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Biosensors in Fermentation Applications

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Abstract

Biosensing technology offers new analytic routes to the use and study of fermentations, taking advantage of the high selectivity and sensitivity of the bioactive elements it exploits. Various biosensors had been commercially available today; they provide fermentation processes with convenient, accurate, and cost-effective ways of monitoring for key biochemical parameters. In this chapter, the basic ideas and principles of biosensors, especially applications of the most popular biosensors related to fermentations were highlighted.

Keywords: biosensor, electrochemical techniques, enzyme electrode, amino acid, sugar, alcohol

1. Introduction

Biosensor is a field of interdisciplinary studies and applications, which is underlain by many theoretical and technical fundamentals from life science, physics, analytical chemistry, information technology, and so forth. The study of biosensors is a branch of analytical biology. It is largely aimed to construct rapid, stable, and facile analytical devices and analytical technologies used thereby. As a novel analytical technique, biosensors features small size, high sensitivity, high analytical specificity, and rapid accessibility, ready to realize reagentless analyses. This technology has made its way in great advances and attracted attentions since it was first proposed in 1960s.

The first biosensor was reported to be constructed and succeeded in measuring medical data, a biological electrode by Pro. Clark and co-works in 1962. **Figure 1** is a schematic of it. The electrode is fabricated via fixing a layer of immobilized glucose oxidase (glucose oxidase, EC 1.1.3.4) membrane onto an ion selective electrode that is capable of detecting dissolved oxygen

concentrations. When working, glucose oxidase catalyzes the conversion from the substrates β -D-glucose (the analyte, exists in the solution environment) and molecular oxygen into gluconic acid and hydrogen peroxide (H_2O_2), the products. The electrode could detect oxygen changes in the environment as it is consumed by the enzymatic reaction and transmit the sensing signal into form of voltages, and, in turn, the glucose concentration can be determined, for it is proportional to the dissolved oxygen concentration in certain range. In this method, the high specificity of enzymatic reaction and powerful detecting ability of electrochemical electrode was judiciously integrated so that the biochemical reaction can be monitored through a physicochemical detector. After this exemplification, biosensors had being hot topics among the researchers worldwide. Now, biosensors had found their way in both practical applications and scientific researches in many forms of commercially available products.

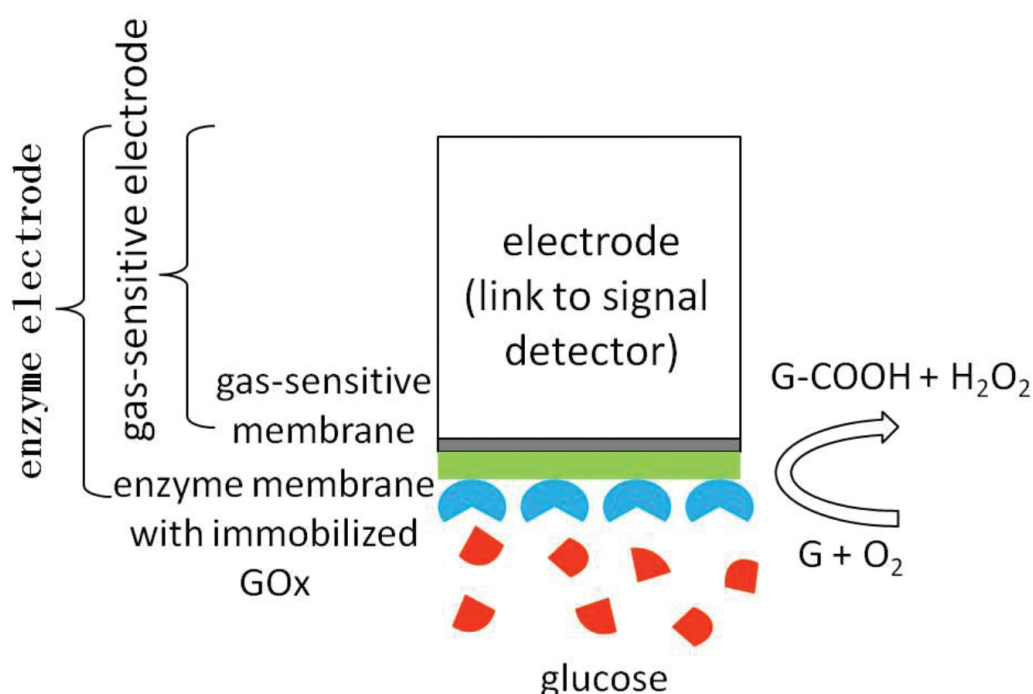


Figure 1. A schematic representation of the classic Clark enzyme electrode with glucose oxidase (Gox) as its biological element.

