethanol	Liquor	LR: 0.43–88.15 mM, LOD: 0.12 mM	Nafion/Graphene-NiNPs/GCE vs. Ag/AgCl	[33]
Ethanol	Ethanol	LR: 0–60 mM, LOD: 16.8 µM	Pd/Ni/Si-MCP	[35]

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Ethanol	Beer	LR: 700–4700 ppm	PtNPs/GCE vs. Ag/AgCl	[37]
Biosensor				
Ethanol	Beer and Wine	LR: 2.5–25 µM	Carboxyl esterase/AOx/GCE vs. Pt	[39]
Ethanol	Rum, Vodka, Raki	LR: 0.085–1.7 mM, LOD: 0.052 mM	P(TBeSe-co-P3CA)/AOx/Graphite vs. Ag/AgCl	[40]
Ethanol	White/Red Wine, Whisky	LR: 250–1500 µM, LOD: 50 µM	PDDA/Carboxylated Graphene/AOx/Graphite vs. SCE	[41]
Ethanol	Beer	LR: 1×10^{-5}–4×10^{-4} M, LOD: 5×10^{-6} M	CA-TBO/GCE vs. Ag/AgCl	[44]
Ethanol	Spirits, Wine, Beer	LR: 0.2–21 mM, LOD: 20 µM	Graphene/ADH/GCE vs. SCE	[46]
Ethanol	Local Beverage	LR: 283–856 mM, LOD: 29 mM	TBO/Nafion/ADH/GCE vs. Ag/AgCl	[45]
Ethanol	Red/White Wine, Raki	LR: 320.2–1000 µM, LOD: 96.1 µM	MWCNTs/AuNPs/PNR/ADH/SPCE vs. Ag/AgCl	[47]

V. Conclusion and Future Perspective

Electrochemical sensors and biosensors have emerged as effective and reliable tools for the detection of ethanol in alcoholic beverages, offering distinct advantages over traditional analytical methods. Electrochemical sensors, particularly those enhanced with metal nanoparticles such as nickel, platinum, and palladium, provide high sensitivity and rapid response times, making them ideal for ethanol detection in complex matrices like alcoholic beverages. These sensors can detect ethanol over a wide concentration range, enabling precise monitoring of fermentation processes. They are also cost-effective and portable, which allows for on-site testing and real-time monitoring. However, electrochemical sensors face challenges, such as susceptibility to interference from other electroactive substances present in complex beverage matrices. Additionally, issues like electrode fouling, limited shelf life, and variability in sensor reproducibility can affect performance during long-term or repeated use.

Biosensors, which rely on biorecognition elements like ADH or AOx, offer distinct advantages in ethanol detection due to their high selectivity for ethanol, minimizing interference from other substances making them very selective tools for ethanol detection even in a complex sample. Their integration into compact and user-friendly platforms makes them suitable for portable applications, although their performance can be affected by environmental variables such as temperature and pH. Additionally, the production and immobilization of enzymes remain relatively costly, posing a barrier to broader adoption. Moreover, practical deployment in real beverage environments poses additional challenges, including matrix effects from sugars, acids, or carbonation; signal interference from colored or turbid samples; and reduced sensor shelf-life due to enzyme degradation.

Despite these challenges, both electrochemical sensors and biosensors hold significant potential for improving the accuracy and efficiency of ethanol detection in alcoholic beverages. Advances in materials science, enzyme engineering, and sensor design continue to address the limitations of these technologies. Researchers are focusing on improving the stability and longevity of the biorecognition elements in biosensors, as well as enhancing the reproducibility and sensitivity of electrochemical sensors. Furthermore, hybrid systems that combine the strengths of both electrochemical sensors and biosensors are being explored to achieve higher levels of detection specificity and reliability. These advances have the potential to revolutionize ethanol monitoring in the beverage industry, offering more efficient and accurate methods for quality control, fermentation monitoring, and regulatory compliance.

For real-world deployment, commercialization of these sensors must address regulatory approval, cost, and integration into existing beverage production workflows. Compliance with food safety standards, validation against conventional methods, and sensor shelf-life are key factors. Collaboration between academia and industry will be crucial to bring these technologies to market.

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