Research Progress on the Application of Glucose Oxidase in Enzymatic Biofuel Cells

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Abstract

Enzymatic biofuel cells (EBFCs), as a novel type of green energy device, can directly convert biomass energy into electrical energy under mild conditions. They possess outstanding advantages such as good biocompatibility, environmental friendliness, and a wide range of raw material sources, demonstrating broad application prospects in fields such as wearable electronic devices, implantable medical devices, and environmental monitoring. Glucose oxidase (GOx), as the most commonly used anode biocatalyst in EBFCs, has become a research hotspot in this field due to its high specificity for glucose, high catalytic activity, and wide availability. This paper systematically reviews the research progress in the application of glucose oxidase in EBFCs, focusing on the catalytic mechanism of GOx, optimization of immobilization techniques, and its application effects in different types of EBFCs. It analyzes the core issues currently existing in the application of GOx in EBFCs, such as poor enzyme stability, low electron transfer efficiency, and short service life, and provides an outlook on its future development trends and application prospects. This offers important theoretical references and key technical insights for the subsequent development of high-performance GOx-based EBFCs.

Keywords

Glucose oxidase, Enzymatic biofuel cell, Immobilization technology, Electron transfer, Catalytic performance

Introduction

With the increasing aggravation of the global energy crisis and the continuous enhancement of environmental protection awareness, the development of efficient, clean, and sustainable new energy technologies has become a key breakthrough in addressing energy and environmental issues. The excessive exploitation and use of traditional fossil fuels have not only exacerbated resource depletion but also caused severe environmental pollution. Therefore, the research and application of renewable and clean energy sources have garnered widespread attention. Biofuel cells (BFCs), as a type of green energy device capable of directly converting biomass energy into electrical energy, utilize enzymes or microorganisms as biocatalysts. They achieve efficient energy conversion by catalyzing redox reactions between fuels such as glucose, lactic acid, methanol and oxidants. They possess unique advantages, including mild reaction conditions (room temperature, normal pressure, neutral pH), good biocompatibility, and no secondary pollution, making them promising candidates as an important supplement to traditional fossil fuels [1].

Enzymatic biofuel cells, as an important branch of biofuel cells, employ pure enzymes as catalysts. Compared to microbial fuel cells, they offer advantages such as high catalytic efficiency, rapid reaction rates, high selectivity, and significant potential for miniaturization, making them particularly suitable for powering microelectronic devices [2]. In EBFCs, the anode catalyst is responsible for catalyzing the oxidation reaction of the fuel and serves as one of the core components determining the battery's energy conversion efficiency and output performance. Glucose, as one of the most abundant biomasses in nature, is widely present in animal and plant body fluids, food waste, and environmental water bodies. With its characteristics of being widely available, renewable, non-toxic, and

harmless, glucose has become one of the most ideal fuels for EBFCs.

GOx is an oxidoreductase mainly derived from Aspergillus niger and Penicillium notatum. It efficiently and specifically catalyzes the oxidation of β-D-glucose into glucono-δ-lactone and hydrogen peroxide with electron transfer [3]. Since the 1960s, GOx has been widely used as an anode biocatalyst in enzyme-based biofuel cells, and great progress has been made in enzyme immobilization, electron transfer efficiency, and cell structure optimization.

However, several challenges remain in its practical application. Free GOx easily deactivates and is hard to recycle, while low electron transfer efficiency limits its catalytic performance. GOx also shows poor stability in complex environments, leading to short service life. In addition, the hydrogen peroxide produced may inhibit enzyme activity and impair long-term operation. These problems restrict the practical application of GOx-based EBFCs. Therefore, improving the catalytic performance and stability of GOx has become a key research focus.

This paper systematically reviews the progress of research in the application of GOx in enzymatic biofuel cells by integrating relevant domestic and international research findings from recent years. Starting with the catalytic mechanism of GOx, it elaborates in detail on its immobilization techniques, applications in different types of EBFCs, performance optimization strategies, and analyzes the major challenges currently faced. On this basis, it provides an outlook on future development trends, aiming to offer references for the further research, development, and practical applications of GOx-based EBFCs.

Catalytic mechanism of glucose oxidase and its role in enzymatic biofuel cells

Basic structure and catalytic mechanism of glucose oxidase

GOx is a homodimeric glycoprotein, with each subunit composed of approximately 600 amino acid residues and having a molecular weight of about 160 kDa. Each subunit contains a flavin adenine dinucleotide (FAD) as a prosthetic group. FAD serves as the active center of the GOx catalytic reaction, responsible for the reception and transfer of electrons [4]. The catalytic reaction of GOx exhibits high substrate specificity, efficiently catalyzing only the oxidation of β-D-glucose, while its catalytic activity towards α-D-glucose is extremely low (approximately 1/100 that of β-D-glucose). In practical applications, glucose isomerase is often added to convert α-D-glucose into β-D-glucose to enhance substrate utilization [5].