1.1. Classification of biosensors

As is recommended by IUPAC in 1999, a biosensor is an independently integrated receptor transducer device, which is capable of providing selective quantitative or semiquantitative analytical information using a biological recognition element [1].

A typical biosensor is made up of two main parts: (a) a biological element that can give any form of detectable signals, enzymatically catalyzed reactions, and biomolecular recognitions are the most referred, among others. Enzymes, antibodies, and nucleic acids are often exploited as the biological element; (b) a transducer by which the signal produced by the biological element can be detected and converted into measurable electrical signal.

To construct a biosensing system, three main elements are often required, they are as follows: a biological element, a transducer, and a signal processing system [2]. A schematic of a typical biosensor system can be seen in **Figure 2**.

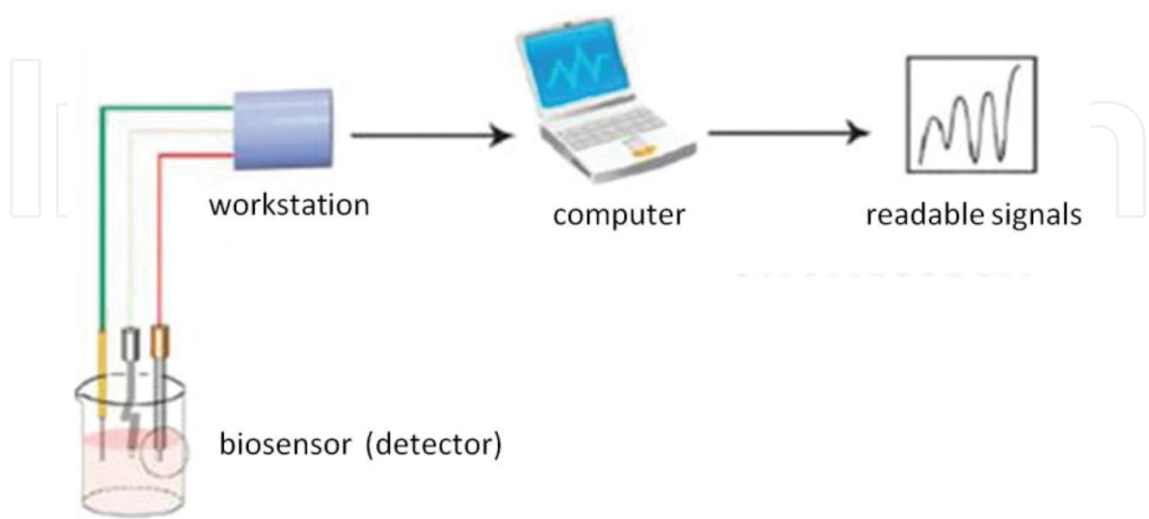


Figure 2. Basic elements to construct a biosensor system.

According to the difference of the biological element and transducer utilized, biosensors can be divided into several categories as what shall we introduced in the following listed in **Figure 3**.

