



Amperometric Biosensors as an Analytical Tool in Food, Dairy and Fermentation Industries

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ABSTRACT

Biosensors are analytical devices that sense the presence of a biological component in the vicinity and generate signals that can be easily detected using a physicochemical detector. With the advent of biosensors, conventional methodologies such as the detection of contaminants in pharmaceutical industries and the diagnosis of diseases via biomarkers have been accelerated to a great extent. The review highlights the use of Amperometric biosensors in three important industries namely, the food, fermentation, and dairy industries, and highlights the potential use of these biosensors in various domains of these industries. These industries can employ the use of amperometric biosensors to control and monitor the presence of various metabolites and constituents that may either be of economic importance or toxic to the consumers. The continuous monitoring of these metabolites is of prime importance as they ultimately determine the quality of the end product. The review also gives a comprehensive description of the modifications done in the amperometric biosensors, using specific biomolecules and chemicals, along with their associated analytical features.

Keywords: Amperometric biosensors, analytical features, industrial applications.

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INTRODUCTION

Biochemical analytical techniques have major importance in the field of medicine, pharmaceuticals, and other industries and with the origin of biosensors, the continuous monitoring of the processes has become easier. An analytical device that is used to convert a sensitive biological response into an electrical signal using a physicochemical detector is called a biosensor.¹ The first biosensor as defined in 1962 by Clark and Lyons.^{2,3} Based on forms of signal transduction, biosensors may be categorized as electrochemical, optical, piezoelectric, and thermal sensors.⁴⁻⁸ based on the type of electrical property measured, electrochemical biosensors are categorized as potentiometric, amperometric, and conductometric sensors.⁹⁻¹¹ The utility of biosensor areas are clinical, quality control, diagnostics, medical applications, process control, bioreactors, agriculture and veterinary medicine, bacterial and viral diagnostics, drug production, wastewater treatment from industries, mining, military defense industry etc.¹²⁻²⁶ Amperometric biosensors are integrated devices that are established based on the estimation of the current generated due to the oxidation or reduction of an electroactive organic component, yielding a distinct quantitative analytical data.²⁷ Amperometric biosensors measure the current

stream between the anode and cathode when a redox response takes place and the resulting current is found to be proportional to the electroactive element in the solution.²⁸ Glucose biosensors are one of the most actively used and researched amperometric biosensors.²⁹

Food production and processing industry and its subsequent industry supply food to the population and play a vital role in the economy of a country. The food examination prospects mainly focus on the quality, safety, and authenticity of the process and the resulting products. Amperometric biosensors have great potential in fermentation industries as analytes like phenols, alcohol, and oligonucleotides need to be continuously monitored at different phases of the process.³⁰ In the food and dairy industry, there is a need for the detection of impurities like allergens, antibiotic residues, pathogenic microbes, and toxins³¹⁻³⁴ and amperometric biosensors are found to be of great importance in this domain. Amperometric Biosensors have high sensitivity compared to the traditional methods, which were earlier used to detect the contaminants. Nutritional content and authenticity analysis can also be measured by amperometric biosensors.^{35,36} Biosensors always undergo constant innovations and shortly, they are expected to be linked with the information technology to assist the users in their decision-making progress.^{37,38}

In the Fermentation industry, tracking of lactate, ethanol, glucose, fructose, lactic acid, and glycerol is essential for the quality of end product and health concerns. Spectrophotometric methods are currently being employed for the analysis. Several oxidases that are immobilized on the electrodes undergo oxidation to produce hydrogen peroxide. Upon oxidation, the current is

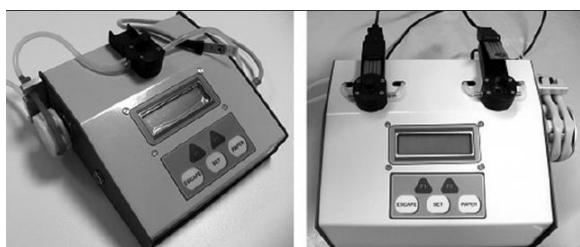


produced, which is proportional to the concentration of the analyte. The analyte concentration can hence be detected using the amperometric biosensor.

The dairy industry is an industry whose pathogen, adulteration, and toxins, pose a grave risk to the consumer. Detection of pathogen and colostrum adulteration hence is of prime importance and this is done by immobilizing antibodies to the electrodes of the biosensor. The screening of the analyte then takes place amperometrically.

The food industry has a lot of demand for biosensors right from production to packaging. Biosensors are already in use for packaging³⁹, but biosensors for industrial processing are yet to be updated. Threats like pathogens, toxins, antibody residues are a risk for human life, and the traditional methods are, however, to be replaced by biosensors, as they are not highly efficient methods for detecting the potential hazards. An amperometric biosensor which is an integration of the sample holder and quantification chamber is designed for industrial employment and is illustrated in **Figure 1**.

This review aims to provide information about the amperometric biosensors developed for industrial applications in recent years. The biosensors mentioned are tested in lab conditions and are modified to work in mass production and processing. Amperometric detection of analytes in different industries is a vast opportunity for economic benefits, and improving food safety, and scientists across the world have been trying new modifications to improve its analytical parameters and increase its efficiency. Many new and modified additional components, like nanomaterials, nanotubes, platinum, hexadecane, gold electrodes, matrices like sodium alginate, resydrol polymer for immobilization of bioreceptors, etc. have been used to upgrade the properties of biosensors like better conductivity and sensitivity. The review highlights the use of various elements incorporated onto the amperometric biosensors for potential applications in the food, fermentation, and dairy industry.