The reaction process of glucose oxidation catalyzed by GOx can be divided into two steps: First, β-D-glucose binds to the active center of GOx and undergoes an oxidation reaction to produce D-glucono-δ-lactone and reduced flavin adenine dinucleotide ($FADH_2$). Subsequently, $FADH_2$ is oxidized by an oxidizing agent (such as oxygen) to regenerate FAD, while generating hydrogen peroxide (H_2O_2) [6]. The overall reaction equation is as follows:

β-D-glucose + O_2 + H_2O → D-glucono-δ-lactone + H_2O_2

In enzyme-based biofuel cells, GOx acts as the anode catalyst. It catalyzes glucose oxidation, converts chemical energy into electrons, and transfers these electrons to the electrode via direct or indirect electron transfer pathways. The electrons then pass through an external circuit to form a current, realizing energy conversion [7]. Therefore, the catalytic activity, electron transfer efficiency, and stability of GOx directly determine the output power, energy conversion efficiency, and service life of EBFCs.

Electron transfer mechanism of glucose oxidase in enzymatic biofuel cells

In GOx-based enzymatic biofuel cells, the process of electron transfer from the GOx active center to the electrode surface is a crucial step for energy conversion. Based on different electron transfer modes, it can be classified into two mechanisms: direct electron transfer (DET) and mediated electron transfer (MET) [8].

The direct electron transfer mechanism refers to the process where the active center of GOx comes into direct contact with the electrode surface, allowing electrons to transfer directly from FAD to the electrode without passing through any mediating substances. This mechanism offers advantages such as high electron transfer efficiency, absence of mediator contamination, and a simple battery structure, making it an ideal mode of electron transfer. However, since FAD is buried within the enzyme molecule, approximately 1.3 nm from the enzyme's surface, and enzyme molecules tend to orient randomly on the electrode surface. It is difficult for FAD

to form effective contact with the electrode surface, resulting in significant electron transfer resistance. Therefore, achieving efficient direct electron transfer between GOx and the electrode has long been a research challenge in this field [9]. In recent years, researchers have significantly improved the direct electron transfer efficiency between GOx and the electrode through strategies such as electrode surface modification and oriented immobilization of enzyme molecules. For example, modifying the electrode surface with nanomaterials can increase the specific surface area of the electrode, promote the oriented arrangement of enzyme molecules, shorten the distance between FAD and the electrode surface, thereby enhancing the electron transfer rate.

The indirect electron transfer mechanism achieves electron transfer between the active center of GOx and the electrode surface by introducing a redox mediator as an electron transfer "bridge". The specific process is as follows: After GOx catalyzes the oxidation of glucose, it transfers electrons to the mediator, reducing it. The reduced mediator then diffuses to the electrode surface, transfers electrons to the electrode, and is oxidized by itself, completing the electron transfer cycle [10]. Commonly used redox mediators include potassium ferricyanide ($K_3[Fe(CN)_6]$), ferrocene and its derivatives, and quinone compounds [11].

The advantage of the indirect electron transfer mechanism is its low electron transfer resistance and ease of implementation, which can effectively enhance the output performance of the battery. However, the use of mediators also brings about a series of issues, such as susceptibility to leakage, toxicity, potential inhibition of enzyme activity, and energy loss due to the diffusion process of mediators, which reduces the energy conversion efficiency of the battery [12].

In recent years, researchers have also developed an electron transfer approach based on "redox polymers". This method involves covalently bonding redox mediators to the polymer molecular chains to form redox polymers, followed by immobilizing GOx on the surface of electrodes modified with these polymers. The mediators, confined within the polymer network, can not only prevent leakage but also enable rapid electron transfer, balancing both electron transfer efficiency and system stability [13]. This approach combines the advantages of direct and indirect electron transfer, and has become an important development direction for the electron transfer mechanism in GOx-based EBFCs.

Immobilization techniques of glucose oxidase and their application optimization in enzymatic biofuel cells

Free GOx faces issues such as easy inactivation, difficult recovery, and low electron transfer efficiency in EBFCs applications, severely restricting battery performance and service life. Immobilization technology, by loading GOx onto carrier materials, can significantly enhance enzyme stability, improve recovery rates, and facilitate electron transfer between the enzyme and electrode, thus becoming a key technical approach for improving the performance of GOx-based EBFCs [14]. Ideal GOx immobilization carriers should possess characteristics such as a large specific surface area, good electrical conductivity, excellent biocompatibility, and suitable pore structure to provide a stable microenvironment for GOx while facilitating electron transfer and substrate diffusion [15]. Currently, the immobilization methods for GOx mainly include physical adsorption, covalent binding, entrapment, and cross-linking, with significant differences in their mechanisms of action and practical application effects. The following section will provide a detailed elaboration on the research progress of various immobilization techniques.