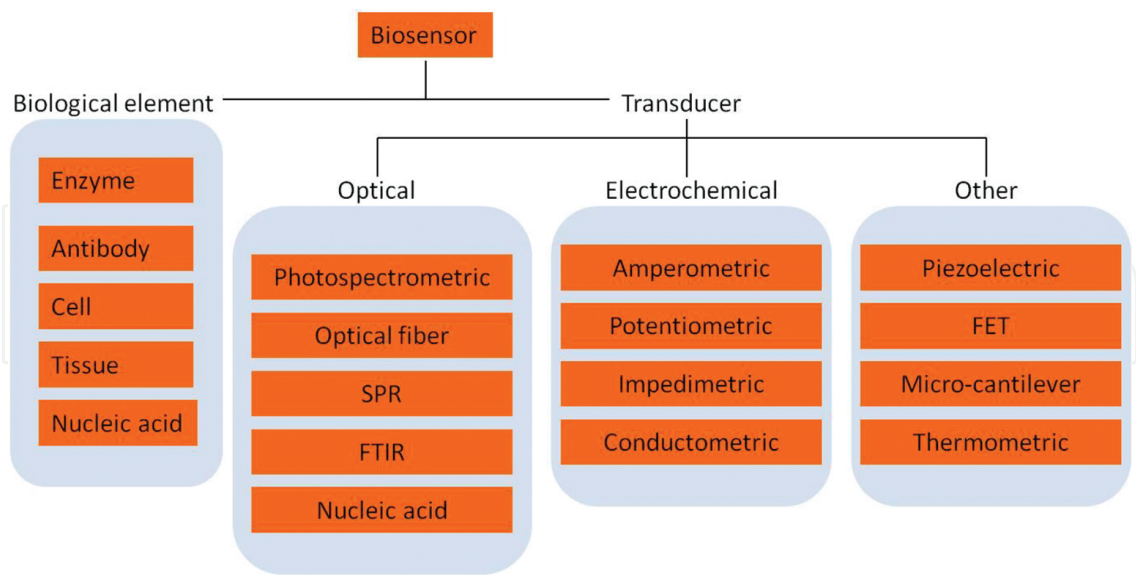


Figure 3. Classifications of common biosensors.

As is shown in the figure, electricity, light, sound, heat, and force, almost all physical dimensions have been available to be used as the target property for constructing a biosensor.

Biosensors based on different physical principles have both their advantages and disadvantages and benefit various kinds of analyzing targets. Electrochemical biosensors are inexpensive, easy to be prepared, and available to meet various ranges of analyte concentration [3]; this strategy is most often used in both fermentation and other practical applications. Optical ones can detect biological parameters with UV-visible [4], infrared [5], fluorescent [6], and chemiluminescent [7] lights. Thermal ones detect heat released in physicochemical processes. For fermentation uses, heat is released by both cellular and non-cellular processes to facilitate monitoring the fermentation progress [8]. Other strategies are often seen in comparatively highly specific uses, for example, SPR and microcantilever sensors are good choices to facilitate biomolecular researches [9], although they are often cost non-saving and high in instrumental and operative requirements.

Biosensors, other than those fit into the definition given above, and some sensing technologies that are aimed at detecting biologically related parameters yet contain not any biologically active elements are also counted, and they are usually called “generalized biosensors.” Examples of generalized biosensors include for instance: mass spectrometric measurements used in off-gas analyses in fermentation processes [10, 11] and cytometry for fermentation process controls [12].

Out of its prevalence, robust analyzing capability, cost-saving, and facile instrumenting, in this text, we put the most focus on the most studied and relatively mature in practical applications such as electrochemical biosensors.

1.2. Basic principles

Electrochemical biosensors are based on various kinds of electrodes by which electrical signals can be produced and sensed. There are three main techniques widely used for electrochemical biosensors [13]:

1. *Potentiometric*. It includes zero-current potentiometry and techniques of applying amplitude controlled current onto the working electrode. In these methods, electrical potential is detected for measurements.
2. *Amperometric*. Amperometric method is based on detecting current produced by applying a known potential. There are mainly two forms of amperometric methods: constant-potential amperometry and amperometry with applying various potential waveforms. Most of the biosensors used in fermentations are of amperometric type.
3. *Impedimetric*. It is also called impedance spectrometry, which is based on detections of impedance, conductance, and capacitance of a certain electrochemical system. It is more often used in theoretical analyses of the sensing surface.

2. Applications

2.1. Amino acid detection

Amino acid is one of the most important biomolecules for life, and they act as building blocks of numerous proteins, precursors in the synthesis of many biologically functional molecules and energy resource, in some cases. For human beings, counting in the eight essential and two semi-essential ones, in total about 22 natural amino acids are required to maintain a healthy human body. In food and medicine applications, amino acid is a vital material.