1. AMPEROMETRIC BIOSENSORS EMPLOYED IN THE FERMENTATION INDUSTRY:

Sugars present in the raw materials are converted into ethanol and CO₂. This process is called fermentation. Fermentation is controlled to decide the quality of the output.⁴⁰ The industry which involves fermentation is a major part of the food processing industry, but the demand for a biosensor in this particular sub-industry is

high. The parameters like levels of starch, glucose, lactate, and ethanol need constant monitoring in this industry for obtaining exceptional quality end products and for other economic reasons.⁴¹ **Figure 2** illustrates a glucose biosensor, which monitors the fermentation process of hardwood hydrolysate by glucose monitoring using glucose oxidase as the recognition element. The microbial load present in the primal matter and the range of raw products also decide the grade of the final product.⁴² Certain substrates that are specific to the analytes like, oxidases are immobilized on the electrode surface, which binds and reacts with the analytes and produces H₂O₂, whose oxidation produces current, measured by the amperometric transducer. The reaction is as follows:

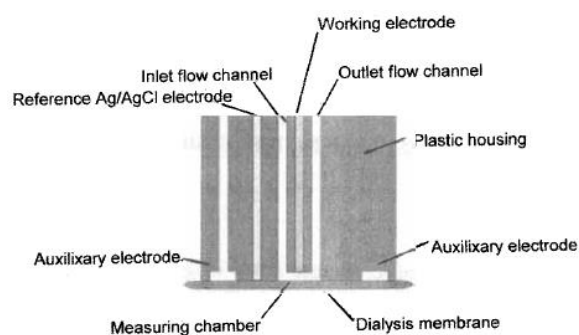
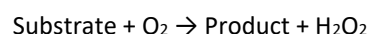


Figure 2: Diagrammatic representation of a glucose biosensor, which monitors the fermentation process of hardwood hydrolysate.²⁸

(i) Detection of ethanol concentration in alcoholic beverages:

Ethanol determination is significant because the level of its concentration determines its economic importance and also determines the nature of the final product. A lot of amperometric biosensors are developed and used recently for ethanol determination. There have been very recent modifications in the sensors for an increase in selectivity and other characteristics like sensitivity, measurement range, accuracy, repeatability, and response time.⁴³

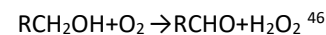
- (a) *ADH based biosensor for ethanol determination:* M. Boujtita et al. reported an ADH based ethanol biosensor which focuses on the oxidation of ethanol, resulting in acetaldehyde. NAD⁺ catalyzes this reaction. NAD⁺ is converted into NADH and the oxidation of NADH takes place, releasing electrons and is monitored by the biosensors amperometrically.⁴⁴ The biosensor uses alcohol dehydrogenase (ADH) and NAD⁺ modified carbon paste electrodes. The research also shows the response of the sensor studied with different types of pasting liquid, which includes analyzing the concentration of ethanol in wine, beer, and it was compared favorably with spectrophotometry. The hexadecane electrode is used because it showed more sensitivity and linearity. The matrix has minimal effect on the amperometric current measured and

does not interfere in the result of the ethanol analysis given by the carbon paste electrode modified with ADH/NAD⁺. The measurement time is 20 seconds, and the sample needs a preparation i.e. dilution because the enzyme electrode depends on the dilution factor. The ADH based biosensors have limited operational strength and are dependent on the coenzyme NAD⁺, which needs persistent recovery in the analysis, which increases the general expense of the production of biosensors.⁴³

(b) *Amperometric Biosensor for the detection of ethanol, based on ethanol oxidase (AOX) based Polyvinyl chloride (PVC) cell:* This is an amperometric bio-enzymatic biosensor reported by Vinita Hooda et al. These biosensors are based on alcohol oxidase integrated with the PVC reaction cell. For the rapid quantification of alcohol, nanomaterials modified working electrodes are used. Alcohol oxidase based alcohol biosensors work by utilizing just molecular oxygen to oxidize ethanol into the corresponding aldehyde with the arrival of H₂O₂, without the addition of any other cofactor. The use of multi-walled carbon nanotubes (MWCNTs) and silver nanoparticles (AgNPs) facilitates the direct transfer of electrons between the Horseradish Peroxidase (HRP) enzyme and the electrode. The immobilized AOX onto the outside of the PVC container holds great electrocatalytic movement at physiological pH, and detection takes place amperometrically. Electrochemical cells are constructed using PVC because of its properties like reusability, improved analytical performance, and stability. The currently used alcohol biosensor was compared with this biosensor by conducting experiments with wine samples, and the analytical parameters were improved. Response time is 8 seconds at pH 7.5 at 35°C incubation temperature. The result was compared with that of standard analytical methods.⁴⁵

(c) *Microbial amperometric biosensor:* Many microbial amperometric biosensors are reported for ethanol determination, based on microorganisms like *T. brassicas*, *S. cerevisiae*, *Acetobacter acetic*, or *Acetobacter xylinum*.^{46,47} L. Rotariu et al. reported a microbial amperometric biosensor based on yeast cells (*Saccharomyces ellipsoideus*) immobilized on Clark based oxygen electrodes. This biosensor determines the ethanol by the products released by the respiratory activity of the microorganism. The reaction time is 2 minutes. This biosensor utilizes a second Teflon membrane, which shields the biocatalytic yeast layer, which helps for selective determination of ethanol in the presence of glucose. Incubation with ethanol was reported to improve the selectivity of the biosensor. The selective biosensor was used to determine ethanol concentration in alcoholic beverages. The results were compared with the spectrometric method with alcohol

dehydrogenase and a good correlation was observed.⁴⁸



Detection of lactate concentration in wine

Secondary fermentation is called malolactic fermentation, and it is performed by LAB (lactic acid bacteria). Lactate is formed after a series of biochemical reactions performed by LAB. This process can influence the taste and other qualities and attributes of the beverage and hence, it is essential to control its levels. Various innovations in lactate biosensors are reported from all over the world. New trends in biosensors stated below are all tested with the results of the traditional methods and functional correlations have been found. The current measured by the amperometric biosensor is proportional to the concentration of lactic acid. Amperometric biosensors are divided into two types: lactate oxidase-based biosensor (LOx) and lactate dehydrogenase (LDH)-based biosensor.⁴⁹