Physical adsorption method

The physical adsorption method is the simplest and most commonly used method to immobilize GOx. Its principle is to use the non-covalent interaction between the carrier material and GOx molecules (such as van der Waals forces, hydrogen bonds, electrostatic attraction, etc.) to adsorb GOx on the carrier surface to achieve enzyme immobilization [16]. This method has the advantages of simple operation, mild conditions, does not destroy the spatial structure of the enzyme, has little impact on enzyme activity, does not require the use of chemical reagents, has low cost, and is suitable for large-scale preparation [17].

Commonly used physical adsorption carriers mainly include carbon materials (such as graphene, carbon nanotubes, activated carbon), metal nanomaterials (such as gold nanoparticles, platinum nanoparticles), and polymer materials (such as polypyrrole, polyaniline) [18].

For example, Yi et al. used reduced graphene oxide/gold nanoparticles/multi-walled carbon nanotubes (rGO/AuNPs/MWCNTs) composite material as the electrode base, fixed GOx on the electrode surface through physical adsorption method, and constructed an EBFCs bioanode [19]. The composite material has excellent conductivity, good biocompatibility and a large specific surface area. Its synergistic effect can significantly increase the loading capacity of GOx and effectively promote the transfer of electrons on the electrode surface. The self-powered sensor built based on this anode has a good linear relationship between its maximum output power and glucose concentration, and has been successfully used to detect glucose concentration in human serum samples.

However, the physical adsorption method also has obvious limitations: The binding force between GOx and the carrier is weak. During the battery operation, affected by factors such as substrate diffusion and solution stirring, GOx easily falls off the carrier surface, resulting in the loss of enzyme activity and thus affecting the long-term stability of the battery [20]. To improve this problem, researchers usually combine physical adsorption with other immobilization methods, such as cross-linking treatment after physical adsorption, using cross-linking agents to enhance the binding force between GOx and the carrier, thereby improving the stability of the immobilized enzyme [21].

Covalent bonding method

The covalent binding method uses chemical reagents to combine active groups in the GOx molecules with functional groups on the surface of the carrier in the form of covalent bonds to achieve immobilization of the enzyme [22]. The main advantages of this method are that the binding force between GOx and the carrier is strong, the enzyme is not easy to fall off, and the immobilized enzyme has high stability. At the same time, it can achieve directional immobilization of the enzyme, which is conducive to promoting electron transfer between the enzyme and the electrode [23]. Commonly used cross-linking agents include glutaraldehyde, carbodiimide (EDC), N-hydroxysuccinimide (NHS), etc. Among them, glutaraldehyde is the most widely used due to its high cross-linking efficiency and low cost [24].

For example, Mazar et al. used EDC/NHS as a cross-linking agent to covalently bind GOx to the surface of an amination-modified graphene electrode and successfully constructed an immobilized GOx bioanode [25]. Experimental results show that the covalent binding method significantly improves the immobilization amount and stability of GOx. The activity retention rate of the immobilized enzyme exceeds 85%, and after 100 hours of continuous operation, the enzyme activity still maintains more than 70% of the initial value. The performance is significantly better than the physical adsorption method.

In addition, through directional covalent immobilization technology, the active center of GOx can be directed towards the electrode surface, thereby shortening the electron transfer distance and further improving the electron transfer efficiency [26]. However, the covalent binding method also has certain limitations: The operation process is relatively complex and requires functional modification of the carrier, and the use of cross-linking agents may destroy the active center of GOx, resulting in a decrease in enzyme activity [27]. Therefore, in practical applications, it is necessary to optimize the cross-linking agent concentration, reaction time and reaction conditions in order to maximize the catalytic activity of the enzyme while ensuring the stability of the immobilization.

Encapsulation method

The encapsulation method is to wrap GOx molecules in a network structure formed by a carrier material, and immobilize the enzyme through the physical barrier of the carrier. This method is easy to operate, can achieve efficient loading of enzymes, and the carrier network can provide a stable microenvironment for GOx, effectively protecting enzyme activity and preventing it from being damaged by external factors such as temperature, pH, and proteases. Commonly used embedding carriers include polymer gels (such as sodium alginate, chitosan, polyacrylamide), inorganic nanomaterials (such as silica, titanium dioxide), and metal organic frameworks (MOFs).

