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Review Article

Membranes, immobilization, and protective strategies for enzyme fuel cell stability

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Abstract

Enzymatic biofuel cells (EBFCs) for direct biochemical energy conversion are a promising candidate for addressing the growing power demands for low-power implantable and wearable devices. EBFCs comprise electrodes modified with biorecognition elements that produce bioelectrical energy from the redox activity of an organic fuel (sugars, alcohols) and an oxidant at the surface of the anode and cathode. The biorecognition layers are carefully constructed using enzymes immobilized on the electrode via surface modification strategies to increase the enzyme loading and hence the turnover rate. In addition, a polymer encapsulation membrane is implemented to create a protective microenvironment for the enzymes to enhance the biofuel cell's productivity. In this brief review, the different methods carried out to improve the stability of the EBFC system are discussed. New trends and key challenges are presented to illustrate the importance of the various materials implemented in extending the operational lifetime of EBFCs.

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Introduction

Biofuel cells are classified by the type of the biocatalyst used, such as enzymatic biofuel cells (EBFCs) [1], microbial fuel cells [2], and organelle biofuel cells [3]. Compared with microbial fuel cells and organelle biofuel cells, EBFCs can offer higher power efficiency because the enzymes directly catalyze the reaction without any bio-inhibitions [1,4]. The first EBFC was invented by Yahiro in 1964 using a glucose oxidase (GOx)—modified bioanode and platinum cathode [5]. Since then, significant research has been carried out to promote the development of EBFCs as alternative technologies for power generation from organic fuel. The high demand for alternative energy sources has resulted in extensive research on renewable energy worldwide [5–9]. EBFCs use organic fuel sources such as sugars or alcohols, and the biochemical energy of these analytes is converted into electrical energy via redox reactions. EBFCs are generally lightweight and small, thereby making them an excellent candidate for portable power sources.

Earlier work focused on the dispersion of bacterial methanol dehydrogenase in a liquid medium for the conversion of the biochemical energy of methanol into electrical energy [10]. However, the redox reaction of the dehydrogenase enzyme in the liquid media was highly unstable in the transport of the generated electrons to the electrode surface. As the solution-borne enzymes exhibited poor reusability in addition to very low power generation, Persson et al. [10] found that the performance of EBFCs can be improved when enzymes are immobilized on the electrode surface. Although recent investigations report that effective enzyme immobilization on electrode surfaces results in increased performance and operational lifetime, some limitations remain [11,12]. The grand challenge in the development of an EBFC is to improve the effective electron transfer between the enzyme active site and the electrode surfaces to generate higher power densities. EBFCs use direct electron transfer (DET) or mediated electron transfer (MET) to transmit electrons to the electrode surface. The DET approach uses enzymes directly attached to the electrode surface to enable the electrons to be transmitted between the enzyme active site and the electrode surface. The MET approach uses small molecules also known as the redox mediator to shuttle electrons from the active site of the enzyme to the electrode surface [1]. Although the electron transfer rate is much higher when using MET, this approach increases the complexity of the biofuel cells as well as introduces an additional overpotential.

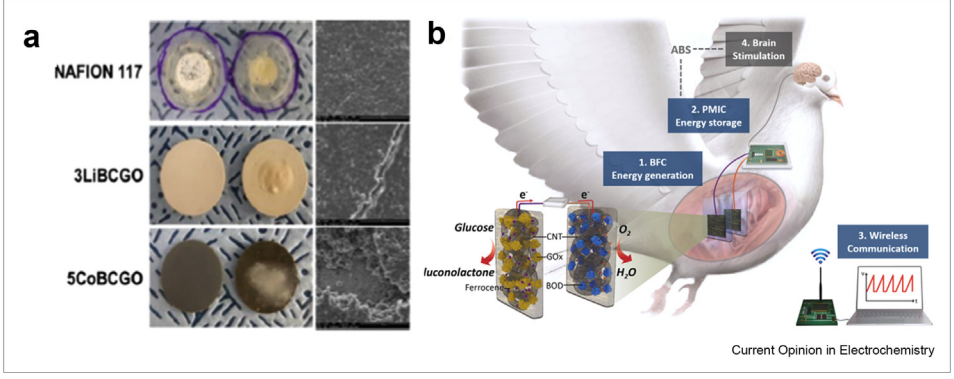
Enzyme immobilization and stabilization methods include physical adsorption, cross-linking, encapsulation,

entrapment, electrochemical polymerization, and layer-by-layer assembly [13]. The immobilization method used in the design of EBFCs depends on the type of the enzyme and the electrocatalytic process. Therefore, the selection of the appropriate electrode support material is a critical challenge to overcome as the electrode support material may exhibit significant influence on the enzyme's biocatalytic properties. Porous materials have been used to immobilize enzymes via physical adsorption [14], whereas covalent immobilization has been shown to improve enzyme stability and produce higher current densities in EBFCs [15]. Because encapsulation methods are known to enhance enzyme stability and operational lifetime, the electrochemical polymerization technique has gained attention as an alternative method to encapsulate enzymes on the electrode surface [13]. This encapsulation process provides a 'protective' microenvironment for the enzyme by either entrapping the enzyme within the polymeric structure or by attaching the enzyme to the backbone structure of the polymer. Layer-by-layer enzyme assembly can also help retain the enzyme catalytic activity over a long period of time because of their three-dimensional structures [16]. In this review, the different methods of enzyme immobilization to improve EBFC long-term operational stability via support membranes are discussed to show their role as protective layers in extending the lifetime of EBFCs.