(a) *Bioenzymatic biosensor for l-lactate concentration determination:* Sandra Perez et al. reported an Amperometric enzymatic biosensor that uses a fusion of Horseradish Peroxidase (HRP) and Lactate Oxidase (LOx) enzymes into a carbon nanotube/polysulfone film by the phase inversion technique and screen-printed electrodes (SPEs) are obtained. Horseradish Peroxidase is a biochemical used as an enzyme in this research.⁵⁰ Ferrocene was applied to the layer as they reportedly improve sensibility, working potential, and permit a decrease of H₂O₂ at 100mV. The report has mentioned the estimations done in phosphate buffer solution at pH 7.5 under batch conditions. The reaction time of the biosensor was 20 seconds. Detection has been conducted on various wine and beer samples. They have compared the outcomes acquired with the biosensor and the results from the spectrophotometric measurements and obtained functional correlations. This device was found to have high accuracy, high sensitivity, low limit of detection, and a quick throughput. However, it is also reported that the shelf-life stability isn't very high and needs improvement in future trials.⁵¹

(b) *Lactate oxidase-based amperometric biosensor using platinum electrode:* This amperometric biosensor is intended for the determination of lactate. The report analyzes two techniques involving SensLab platinum printing anodes by immobilizing lactate oxidase on its surface with different types of absorption. The sensor with lactate oxidase immobilized by physical adsorption in Resydrol polymer appears to have both a smaller dynamic range (0.004–0.5 mM lactate) and more sensibility (320 nA/mM). The immobilization strategy has no impact on results, and this report was obtained when these biosensors were tested. The data obtained by analyzing the lactate concentration in wine and the must during fermentation using

amperometric lactate biosensor and standard chromatography techniques have a reasonable correlation. The report by L.V. Shkotova et.al. also suggest that the biosensor created can be utilized in the food industry for improvement and also helps in the supervising the procedure of wine aging .⁵²

- (c) *Lactate detection with biosensor integrated with nanoparticles:* Nanoparticles are best described as a cluster of atoms, and their size ranges from 1-100 nm. Kang, H et al. have effectively explained the application of nanoparticles in biosensors to increase its working efficiency.⁵³ Amperometric biosensors have been modified and tested with palladium and platinum nanoparticles by L. Shkotova et. al. for analysis of the final product for lactate concentration during fermentation. They observed an increase in bio selectivity upon the incorporation of Pd and Pt NPs. This modified biosensor finds effective application for maintaining the excellent quality of wine and for selective detection of lactate in raw material during fermentation. Research has shown that the lactate biosensor function of amperometric transducers has improved sensitivity and selectivity with the incorporation of Pt And Pd nanoparticles along with the Nafion protective membrane.⁵⁴

(iii) Detection of multiple enzymes:

Multiple analytes are to be maintained in the industrial fermentation process, so multiple oxidase-based biosensors are developed to serve this purpose.

- (a) *Based on platinum (printed) electrodes and immobilized enzymes:* Goriushkina T. B et al. reported a biosensor for application in wine analysis that concentrates on the determination of the concentration of ethanol, glucose, and lactate by immobilization of respective oxidases on printed platinum electrodes. The immobilized oxidases react with the analyte and produce H₂O₂ upon oxidation. The current produced is measured by the amperometric transducer. The data of linear response to ethanol, glucose, and lactate within the concentration range is mentioned in **Table 1**. It was seen that the biosensor activity did not alter even after two months of fabrication for ethanol and glucose. However, for lactate biosensor activity, the operational stability lasted for four days. Research also showed high selectivity to the substrate and commercial applicability during the fermentation of wine, must for analysis of ethanol, glucose, and lactate in wine and must during its fermentation. Research has also reported the correlation of results of various types of wine like red, white, and rose wine and also dry, strong, and sweet forms and must with other amperometric biosensors and the traditional methods.⁵⁵
- (b) *Screen printed biosensors for real-time monitoring of alcoholic fermentation:* Screen-printed biosensors

for the determination of glucose, ethanol, and fructose are combined with convenient instrumentation for real-time monitoring. S. Piermarini et.al reported the application of these biosensors to screen micro alcoholic fermentation (micro ALFs) processes in red wine. The ethanol and glucose biosensors fabrication was done on graphite screen-printed sensors and further altered with Prussian Blue coupled with oxidase enzyme. The electrochemical mediator permits a delicate and particular location of H₂O₂, at applied potential (50 mV). For fructose, a normal screen-printed sensor was coated with fructose hydrogenase and an electrochemical mediator phenazine methosulphate was used for acting as an enzymatic co-substrate. They have reported the working range, reproducibility of probe fabrication, and its stability. The research shows that they have collected samples of fortified must-wine, which were tested to monitor the micro-ALFs in the samples induced by two strains of yeast. The data were also compared with spectrophotometry, and a good correlation was reported. The fermentation profiles were analyzed at different time intervals, and the data was able to determine the typical fermentation phases. They also indicated the differences in the metabolic behavior of the two strains.⁵⁶