Biosensing tactics for amino acids are mainly realized by using enzyme biosensors. The enzymes are often amino acid oxidases as L-amino acid oxidase [14], glutamate oxidase [15–17], leucine dehydrogenase [18], tyrosinase [19, 20], and L-phenylalanine dehydrogenase [21].

Glutamate is very important in medical uses [22, 23], and sodium salt is a widely used seasoning additive, which is now mainly produced by large-scale cellular fermentations. Amperometric enzyme electrodes that detect glutamate is one of the most widely and maturely used biosensors. In majority of the now commercially available glutamate biosensors, a typical strategy is to integrate a glutamate oxidase onto a platinum electrode. When use, preset potential is applied upon the electrode to electrochemically catalyze the oxidation of the enzymatic reaction's product—hydrogen peroxide. Computer records the electrical current produced in this process and translates it into the corresponding concentration as the readout [24]. In a research, glutamate oxidase (EC 1.4.3.11) and NADP⁺-dependent glutamate dehydrogenase (EC 1.4.1.3) were co-immobilized onto an oxygen electrode to fabricate an enzymatic MSG detector for food uses. By exploiting those two enzymes, monosodium salt of glutamate and glutamic acid can be distinguished to perform more accurate measurements [25]. Rita et al. [26] constructed a glutamate enzyme sensor by immobilize L-glutamate oxidase (GLOD, EC 1.4.3.11) and Gox on glass carbon electrode. To minimize the interference, the enzyme electrode was then modified with the polymer Nafion, a very widely used material to improve the sensing selectivity of amperometric enzyme sensors. The electrode can perform simultaneous measurement of L-glutamate and glucose without any obvious interference. Tang et al. [27] constructed glutamate enzyme electrode with NAD⁺-dependent glutamate dehydrogenase (EC 1.4.1.3). To improve its sensitivity, the electrode was modified with nanocomposite. The electrode has a very rapid response and good stability (remains 85% sensing intensity after 4 weeks). For quantifications of total L-amino acid, enzyme electrode based on immobilized L-amino acid oxidase can be a very good choice [28].

Stasyuk et al. [29] used recombinant yeast cells as the arginine activity source to establish an amperometric biosensor along with immobilized urease. The cell-enzyme coupled sensor reportedly exhibited a linear range cross 3 orders of magnitude up to 0.6 mM and can give the result within no more than 1 min. Another strategy is by coupling arginase (EC 3.5.3.1) and urease (EC 3.5.1.5). An arginine biosensor was constructed on ion-selective field effect transistors (ISFETs) surface via co-immobilizing arginase and urease. When working, arginine catalyzes the conversion of arginine into ornithine with release of urea, which, in turn, is

degraded by urease to produce ammonium ions. Production of ammonium is accompanied by the subtle change of pH and thus can be detected by the transmitter [30].

All amino acids exist in humans are of L-form, for enzyme dealing with D-amino acids are in lack in human body. Therefore, were there D-amino acids in food or medical products, it is of danger to cause problems of safety and monitoring of their presence is often one part of many fermentation products' quality control. To meet this need, an enzyme electrode was developed by co-immobilizing D-amino acid oxidase (DAAO, EC 1.4.3.3) and peroxidase onto polymer electron mediator modified electrode [31]. Zain et al. devised a D-serine-sensitive electrochemical detector via immobilizing DAAO onto polymer functionalized metal electrode. The detector is reported to have ideal interference resistance toward most of the neurochemicals and can be used for *in vivo* D-serine detections [32].

Other than enzyme-biosensing methods, amino acid measurement can also be accomplished by enzyme-free techniques. Dai et al. [33] prepared nanoporous nickel-modified boron-doped diamond electrode with electron-assisted hot filament chemical vapor deposition method, and the electrode can capture redox processes of L-alanine and can perform anti-interference, sensitive detections of it. Seki et al. developed a tryptophan sensitive, potentiometric detector with microbial cells as the biological element. In the scenario, an auxotrophic bacterial strain *Escherichia coli* WP2—a mutant requiring tryptophan for its growth was monitored with a light-addressable potentiometric sensor. When L-tryptophan is in present, the bacterial metabolic result of it will cause pH changes, which can be detected and used for quantification by the sensor [34].