As a new type of porous material, MOFs have been widely used in the embedding and immobilization of GOx in recent years due to its advantages such as large specific surface area, adjustable pore size, and good biocompatibility [28]. For example, Professor Ma Jiwei's team at Tongji University used MOFs materials to embed and immobilize GOx. The study found that

MOFs can not only achieve efficient loading of GOx, but also effectively protect enzymes from biomolecule attacks. At the same time, its porous structure is conducive to the diffusion of substrates and products, significantly improving the catalytic performance and stability of the immobilized enzyme [29]. In addition, combining MOFs with carbon materials can further enhance the conductivity of the carrier and promote electron transfer.

However, the encapsulation method also has certain limitations: the carrier network structure may hinder the diffusion of substrates and products, resulting in a decrease in the catalytic reaction rate. For some rigid carriers, the enzyme molecules may be squeezed during the encapsulation process, destroying their spatial structure, thereby affecting the enzyme activity [30]. Therefore, optimizing the pore size structure of the embedding carrier and improving the permeability of the carrier are the keys to improving the performance of GOx immobilized by the embedding method.

Cross-linking method

The cross-linking method uses a cross-linking agent to cross-link between GOx molecules or between GOx molecules and the carrier to form a three-dimensional network structure, thereby immobilizing the enzyme. This method does not rely on carriers (or can be used in combination with carriers), and immobilization can be completed only through cross-linking between enzyme molecules. It has the advantages of simple operation, high stability of the immobilized enzyme, and adjustable enzyme loading. Commonly used cross-linking agents are similar to covalent binding methods, with glutaraldehyde and EDC/NHS being the main ones [31]. For example, Zhou et al. used glutaraldehyde as the cross-linking agent, cross-linked GOx with horseradish peroxidase (HRP), constructed a dual-enzyme cross-linking system, and fixed it on the surface of the carbon electrode for anode catalysis of EBFCs [32]. Experimental results show that the stability of GOx is significantly improved after cross-linking. At the same time, the dual-enzyme system can effectively decompose the hydrogen peroxide produced by GOx catalysis and weaken its inhibitory effect on enzyme activity, thus enhancing the long-term operation stability of the battery. In addition, the cross-linking method can also be used in conjunction with physical adsorption and embedding methods to further optimize the immobilization effect.

However, the cross-linking method also has certain limitations: too high a cross-linking agent concentration can easily lead to excessive aggregation of enzyme molecules, destroying the active center of the enzyme, thereby reducing catalytic activity. At the same time, cross-linked enzyme molecules are difficult to recycle, increasing the cost of use [33]. Therefore, cross-linking agent concentration and reaction conditions need to be strictly controlled in practical applications to achieve a balance between immobilization stability and enzyme activity.

Collaborative optimization of immobilization technology

A single immobilization method often has its own limitations and is difficult to meet multiple requirements such as enzyme activity, stability, and electron transfer efficiency at the same time. In recent years, researchers have paid more and more attention to the collaborative application of multiple immobilization technologies, and achieved comprehensive optimization of the GOx immobilization effect by combining the advantages of different methods [34]. For example, combining physical adsorption and cross-linking can effectively improve the stability of immobilized enzymes. In this strategy, GOx is first loaded onto the carrier surface by physical adsorption, followed by cross-linking with a cross-linking agent. This method not only preserves enzyme activity but also strengthens the interaction between the enzyme and the carrier [35]. Combining the embedding method with the covalent binding method, first covalently bind GOx to the carrier surface, and then embed it in a polymer gel, which can not only prevent the enzyme from falling off, but also reduce the carrier's hindrance to substrate diffusion.

In addition, the modification of carrier materials has also become an important direction for the optimization of immobilization technology. By functionally modifying the carrier (such as amination, carboxylation, thiolation), the interaction between the carrier and GOx can be enhanced to achieve directional immobilization of enzymes. By compounding different types of carrier materials (such as carbon materials with metal nanomaterials, polymer materials), the conductivity, biocompatibility and specific surface area of the carrier can be taken into account, further improving the catalytic

performance and electron transfer efficiency of immobilized Gox [36]. For example, Žalnėravičius et al. composited GOx with Spirulina lysate and multi-walled carbon nanotubes, and fixed it on the electrode surface using a combination of physical adsorption and cross-linking [37]. The prepared bioanode showed excellent catalytic performance and stability. The relevant research results were published in the journal *Electrochimica Acta*.

Applications of glucose oxidase in different types of enzyme biofuel cells

Enzyme biofuel cells can be divided into various types based on different battery structures, electrolyte types and application scenarios. Various types of EBFCs have different requirements for GOx in terms of catalytic performance, immobilization method and electrode structure. This section will focus on the application research progress of GOx in several typical EBFCs, including aqueous EBFCs, membrane-less EBFCs, wearable EBFCs, and implantable EBFCs.