- (c) *Detection of enzymes using carbon film resistors:* Multiple oxidase-based enzyme electrode biosensors use Carbon film resistors as transducers for their development. The resistor electrodes were altered with Prussian Blue (PB), and then a layer of enzymes was immobilized covalently. The substrates of the enzymes are detected by the reduction reaction of hydrogen peroxide against Ag/AgCl in response time of 1-2 minutes. Finally, the electrochemical biosensors were used to analyze complex food matrices—yogurt, wine, and must. The above experiment was compared with the spectrophotometric reference method, which indicated a good correlation with the results and indicated that it is suitable for wine and food analysis. These biosensors have some unique properties which make this an excellent material for disposable or easily renewable sensors. Voltammetric techniques and EIS have been used to test its stability towards carbon resistor substrates. This research mainly focuses on wine process control as the electrodes are studied and analyzed in wine samples. The detection limits of these biosensors are comparable with screen-printed biosensors and platinum-based biosensors in terms of sensitivity. They are low cost and are being tested for other analytes. They are hence, found to be suitable for food screening. The different designs allow swift immobilization of biological substrates and compounds. The research has also shown that the

enzyme biosensors with carbon film resistors are suitable for complex matrices.⁵⁷

The various important analytical characteristics of the biosensors used in the fermentation industry are mentioned in **Table 1**.

Table 1: The analytical characteristics of amperometric biosensors used in the fermentation industry.

The electrode used in the Biosensor	Analyte	Limit of detection	Linear detection range	Potential	Sensitivity(A/mM)
Alcohol dehydrogenase (ADH) on NAD ⁺ modified carbon paste electrode. ⁴³	Ethanol	-	0 M to 11 mM	0.7V	-
Alcohol oxidase coupled PVC reaction cell on nanomaterial modified electrodes. ⁴⁵	Ethanol	0.0001 μ M	0.01mM to 50mM	-0.75V to 1.2V	155 μ Am/Mcm ²
Yeast cell immobilized on Clark type oxygen electrode. ⁴⁸	Ethanol	1.5mM	3mM to 50mM	-650mV	-
LOx and HRP in Carbon nanotube on SRP. ⁵¹	L-lactate	5.6 X 10 ⁻⁷ M	1.1x10 ⁻⁶ M to 5.6 X10 ⁻⁴ M	-100mV	1168.8 μ A/mM
LOx in resydrol polymer on platinum printed electrode. ⁵²	Lactate	-	0.004mM to 0.5mM	0V to 3000mV	320nA/mM
Platinum and palladium nanoparticles. ⁵⁴	Lactate	0.1 μ M	0.05mM to 0.8mM	0V to 1.0V	3.03 nAm/Mcm ²
Oxidases immobilized on electrodes. ⁵⁵	<ul style="list-style-type: none"> • Ethanol • Glucose • Lactate 	<ul style="list-style-type: none"> • 0.03mM • 0.04mM • 0.008mM 	<ul style="list-style-type: none"> • 0.3mM to 40 mM • 0.04mM to 2.5mM • 0.008mM to 1mM 	0 to 600mV	-
Graphite screen-printed sensors modified with Prussian Blue coupled with oxidase enzyme. ⁵⁶	<ul style="list-style-type: none"> • Glucose • L-Glutamat • L-Lactate • Ethanol 	1 μ M	<ul style="list-style-type: none"> • 10μM –800 μM • 10μM–700μM • 10μM–500μM • 10μM–700μM 	-	<ul style="list-style-type: none"> • 8μA/mM • 7μA/mM • 10.4μA/mM • 5.5μA/mM
Screen-printed sensors modified with Prussian Blue coupled with oxidase enzyme with carbon film resistors as transducers. ⁵⁷	<ul style="list-style-type: none"> • Glucose • Fructose • Ethanol 		<ul style="list-style-type: none"> • 0.02mM–0.7 mM • 0.05mM–0.5 mM • 0.05mM–0.5 mM 	70mV	-

'-' Refers as not mentioned

Amperometric Biosensors used in Dairy Industry

The dairy industry includes the harvesting of milk from cattle and processing of the milk to produce different products like butter, curd, etc. A variety of adulteration and pathogen contamination takes place during the harvesting of milk. Therefore, biosensors have been developed, modified, and experimented for replacing the traditional methods and detecting impurities with higher

accuracy. For instance, animal-specific IgG is detected in milk to determine milk adulteration using the antibody immobilized immunosensor, which is depicted in **Figure 3**. All the analytical properties are stated in Table 3.

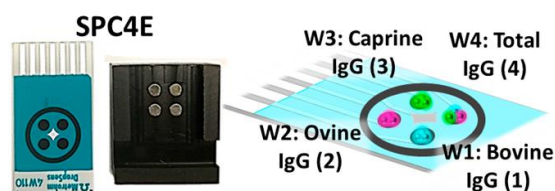


Figure 3: Schematic representation of the immunosensor with the platinum screen printed electrodes to detect animal-specific IgG in milk.⁷⁶

Lactose detection in milk

Lactose detection in milk is essential because the lactose level is a key marker for the assessment of milk and the discovery of abnormalities. For instance, milk from cows struggling with mastitis has lower lactose levels.⁵⁸ Also, lactose is a fundamental parameter in wastewater management and veterinary medicine.⁵⁹ Gastrointestinal malignancy stipulates a greater amount of lactose in blood.