2.2. Sugar detection

In fermentation processes, sugars can either a vital substrate for cellular fermentations or in some cases the target product (e.g. oligosaccharides in isomalto-oligosaccharide [35] and chito-oligosaccharide preparations [36]; glucose from enzymatically degraded starch). The most commercially available and under-research biosensors for sugars are aiming at detections of monosaccharides. For oligo- and polysaccharides, very mature sensing platform is rarely seen mainly because accessible biological elements that exhibit good biorecognitions toward them are difficult to obtain.

Up to now, glucose is the target analyte in most biosensor researches during which its medical uses are most concerned. For fermentation uses, there had been many kinds of commercial biosensors for choice; the majority of them are operated under off-line mode.

A very widely applied glucose biosensor is much the same like the typical glutamate enzyme electrode aforementioned, with the only difference being that the enzyme alters into usually glucose oxidase (Gox). Although the rare metal-based enzyme glucose biosensors are easy to prepare, cost acceptable, and often renewable (in a typical mode of Pt-based Gox electrode, the enzyme is immobilized onto a polymer permeable membrane), biosensor is achieved by covering the enzyme laden side tightly to the Pt electrode surface. When the detected signals are seen obviously declined after a period of use, operators need to only replace the enzyme membrane with a new one from the same manufacturer to refresh the sensor. Pt base is

electrochemically inert and thus is resistant to repeated use (**Figure 4**). Difficulties in modifying the base electrode to functionalize it, high cost of the electrode hinders it from altering into disposable forms, etc., are often motives for researchers to develop more sophisticated ones.

Commercial blood glucose biosensor had been used to monitor glucose concentration in the fermentation broth. The result shown the method is potent to fill formation needs and is a good alternative for HPLC analysis and reducing sugar assay [37]. White et al. used screen-printed Gox electrode as the glucose detector to perform real-time fermentation control [38]. The electrode is a classical form of electrochemical detector, which is extremely inexpensive, highly reproducible, and repeatable, and very large-scale production is suitable. To overcome the problem of on-line sensing, the researchers introduced flow injection analysis system to rid the gap between sampling and the sensor.

In fermentations, high-temperature processing is often inevitable for preventing the fermentation processes from biological contaminations. However, biosensors are in most cases non-high-temperature tolerant. To tackle the problem, Phelps et al. proposed a glucose-sensing device that is autoclavable and can be repeatedly used [39]. Unlike the conventional immobilized enzyme electrodes, the electrode consists largely of a semipermeable membrane in protecting the sensing surface from fouling by the fermentation broth, a Pt electrode functions as its conventional versions, and a chamber with conduits through which electrolyte and enzyme solutions can be filled or discarded. The design circumvented the contact between the intense autoclaving conditions and the temperature vulnerable enzyme.