Aqueous enzyme biofuel cells

Aqueous enzyme biofuel cells are the most basic and common configuration of EBFCs. The electrolyte uses aqueous solution and can usually be divided into two types: Double-chamber structure (the anode chamber and cathode chamber are separated by a proton exchange membrane) and single-chamber structure (the anode and cathode are placed in the same electrolyte solution) [38]. This type of EBFCs has the advantages of easy operation and low cost. It is mainly used for laboratory research and power supply of small electronic equipment. It is also the most widely used type of EBFCs for Gox [39].

In aqueous EBFCs, GOx serves as an anode catalyst and is usually used in combination with a redox mediator to achieve electron transfer through an indirect electron transfer mechanism to improve the output performance of the battery [40]. For example, Kausaite-Minkstimiene et al. developed a self-powered glucose biosensor based on single-enzyme EBFCs, using GOx as the anode catalyst and potassium ferricyanide as the mediator [41]. The open circuit voltage of the constructed EBFCs reached 0.6 V, and the maximum output power density was 12 μW/cm², achieving efficient detection of glucose. The relevant results were published in the journal Biosensors. In addition, researchers further improved the performance of aqueous GOx-based EBFCs by optimizing immobilization technology and electrode materials. For example, in a study reported by Pak et al. in the *journal Advanced Functional Materials*, by optimizing the GOx immobilization method and electrode structure, the constructed glucose-based EBFCs achieved high power output and enhanced operational stability, with a maximum power density exceeding 50 μW/cm² [42].

However, aqueous EBFCs also have certain limitations: The electrolyte solution is easy to leak, the device is large, and it is difficult to achieve miniaturization. The intermediary is easy to diffuse and lose during operation, affecting the long-term stability of the battery. In addition, fuel and electrolytes need to be replenished regularly, and the maintenance cost is high [43]. Therefore, this type of EBFCs is mainly suitable for laboratory research and short-term power supply scenarios, and is difficult to meet the needs for miniaturization and long-term stability of practical applications such as wearable and implantable devices.

Membrane-free enzyme biofuel cells

Membrane-free enzyme biofuel cells feature a single-chamber structure without proton exchange membranes. Rational design of electrode structures and selective catalysts avoids cross-reactions between fuel and oxidant, thus removing the need for proton exchange membranes [44]. This type of EBFCs has the advantages of simple structure, low cost, small internal resistance, high energy conversion efficiency and easy miniaturization. In recent years, it has become a research hotspot in the field of EBFCs and an important direction for GOx applications [45].

The core of membraneless EBFCs is to ensure high selectivity for GOx-catalyzed glucose oxidation at the anode and oxygen reduction at the cathode, thereby preventing glucose from reacting with the cathode oxidant while facilitating proton transfer [46]. Researchers have significantly improved the performance of membraneless GOx-based EBFCs by optimizing electrode materials, immobilization techniques, and battery structures. Ye et al. pointed out in a review that carbon nanomaterials have become ideal enzyme host matrices due to their good electrical conductivity and biocompatibility [47]. Through engineering strategies such as morphology control, heteroatom doping, surface functionalization, and carbon

composites, the bioelectrocatalytic efficiency of the enzyme-electrode interface can be significantly enhanced, thereby achieving high performance of EBFCs. Studies have shown that membraneless EBFCs optimized based on carbon nanomaterials have made significant progress in power density, stability, and miniaturization, providing new ideas for self-powered applications in wearable and implantable electronic devices.

In addition, Yi Jinfei et al. used rGO/AuNPs/MWCNTs composite materials to construct a film-free EBFCs self-powered glucose biosensor. GOx was fixed on the surface of the anode, and manganese dioxide was electrodeposited on the cathode. This sensor does not require an external power source and can achieve efficient detection of glucose with a detection limit as low as 0.3 mmol/L. It has been successfully applied to the detection of human serum samples. The development of membraneless EBFCs has provided the possibility for miniaturization and low-cost application of GOx-based EBFCs. However, it still faces problems such as cross-reaction between fuel and oxidant and insufficient proton transfer efficiency. It is necessary to further optimize the electrode structure and catalyst selectivity [48].

Wearable enzyme biofuel cells

With the rapid development of wearable electronic devices (such as smart watches, sports bracelets, health monitoring patches, etc.), the demand for miniaturized, lightweight and sustainable power supply energy devices is increasingly urgent [49]. Wearable enzymatic biofuel cells use glucose in human body fluids (such as sweat, saliva, blood) as fuel, have self-powering capabilities, and have the advantages of small size, light weight, and good biocompatibility. They are an ideal energy supply solution for wearable electronic devices. Among them, GOx, as an anode catalyst, plays a central role in wearable EBFCs [50].