(a) *Lactose detection using a gold electrode:* The lactose biosensor was assembled by β -Gal, GOD, HRP immobilization, and the mediation of Tetrathiafulvalene (TTF) on the gold disk electrode. Glucose is produced when β -Gal catalyzed lactose hydrolysis takes place. Glucose then oxidizes into gluconic acid and H_2O_2 . The product is then reduced with the involvement of HRP, mediated by TTF. TTF reduction results in the production of amperometric signals proportional to lactose concentration. It shows good analytical performance, including simple and rapid analytical technologies with amperometric detection. The lifespan of this biosensor is 28 days. The effect of potential interference has been evaluated by F. Conzuelo et. al. The biosensors have been applied for lactose determination in milk and other edible items like chocolate, cheese, butter, yogurt, and mayonnaise. The results were compared with traditional methods and commercial enzyme kits and a good correlation was found.⁶⁰

(b) *Lactose determination by biosensor in raw milk:* Lourenço et al reported raw milk lactose determination using a biosensor. The reported new amperometric biosensor was established through the concurrent immobilization of enzymes namely galactose oxidase and β -galactosidase on derivatized polyethersulfone membrane.⁶¹ The biosensors can be stable for 20 days. The β -linkages of galactose and glucose develop lactose as it is a disaccharide. β -galactosidase and galactose oxidase act as a catalyst. The former catalyzes the lactose hydrolysis which results in galactose and glucose and the latter catalyzes the oxidation of galactose into galactonic acid and H_2O_2 . A constant voltage is supplied to the biosensor and variation of current is calculated and hence the concentration of lactose is determined.⁶²

Amperometric lysine biosensor by using two different electrodes

Lysine is an essential amino acid, which is popularly used as a nutritional supplement in the animal feed industry as well as in human food products.⁶³ Diseased conditions like tiredness, bloodshot eyes, inability to concentrate, retarded growth, anemia, hair loss, and reproduction problems show a low level of lysine in the blood and other body fluids.⁶⁴ Due to the clinical, dietary, and pharmaceutical criticalness of this amino acid, quick strategies are required for the location of lysine in different frameworks.^{65,66}

Two amperometric L-lysine biosensors were reported by Nidhi Chauhan et al., who distinguished them using instruments like SEM. The electrodes used in biosensors were modified by two different components namely Polyaniline (PANi) and 3,3'-Diaminobenzidine (DAB), in different apparatus, and were stored at 4°C. Eventually, the modified biosensors have experimented with milk samples. After 100 uses, it was seen that the PANi modified electrode was stable for 120 days and the DAB modified electrode was stable for 90 days. Ideal material for biosensors is already initiated by Gold nanoparticles (AuNPs), especially for those using electrochemical detection, because the gold surface is preferable for the binding of biomolecules and direct electron transfer.⁶⁷ When lysine is oxidized in the presence of LOx, a-keto-3-aminocaproate, H_2O_2 , and NH_3 is formed. The oxidation of H_2O_2 then results in the electron flow and current generation in LOx/AuNPs/c-MWCNT/PANi/Au electrode. They reported that the electrode modified by PANi gives better systematic properties than the DAB modified electrode; it was utilized for the identification of lysine in real samples like milk, pharmaceutical tablets, and serum proteins. Samples from milk were absorbed for 6 N HCl in 15 min, following which the samples were escorted to pH 7.0 with 1 M KOH, and then the samples were evaluated for lysine content. The use of PANi/DAB and nanoparticles (AuNPs) in the manufacturing of lysine biosensors gave the results in its much-improved system performance than the earlier used biosensors concerning the response of time (2s and 4s), detection limit (5 and 20 μ M), higher stability (up to 4 months) and no involvement from different serum substances. They also conducted experiments and found the PANi electrode has better performance than the DAB electrode.⁶³

Discrimination of milk with a multisensor system

Lactose intolerance or weight problems might cause people to utilize lactose-free milk and coffee fats milk for their daily needs.⁶⁸ Electrochemical biosensors display excessive selectivity and may play an essential function in enhancing the effective manipulation of milk on lactose levels.^{69,70}

Coral Salvo-Comino et al. developed a layer-by-layer technique for the analysis of milk using a bi-sensor system because of its low cost and intrinsic simplicity.⁷¹ There are

two sensing units present in this system. The first sensor consists of anodic and cathodic parts: anodic part is copper sulfonate phthalocyanine (CuPcS), which acts as an electrocatalyst. The cathodic part consists of a mixture of an ionic liquid (IL) (1-butyl-3-methylimidazolium tetrafluoroborate), which expands the active site, along with CHI (chitosan) which increases the efficiency of the electron transfer and the conductivity. It is represented as [CHI+IL/CuPcS]₂. The immobilization of galactose oxidase acts as the second sensing unit. The biosensor is denoted as ([CHI+IL/CuPcS]₂-GAO). Sensors were blended to shape into electronic tongues for milk analysis. The enzyme GAO acts as an electron mediator. Principal evaluation of the biosensor is the capacity of the sensor system to distinguish among milk samples with distinctive lactose content, but it was also tested with galactose. The sample milk was collected from local stores with different lactose and fat content, and they successfully experimented and reported the spoilage and lactose content. The biosensor has been tested on milk samples, and the results have been correlated with the traditional methods. They also experimented with the biosensor for the detection of lactose, fat, protein content, and acidity by using PLS-1.⁷²

Amperometric immunosensor for the adulterant detection in milk

Milk, olive oil, honey, orange juice, saffron, coffee, and apple juice are prominently the most frequent targets for adulteration.⁷³ Milk is harvested from cattle like bovine, ovine, and caprine, and these kinds of milk are similar, therefore milk adulteration is hard to identify.^{74,75}