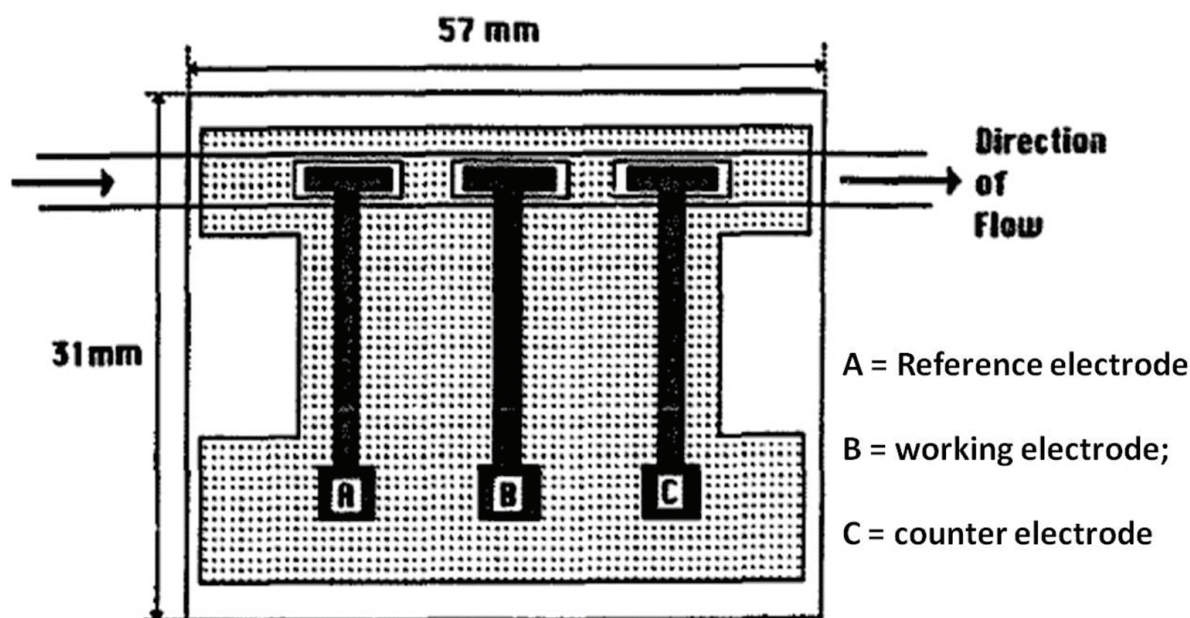


Figure 4. A schematic representation of screen-printed electrode. The base electrode is derived from a printing technology called “screen printing,” which uses a mesh with predrawn patterns hollowed out and let the printing ink to through it so that the pattern is printed onto the base material. Enzyme electrodes prepared with this method are near two dimensional so they are potable. The high reproducibility of its preparing progress grants high reproducibility to electrodes made in the same batch.

Another strategy aiming at on-line biosensor design can be seen in a research that uses non-immobilized, liquid Gox as its biological element. The base electrode is not in direct contact with the enzyme, and it only detects the catalytic product in the enzyme solution [40]. Other than the on-line uses, this design opens a new way to devise biosensors that are targeted at measuring high-concentration glucose in consideration that it is especially useful in fermentations. In large number cases of fermentation processes, glucose concentrations at the initial and early period are often too high, which exceed the upper detection limit of most enzyme-immobilized sensors. Therefore, continuous monitoring is hard to be realized without gradient dilutions that are performed, yet the process is often the key source of sampling error. The enzyme-injected mode can allow the electrode to direct detection of high glucose concentration broths.

Development of enzyme electrode has gone through three main stages, in which Gox electrode is a very good example:

Stage I: Gas-sensitive electrodes represented by Clark enzyme electrode. The measurement is performed in potentiometric method by detecting changes of dissolved oxygen or any acid or base produced in the enzymatic reaction. The problem of this strategy is as dissolved oxygen consumed, measuring results are liable to become awry of the electrode's linear range and as a result brings huge errors to the quantification.

Stage II: Electron mediator-functionalized electrodes overcome the shortcut of gas-sensitive electrodes and established new strategy for enzyme electrode designs. Electron mediators were found by researcher that when they were integrated into the biosensing interfaces, electrons produced by the enzymatic reaction can be relayed by the mediator, which, in turn, is oxidized by the base electrode. By this way, electrochemical detection can become oxygen consumption independent. Electron mediators can be either natural substances, for example cytochromes and co-enzymes, or artificial ones as some organic dyes, ferrocene and its derivatives, metal complexes, and some conductive polymers. By using electron mediators, detecting potentials can be effectively lowered so that much interference would thus be eliminated.

Stage III: Direct enzyme electrode was proposed after phenomenon that some proteins can make direct electrochemical communications with the electrode, which is represented by the finding of reversible cyclic voltammetric response on the electrode. Principle of the phenomenon is suggested to be when the redox center of the protein molecule is in close adjacent to the electrode surface, electrons can transport between them directly without the aid of any mediators. By using this mechanism, an obviously improved electron transfer efficiency can be obtained and the sensing capability is enhanced. However, it is often difficult to achieve the required conditions for enzymes. Taking Gox for example, its electrochemical active center —FAD⁺ cofactor— is wrapped into the space formed by the dimer subunits and therefore is difficult to build direct electrochemical communication between the electrode and the enzyme. When Gox is tightly adsorbed onto some electrochemical active materials such as carbon nanotubes and graphenes, the direct electron transfer can be observed. Researchers have to pay more attention and efforts to develop direct enzyme electrodes, as it is a promising strategy to construct more rapid, sensitive, and reagentless biosensors.