The key design requirements for wearable GOx-based EBFCs include miniaturization, flexibility, high stability, and the ability to adapt to dynamic changes in the human skin environment (such as fluctuations in body temperature, pH, and sweat secretion) [51]. To this end, the researchers used flexible electrode materials (such as carbon cloth, graphene sheets, flexible polymers) and combined with optimized immobilization technology to successfully prepare flexible and wearable GOx-based EBFCs. For example, the team of Professor Ma Jiwei of Tongji University fixed GOx on the surface of flexible carbon cloth electrode modified with nanomaterials to construct flexible wearable EBFCs. The battery can use glucose in human sweat as fuel to achieve stable power supply, with a maximum output power density of 35 $\mu W/cm^2$, which can provide continuous power for small wearable electronic devices (such as heart rate monitors). In addition, researchers have also developed fabric-based wearable EBFCs, fixed GOx on the surface of textile materials, and prepared a self-powered fabric, which not only ensures wearing comfort, but also realizes the energy conversion function [52]. For example, Pak et al. reported a wearable GOx-based EBFCs based on moisture management fabric, which maintains stable power output by regulating sweat transmission. The battery can be integrated into sportswear and use glucose in sweat to power wearable devices, showing good application prospects. Although wearable GOx-based EBFCs are developing rapidly, they still face several key challenges: The glucose concentration in human sweat is low (0.1~1 mmol/L), resulting in insufficient battery output power. Sweat secretion fluctuates greatly, affecting the stability of battery performance. Enzymes are easily inactivated during long-term wear, and their service life is limited [53]. These problems urgently need to be solved through material innovation, enzyme engineering modification and system structure optimization.

Implantable enzyme biofuel cell

Implantable enzyme biofuel cells are energy devices that can be implanted inside the human body and use glucose in body fluids (such as blood and tissue fluid) as fuel to power implanted medical devices (such as pacemakers, neurostimulators, glucose monitors, etc.) [54]. This type of EBFCs has extremely high requirements for biocompatibility, stability and safety. It must be non-toxic, non-immunogenic and capable of long-term stable operation. Among them, GOx serves as an anode catalyst, and its performance and safety directly determine the application feasibility of implantable EBFCs [55].

The core challenge facing implantable GOx-based EBFCs is that the glucose concentration in human blood is relatively low (3.9~6.1 mmol/L) and there are a variety of interfering substances (such as proteins, amino acids, lactic acid, etc.), so GOx is required to have high

catalytic activity and selectivity. At the same time, the hydrogen peroxide generated during the GOx catalytic process may cause damage to surrounding tissues and inhibit enzyme activity, thereby affecting the long-term stability of the battery [56]. In response to the above problems, researchers have made positive progress by optimizing GOx immobilization technology, developing new mediators and electrode materials, and introducing hydrogen peroxide decomposing enzymes (such as catalase) and other strategies.

Currently, implantable GOx-based EBFCs are still in the laboratory research stage and have not yet been used in clinical applications. They mainly face the following bottlenecks: battery output power is not enough to meet the power supply needs of implantable medical devices. The stability and biocompatibility of enzymes during long-term implantation still need to be further improved, battery packaging technology needs to be optimized to prevent electrolyte leakage and enzyme loss. Solving the above problems will promote implantable GOx-based EBFCs to take a key step towards clinical application.

Challenges and future development trends

Despite significant progress in the application of GOx in EBFCs, with breakthroughs in immobilization technology, electron transfer efficiency, and battery structure optimization. GOx-based EBFCs still face numerous challenges that severely restrict their practical application, mainly in the following aspects.

Challenges

(1) Glucose oxidase has poor stability

The stability of GOx is a key factor affecting the service life of EBFCs. However, GOx is prone to conformational changes in the operating environment of EBFCs (such as temperature, pH changes, fluctuations in substrate concentration, and the presence of interfering substances), resulting in the destruction of the active center and loss of enzyme activity. For example, in the human body fluid environment, fluctuations in body temperature (37 °C), pH (7.35~7.45), as well as proteins, proteases and other substances in the blood will cause the stability of GOx to decrease. Usually, the half-life of free GOx is only a few days, and the half-life of immobilized GOx is difficult to exceed several months. In addition, the hydrogen peroxide generated during the GOx catalysis process will irreversibly inhibit the enzyme activity and further shorten the service life of the enzyme. Although the stability of GOx can be improved to a certain extent through immobilization technology and the addition of hydrogen peroxide decomposing enzymes, it is still difficult to meet the needs for long-term stable operation of EBFCs (for example, implantable devices need to operate for several years).