Víctor Ruiz-Valdepeñas Montiel et al. reported the use of amperometric immunosensor for the detection of adulterants in milk by using colostrum from various animals. The system is set up by sandwiching the species-specific IgG and the selective antibody pairs, and this is set up on carboxylic acid-modified magnetic beads connected to four screen-printed carbon working electrodes. When the antibodies are present in the milk sample, the sandwich which is conjugated with HRP selectively captures it. The antibody in the sandwich captures the antibodies present in the milk and through the magnetic beads, amperometric transduction takes place near the Ag electrode using the H₂O₂ system. They conducted experiments on milk samples for the screening of antibodies at the level demanded by European legislation. The time taken for detection was 30 minutes. LOD and time duration was less compared to traditional methods like ELISA. The developed biosensor is easy and simple to use by the training staff, and the process can be performed during milk collection or at the dairies.⁷⁶

Amperometric urea biosensor

Urea is a compound found in blood and other biological fluids. Consistent scrutiny of urea in milk can be utilized to foresee the state of the cattle's wellbeing and one can predict the protein necessity in its eating regimen.^{77,78} This strategy includes the utilization of electrochemical urea

biosensors. Urea biosensor is used for tracking the urea content material of adulterated milk. Immobilization of the urease over altered electrodes decides the sensitivity and reproducibility of the biosensor. Urease immobilized electrodes were fabricated by a polymeric membrane, based on polyaniline, and acts as new electron mediating support material. It was reportedly tested at different thicknesses and applied for the screening of urea by amperometry. Florina Brânzoi et al. have reported high sensitivity and other analytical properties by varying and testing the physical properties of the biosensor electrode. The biosensor has been tested on milk samples with urea contamination and good correlation was found with the results of traditional methods.⁷⁹

Pathogen detection in milk (*Listeria monocytogenes* detection)

Listeria monocytogenes (LM) is the most dreadful food-borne microorganism. *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*, are among the most commonly examined food-borne microorganisms.⁸⁰ Despite the fact *L. monocytogenes* is infective to all human population, it has an affinity to cause particularly serious issues in pregnant ladies, children, older and immunosuppressed people.⁸¹

Ying Lu et al. have reported an immunosensor created by immobilizing HRP-anti-*L. monocytogenes* antibodies onto the outside of the multiwalled carbon nanotube fibers with both physical and chemical techniques. The impact of immuno-electrode change techniques on the detection of sensitivity and stability is reported. The experiments conducted with the sample showed high specificity, storage stability, and high reproducibility (RSD < 6.5%). This immunosensor specificity is examined by using a milk sample. The reported biosensor detected *L. monocytogenes* in mixed bacteria of milk sample and showed good specificity, which demonstrated the utilization of immunosensor for the rapid detection of *L. monocytogenes* for applications in the protection of public health.⁸²

Detection of *Escherichia coli* by amperometric biosensor

E. coli O157 is a pathogenic strain and can cause diseases that result in stomach cramps and gentle looseness of the bowels and more deadly complications like hemorrhagic colitis (ridiculous diarrhea), haemolytic uremic disorder (HUS, prompts to kidney failure), and thrombotic thrombocytopenic purpura (TTP).^{83,84} *Escherichia coli* (*E. coli*) O157 is one such bacterial species that brought about numerous principal outbreaks in the past.

N. Dhull et al. proposed the development of amperometric biosensors for the detection of *E. coli* O157. The biosensor was prepared by covalently binding antibodies on a nanostructured Nickel oxide (NiO) thin film using the Rf magnetron sputtering technique⁸⁵ and further on conducting ITO coated glass substrates (NiO/ITO). NiO helps in quick charge transfer providing support to the antibodies increasing the target capture efficiency and



hence detection of *E. coli*. The biosensor was tested in dilute milk samples and results were correlated with traditional methods like PBS buffer⁸⁶ and Plate count method⁸⁷ and the output was in deviation in the range of 10%. Experiments have been conducted and reported showing that the biosensor has high specificity and

selectivity towards the target pathogen even in mixed culture.⁸⁸

The important analytical characteristics of the biosensors used in the dairy industry are mentioned in **Table 2**.

Table 2: Analytical properties of the amperometric biosensors used in the dairy industry

The electrode used in the Biosensor	Analyte	L.R [M]	Sensitivity[nA/M]	LOD [M]	Potential [V]
β -Gal, GOD, HRP and the mediation of TTF on gold disk electrode. ⁶⁰	Lactose	$1.5 \times 10^{-6} \text{ M}$ to $1.2 \times 10^{-4} \text{ M}$	6.04×10^5	$4.7 \times 10^{-7} \text{ M}$	+0.00V
β -galactosidase and galactose oxidase on polyethersulfone membrane. ⁶²	Lactose	0.0043 mol dm ⁻³ and 0.031 mol dm ⁻³	6.81	-	+700V
LOx/AuNPs/c-MWCNT/PANi/Au electrode. ⁶³ 'LOx/AuNPs/c-MWCNT/DAB/Au electrode. ⁶³	Lysine [PANi] Lysine [DAB]	$5 \times 10^{-6} \text{ M}$ to $6 \times 10^{-4} \text{ M}$ $25 \times 10^{-6} \text{ M}$ to $6 \times 10^{-4} \text{ M}$	-	<ul style="list-style-type: none"> $5 \times 10^{-6} \text{ M}$ $25 \times 10^{-6} \text{ M}$ 	0.4V
Carboxylic acid-modified magnetic beads connected to four screen-printed carbon electrodes. ⁷⁶	Bovine-[IgG] Ovine-[IgG] Caprine-[IgG]	2.6-250 ng m/L 2.7-250 ng m/L 2.2-250 ng m/L	-	0.74 ng m/L 0.82 ng m/L 0.66 ng m/L	-0.2V
HRP-anti-L. monocytogenes antibodies over multiwalled carbon nanotube fibers on immuno-electrode. ⁸²	L. monocytogenes	10^2 to 10^5 cfu/mL	-	1.07×10^2 CFU/mL	-
Antibodies on a NiO film on ITO coated glass substrates (NiO/ITO). ⁸⁸	<i>E. coli</i>	10^1 to 10^7 cells/mL	10^0 cell/mL	-0.3V to 0.8V	-