2.3. Alcohol detection

Alcohol content in fermentation broths can be realized by many conventional methods, for example hydrometry and gas chromatography. Considered the error limit or high expense or time-costing procedures of them, biosensor is a good alternative.

A colorimetric biosensor was proposed by Kuswandi et al. The sensor was constructed by polyaniline film immobilized alcohol oxidase. When ethanol is in presence, a color change from green to blue can be observed due to the oxidation of polyaniline by the enzyme reaction product H_2O_2 . Through the computer processing software, the method can determine alcohol quantitatively range between 0.01 and 0.8% [41]. Gotoh et al. devised an amperometric alcohol sensor based on co-immobilized alcohol dehydrogenase and coenzyme NAD^+ , the enzyme electrode shown linear response to solution contains ethanol between 0.05 and 10v/v%. As a reagentless enzyme sensor, it can stand at least weeks of continual detections without addition of the coenzyme [42].

3. Future perspectives

In the applications of fermentation processes, although many tangible advances have been achieved and a bunch of biosensors are now commercially accessible, many questions are still in need of further studies.

First, due to the biologically active species that can serve as biological elements are still in a limited range, the parameters detectable for biosensor in fermentation processes are restricted to the several kinds of target constituents. This, in one hand, can be gradually extended by finding and isolating new suitable biological constituents from the natural world. With respect to enzyme biosensors, dehydrogenase is becoming the most widely used. Over 400 dehydrogenases have been discovered or isolated; many biological constituents would be allowed to be detected by dehydrogenase sensors. Dehydrogenases often have their isozymes, and they require NAD^+ or $NADP^+$ or quinones as the cofactors. Comparing to oxidases, the redox center of dehydrogenases is not wrapped tightly by the protein components so more liable to establish direct electronic communications with electrodes. One of the most thorny problems is to establish methods for cofactors' immobilization to realize the reagentless biosensors. On the other hand, new generations may provide opportunities. One technical route is developing molecular imprinting sensors, in which artificial polymers that mimic the structure of natural enzymes, antibodies, or antigens to produce the alike high specificity can be synthesized. Even though, in some examples, the potent polymers are obtained, there still a long way ahead of the application of the technique. Another route is to establish aptamers, usually short nucleic acid chains or peptides judiciously devised and synthesized. Aptamers are alternatives yet can provide the same biorecognition functions to their natural forms. Many usable aptamers have been established, and it is a critical mission to its applications establishing a high-throughput selecting technology to accelerate the discovery of new aptamers.

Second, endeavor to improve the base electrode is never ended, and it is one of the core topics of electrochemistry. By modification, electrodes can be endowed with new functions or get their

sensor capacities enhanced. The most promising materials for electrode functionalization include nanocomposites, conductive polymers, novel electron mediators, liquid ions, and so forth. Among others, nanocomposites such as carbon nanotubes, graphene, and metal nanoparticles are attracting most of researchers' interests as they have both robust physical and chemical properties that are useful for improving sensing capabilities (e.g., limit of detection, sensitivity, selectivity, anti-interference, and electrochemical stability) and huge potential to exploit for conducting immobilization of biological elements. Nanocomposite per se is also a platform for preparing complex composites via combination of the materials mentioned above.

Third, miniaturization, integration, and automation of biosensors in fermentation uses are still at its preliminary stage. Although many commercial biosensors have, to a great extent, facilitated the detection of several kinds of constituent, it is uneasy to realize multiple parameter automatic controls for the whole fermentation process. Aside of developing more and more diverse biosensors fit for different targets, testing conditions, microfabrication technology, and Internet of Things are promising tools for achieving this goal.

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