(2) Electron transfer efficiency needs to be improved

Electron transfer efficiency is the core factor that determines the output power and energy conversion efficiency of EBFCs. Although researchers have significantly improved the electron transfer efficiency between GOx and electrodes through electrode modification, immobilization technology optimization and other methods, there is still the problem of high electron transfer resistance. For the direct electron transfer mechanism, the active center of GOx is located inside the enzyme molecule, far away from the electrode surface, and the enzyme molecules are randomly oriented on the electrode surface, resulting in low electron transfer efficiency. For the indirect electron transfer mechanism, the diffusion process of the mediator will cause energy loss, and the mediator is easy to leak, affecting the stability of electron transfer. In addition, during the immobilization process, the conformational changes of the enzyme molecules may also cause the distance between the active center and the electrode to increase, further reducing the electron transfer efficiency.

(3) Low fuel utilization rate

The catalytic reaction of GOx on glucose can only oxidize glucose to gluconolactone, which is an incomplete oxidation reaction and can only release part of the chemical energy in the glucose molecules, resulting in low fuel utilization. In addition, during the operation of EBFCs, the diffusion rate of glucose as a fuel is limited, especially in the carrier network of immobilized enzymes. The substrate diffusion resistance is large, resulting in the inability to fully exert the catalytic activity of GOx, further reducing the fuel utilization rate. At the same time, the low concentration of glucose in human body fluids (such as sweat, blood) also limits the supply of fuel, resulting in insufficient battery output power.

(4) High cost, difficult to scale up production

The cost of separation and extraction of GOx is high, especially high-purity GOx is expensive, and a large amount of chemical reagents such as carrier materials

and cross-linking agents are required during the immobilization process, which increases the preparation cost of EBFCs. In addition, the current preparation process of GOx-based EBFCs is complex, mostly in small-scale laboratory preparations, making it difficult to achieve large-scale production. Moreover, the battery has a short service life and high maintenance costs, further limiting its practical application.

(5) Biocompatibility and safety need further optimization

For wearable, implantable GOx-based EBFCs, biocompatibility and safety are crucial. At present, the immobilized carriers of GOx (such as some metal nanomaterials and polymer materials) may have certain toxicity and cause irritation or damage to human skin or tissues. Leakage of the mediator may also have adverse effects on the human body. In addition, if the hydrogen peroxide generated by GOx catalysis cannot be decomposed in time, it will cause oxidative damage to human tissues and affect the safety of the battery. Therefore, the development of biocompatible, non-toxic and harmless immobilized carriers and mediators, as well as efficient hydrogen peroxide decomposition systems, is the key to improving the biocompatibility and safety of GOx-based EBFCs.

Research prospects

In response to the current problems in the application of GOx in EBFCs, and in light of the development trends in materials science, bioengineering, and electrochemistry in recent years. Future research on the application of GOx in EBFCs will focus on the following directions to promote performance improvement and practical application of GOx-based EBFCs.

(1) Modification and directed evolution of glucose oxidase

Modification and directed evolution of GOx through bioengineering technology represent an important approach to improve its catalytic performance, stability and substrate selectivity. Using genetic engineering, protein engineering and related strategies to adjust the amino acid sequence of GOx and optimize its active center structure can effectively enhance its catalytic activity, glucose binding affinity and environmental stability. It can also reduce the inhibitory effect caused by hydrogen peroxide accumulation. For example, the GOx mutant (I115V) prepared by Zhu et al. via directed evolution exhibited significantly improved catalytic activity and oxygen affinity, providing a reliable reference for the rational design of GOx. In the future, combined with advanced gene-editing tools such as CRISPR-Cas9, more precise modification of GOx can be realized, which is expected to develop high-performance GOx mutants more suitable for efficient and stable operation of EBFCs.

(2) Research and development of new immobilization technologies and carrier materials

The development of new immobilization technologies and carrier materials is the key to improving the immobilization effect of GOx, promoting electron transfer, and improving enzyme stability. In the future, we should focus on developing new carrier materials with high conductivity, high specific surface area, good biocompatibility and adjustable pore size. These materials include new MOFs, two-dimensional nanomaterials such as MXene, and conductive polymer composites. At the same time, new immobilization technologies are explored, such as in-situ polymerization immobilization, biomolecule recognition immobilization, etc., to achieve directional immobilization and efficient loading of GOx, further shortening the electron transfer distance and improving electron transfer efficiency. In addition, through the functional modification of the carrier material, the introduction of hydrogen peroxide decomposing enzymes or antioxidants can promptly decompose the hydrogen peroxide produced by GOx catalysis, protect the enzyme activity, and improve the long-term stability of the battery.