'-' Refers as not mentioned

USE OF AMPEROMETRIC BIOSENSORS IN

Food Industry

The food industry involves the use of biosensors at each step of the processing and packaging stages as these biosensors effectively detect the toxins, pathogens, allergens, and other contaminants along with their concentration. **Figure 4** depicts an immunosensor that detects a common allergen, Ara h 1 (peanut allergen).

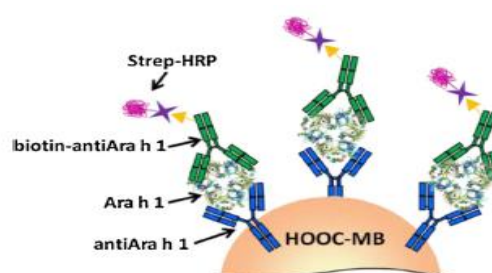


Figure 4: Schematic representation of the Ara h 1 immunosensor constructed with antiAra h 1 antibodies and COOH modified magnetic beads.¹²⁷

Detection of tyramine (1-hydroxy-4-ethyl aminobenzene)

Tyramine is a type of biogenic amine. There are many commercial or home purified based enzymes for which biosensors have been made to determine tyramine content.^{89,90} The biogenic amines are considered as quality markers of nourishment in human beings based on the levels of their presence in our diet.⁹¹⁻⁹⁵

Irina Mirela Apetreia et al. have reported a stable amperometric biosensor for tyramine detection in an aqueous medium with the electrode immobilized by tyrosinase using the cross-linking method. This immobilization takes place over phosphate-doped polypyrrole film. The biosensor was applied in sauerkraut samples. The polypyrrole slim film protects its biocatalytic movement well. This enzyme is immobilized for selective detection, whose action is towards the phenolic group of tyramine. They have also reported the characteristics of this biosensor like linear range, detection limit, which has been mentioned in Table 3. High repeatability and long-term stability and other characteristics are reported after conducting experiments in this biosensor. They also have demonstrated the suitability of amperometric detection of tyramine in the salted sauerkraut samples.⁹⁶

Detection of *Salmonella pullorum* contamination

Salmonella, which is the most often announced reason for the food-borne sickness in the world, is broadly dispersed into creatures and conditions.⁹⁷⁻⁹⁹ The traditional methods to detect Salmonella contamination like plate count techniques require 3-4 days for hypothetical outcomes and 5-7 days for affirmation.¹⁰⁰

Guoyan Liu et al. demonstrated an amperometric biosensor that utilizes screen printed electrodes immobilized by anti-Salmonella polyclonal antibodies on the cellulose nitrate layer. This amperometric biosensor has improved selectivity and sensitivity for the *Salmonella pullorum*. The disproportion of hydrogen peroxide is catalyzed by bacterial catalase and the product obtained, when added on the reaction area of the SPEs, helps in easy and rapid detection of *S. pullorum*. Bacterial catalase (a biomarker for the bacterium) is responsible for the rapid detection of the microbe and the detection time was found to be 10 minutes. The chicken samples were obtained from different markets and were experimented with the biosensor and improved analytical characteristics were reported. This biosensor has rapid detection properties and is cost-effective.¹⁰¹

Determination of the egg white allergen, ovomucoid (OM) at trace levels in fresh and baked food

Eggs, along with milk, are recognized as allergenic components in European youngsters. Egg hypersensitivity principally influences children under three, but the majority of them grow out of their hypersensitivity by the early school years.¹⁰² This strong allergenicity is accepted to rely upon the security of OM against denaturation, accumulation, and proteolytic debasement.¹⁰³⁻¹⁰⁹

Ovomucoid, an egg white allergen, is determined by the biosensor reported by S. Bened et al. It is commonly detected by commercial ELISA kits. This biosensor has been tested and employed on low-level egg residues like unprocessed food samples of eggs, wheat flour, and baked food samples like bread. In this biosensor, immunocomplex is formed by antibody, target protein allergen, and the HRP labeled detector; this is captured by carboxylic acid-functionalized magnetic beads, which results in the formation of a magnetic bioconjugate. This bioconjugate is then immobilized on the surface of a screen-printed carbon electrode (SPCE), and amperometric detection is carried out. They determined the Ovomuroid using the Hydroquinone as an electron transfer mediator and H₂O₂ substrate. Convenient routine screening analysis is possible with the high sensitivity of the sensor for consumer protection. The linearity and other analytical properties of the biosensor have been stated in Table 3.¹¹⁰

Detection of Aflatoxin B1

Aflatoxin B1 (AFB1), a mycotoxin, is the most common and harmful contaminant in human food and animal feed, and hence, its detection in food items is important.¹¹¹ Sodium alginate-altered anodes can give a great microenvironment to catalysts, improving their strength, keeping up their bioactivity, and drawing out the capacity of the biofilm.¹¹²⁻¹¹⁷

Amani Chrouda et al. reported a biosensor for the determination of aflatoxin B1 via inhibition of acetylcholinesterase (AChE). As a sensing bio element, AChE was immobilized in the sodium alginate (herbal biopolymer) matrix-Au electrode. The low detection limit is a promising function of this biosensor in comparison to spectrophotometric techniques for AFB1 detection. The AChE activity inhibition is proportional to the AFB1 concentration. By experiments, it was reported that the sodium alginate composite exhibited a development within the overall performance of the biosensor (storage stability, and reproducibility) and offers a biocompatible host matrix that retains enzyme molecules with the aid of using chemical cross-linking.¹¹⁸

Detection of *Vibrio parahaemolyticus*

V. parahaemolyticus is a Gram-negative halophilic microorganism and a significant food-borne microbe that causes diarrheal illness particularly in people consuming seafood.¹¹⁹⁻¹²¹ The hetero-sandwich electrochemical biosensor¹²² gives an ultrasensitive apparatus for the quantification of an assortment of microbe if fitting antibodies and aptamers are accessible.

V. parahaemolyticus detecting antibody-aptamer-based hetero-sandwich amperometric biosensor is reported by Jun Teng et al. The gold electrode surface contains an antibody that seizes the pathogen. An aptamer containing an ssDNA probe is incubated with the immunocomplex formed. Rolling circle amplification is a process where a circular DNA template is used to amplify a short DNA



strand with the help of various DNA or RNA polymers.¹²³ The hetero-sandwich then formed by an antibody-aptamer allows signal intensification by rolling circle amplification. Methylene blue is added as an electrochemical DNA probe. The amperometric signal is obtained, and the pathogen is detected. This biosensor has experimented in spiked fish samples and high sensitivity was found compared to other sensing techniques.¹²⁴

Detection of Peanut Allergen Ara h1

Since no treatment has been observed for peanut allergy, the peanut is one of the maximum allergic ingredients in the world.¹²⁵ V. Ruiz-Valdepenas Montiel et al. have reported an electrochemical immunosensor for the Ara h1 detection in human saliva samples. Eight A. hypogaea proteins (Ara h 1-8) are now considered to be the most significant peanut allergens.¹²⁶

A disposable amperometric immunosensor primarily based on the usage of magnetic beads (MBs) had been demonstrated for the detection of Ara h1 detection (the fundamental peanut allergen) in 2 hours. The traditional technique involves protein-based assay and PCR. The immunosensor makes use of a sandwich configuration between biotinylated detection antibodies and carboxylic acid-altered MBs (HOOC-MBs). Low detection limit, high selectivity, a terrific reproducibility (RSD = 6.3%) for six various immunosensors, and a beneficial life of 25 days had been reported. V. Ruiz-Valdepenas Montiel et al. reported excellent analytical performance, flexibility, and ease of the MBs and mass-produced electrochemical transducers help in the selective and sensitive detection of food allergens.¹²⁷

The important analytical characteristics of the biosensors used in the food industry are mentioned in **Table 3**.

Table 3: Analytical characteristics of biosensors used in the food industry

Electrode used in the Biosensor	Analyte	Limit of detection	Linear detection range	Potential	Sensitivity
Tyrosinase immobilized phosphate-doped polypyrrole film on an electrode. ⁹⁶	Tyramine	4X10 ⁻⁶ M to 80X10 ⁻⁶ M	5.7X10 ⁻⁷ M	-0.250V	0.1069 A/M
Anti-salmonella polyclonal antibodies immobilized over cellulose nitrate layer on Screen-printed electrode. ¹⁰¹	<i>Salmonella pullorum</i>	1.75X10 ² CFU ml ⁻¹ to 23.63	100 CFU ml ⁻¹	0.55V	-
Antibody-allergen protein-HRP and magnetic beads on screen printed electrodes. ¹¹⁰	Ovomucoid	0.3 to 25 ng ml ⁻¹	0.1 ng ml ⁻¹	-0.20V	-
AChE immobilized on sodium alginate on a gold electrode . ¹¹⁸	Aflatoxin B1	0.1 to 100 ng/mL	0.1 ng/mL	-0.4V to 0.6V	-
Antibody-aptamer sandwich on a gold electrode. ¹²⁴	<i>Vibrio parahaemolyticus</i>	2.2-2.2×10 ⁸ CFU/mL	2 CFU/mL	0.28V	-
Sandwich configuration of antibodies and carboxylic acid altered MBs. ¹²⁷	Food allergen Ara h 1	20.8 and 1000.0 ng mL ⁻¹	6.3 ng mL ⁻¹	-0.20 V	-

‘-’Refers as not mentioned

CONCLUSION

Amperometric biosensors are seen to have promising industrial applications especially because of the incompetence of the conventional methods. Different types of electrodes, matrix, and immobilization techniques have been applied and experimented with samples to improve the biosensor characteristics, enhance the working efficiency, and prepare for mass usage. The demand for biosensors in the food, dairy, fermentation industries is immense. The use of biosensors in the field of medicine has made the diagnosis of diseases very rapid yet reliable and biosensors employed in the pharmaceutical

industries have made a quality analysis of the components very simple and rapid. Biosensors hence have successfully replaced the prevalent time-consuming methodologies of these industries and have shown great potential in all other industrial domains as well. The future of these industries awaits biosensors because of low cost, low LOD, high sensitivity, and other improved analytical properties than classical methods. Many MNCs have invested in biosensors for their application in the industries. The industrial application of these biosensors brings economic and public health welfare, hence enhancing the quality of life. Although the prototype of these biosensors has been



created, tested, and modified for mass applications, industrial employment of these biosensors is yet to be done. Continuous research is however very essential to combat the limitations of these biosensors so that the society can reap the benefits associated and thereby, the ultimate aim to deliver high-quality products to the consumers can be achieved.

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