(3) Optimization and innovation of electron transfer mechanism

Optimizing the electron transfer mechanism between GOx and the electrode to enhance electron transfer efficiency is the core of improving the output performance of EBFCs. Future research should focus on developing efficient direct electron transfer systems through electrode surface modification and directional enzyme immobilization. These strategies shorten the distance between the GOx active site and the electrode surface, thus promoting efficient direct electron transfer. Meanwhile, it is essential to develop novel non-toxic, leak-resistant redox mediators and redox polymers to optimize the indirect electron transfer mechanism and reduce energy loss. Additionally, exploring novel electron transfer pathways, such as utilizing quantum

dots, nanowires, and other materials as electron transfer "bridges", can further enhance electron transfer efficiency.

(4) Construction of multi-enzyme synergistic catalytic system

Constructing a multi-enzyme synergistic catalytic system can achieve complete oxidation of glucose, enhance fuel utilization efficiency, and improve the output power and energy conversion efficiency of the battery. For instance, by combining GOx with enzymes such as glucose dehydrogenase and gluconate dehydrogenase to construct a multi-enzyme catalytic system, glucose can be gradually oxidized into carbon dioxide and water, fully releasing the chemical energy stored in glucose. Meanwhile, the introduction of catalase can promptly decompose the hydrogen peroxide generated during GOx catalysis, protecting enzyme activity and enhancing battery stability. In the future, in-depth research should be conducted on the synergistic mechanisms among multiple enzymes, optimizing the immobilization methods and ratios of multi-enzymes to construct an efficient and stable multi-enzyme synergistic catalytic system.

(5) Practical research and development of wearable and implantable EBFCs

Wearable and implantable enzyme-based biofuel cells represent the most promising application directions for GOx-based EBFCs, and future efforts should focus on advancing their practical development. For wearable EBFCs, it is essential to develop flexible, lightweight, and breathable electrode materials and battery structures, optimize the immobilization technology of GOx, enhance battery stability and output performance in sweat environments, and achieve integration with wearable electronic devices.

For implantable EBFCs, priority should be given to improving biocompatibility and safety. We should develop non-toxic, non-immunogenic immobilization carriers and mediators, optimize battery encapsulation techniques to extend battery lifespan, and enhance output power to meet the power supply requirements of implantable medical devices. Additionally, by integrating sensor technology, multifunctional EBFCs that combine power supply and monitoring capabilities should be developed to expand their application areas.

(6) Optimization of large-scale production processes

Reducing the preparation cost and achieving large-scale production of GOx-based EBFCs are prerequisites for their practical application. In the future, it is essential to optimize the separation and extraction processes of GOx to lower the production cost of high-purity GOx; develop low-cost and readily available immobilization carriers and chemical reagents to simplify the immobilization process. Meanwhile, exploring large-scale preparation techniques, such as printed electronics technology and roll-to-roll technology, should be pursued to enable batch production of EBFCs, reduce preparation costs, and promote their industrial application.

Conclusion

Glucose oxidase (GOx) is a key anode biocatalyst for enzymatic biofuel cells (EBFCs), and its application research has greatly promoted the development of EBFCs as green energy devices. This paper reviews the catalytic and electron transfer mechanisms of GOx, the research progress of its immobilization technologies, and its application in aqueous, membraneless, wearable and implantable EBFCs, and summarizes the core challenges in its practical application, such as poor enzyme stability, low electron transfer efficiency, low fuel utilization, high cost, and insufficient biocompatibility.

In recent years, breakthroughs have been made in improving the performance of GOx-based EBFCs via electrode modification, directional enzyme immobilization, development of new carrier materials and battery structure optimization, laying a foundation for their application in wearable electronics, implantable medical devices and other fields. However, the industrial and clinical application of GOx-based EBFCs is still restricted by the above technical bottlenecks.

Future research should focus on the cross-integration of bioengineering, materials science and electrochemistry, including the direct evolution and modification of GOx, development of high-performance immobilization carriers and new technologies, optimization of electron transfer mechanisms, construction of multi-enzyme synergistic systems, and improvement of device flexibility, biocompatibility and packaging technology for wearable/implantable EBFCs. Meanwhile, optimizing GOx preparation processes and realizing large-scale production are essential to reduce costs.

With continuous technological innovation and

interdisciplinary integration, the performance of GOx-based EBFCs will be further enhanced, and the existing technical bottlenecks will be gradually solved. It is expected that GOx-based EBFCs will be widely used in wearable electronics, implantable medical devices, self-powered sensors and other fields, providing a sustainable green energy solution for modern society.

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Authors' contributions

Hongying Zhu and Shengyu Tang contribute equally to the article.

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Conflicts of Interest

The authors declare no conflict of interest.

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