Role of membranes

Selective membranes play an important role in the development of EBFCs, especially those constructed for the purpose of biological implantation and wearables. A detailed review is provided by Zahra and Slaughter [17]. Membrane encapsulations provide good microenvironment for enzyme immobilization, whereas membrane separators are primarily used in two-chamber compartments to separate the anolyte and catholyte. The separators inhibit the diffusion of chemical mediators and other chemicals from the cathode chamber to the substrates at the anode chamber, thereby reducing unwanted substrate flux from the anode to cathode and ultimately improving the EBFC performance [18]. The most widely used membrane is Nafion because of its excellent ionic conductivity (10^{-2}S/cm) [17,19–22]. Nafion is a sulfonated tetrafluoroethylene copolymer consisting of a hydrophobic fluorocarbon backbone (–CF_2–CF_2–) to which a hydrophilic sulfonate group SO_3^- is attached. The negatively charged sulfonate group SO_3^- is responsible for its high level of proton conductivity. Although Nafion is widely used in the design of EBFCs, it has been shown to be ineffective in improving enzyme stability because of its acidic microenvironment [23]. A suitable alternative to Nafion is Ultrex CMI-7000, a cationic exchange membrane that was reported by Jana et al. [23] and Logan and Regan [24] to be considerably more cost-effective. Anion exchange membranes containing positively charged cation groups such as NH_4^+, NHR_2^+, $NR_2\,H^+$, NR_3^+, PR_3^+, and SR_2^+ have been shown to allow for the passage of negatively charged ions through the membrane [25]. Frattini et al. [26] reported a ceramic membrane separator shown in Figure 1a to minimize biofouling. Ceramic membranes serve as a cost-effective substitute to Nafion and have the capability to lower the diffusion rate of the organic fuel and increase the surface area for biocatalyst loading. Figure 1b depicts the use of a cellulose ester dialysis membrane to drastically improve the implanted EBFC's performance [27]. The metal–organic framework using *in situ* growth on cellulose acetate nanofibers is now being explored as an enzyme encapsulation in the development of a self-powered glucose biosensor [28]. Although this metal–organic framework presents a unique approach to providing a robust support for the anchored enzymes, semipermeable membranes will

Figure 1



(**a**) Photographs and an SEM micrograph of the biomembrane made of barium–cerium–gadolinium oxide (BCGO) perovskites doped with lithium (Li) and cobalt (Co) as an alternate membrane to Nafion in microbial fuel cells [26]. (**b**) A biofuel cell (BFC) using a cellulose ester dialysis membrane is implanted in a pigeon to generate electrical power that wirelessly supplies the power management integrated circuit (PMIC) in the animal brain stimulator [27]. BOD, bilirubin; SEM, scanning electron microscope.

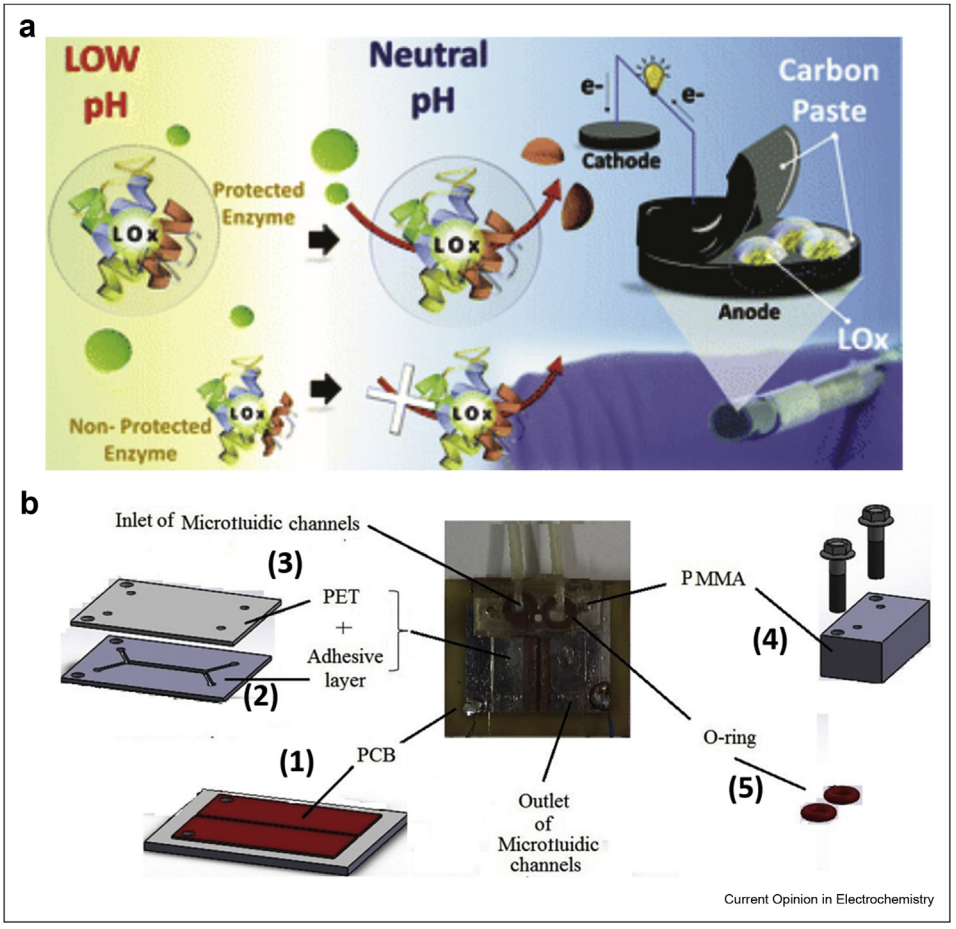
continue to play a crucial role in the performance of EBFCs because of their impact on the transfer rate of different chemical species in the electrolyte.

Immobilization strategies

Immobilization strategies that enable high specific enzyme activity without comprising its stability are critical in the design of EBFCs. Current strategies use the immobilization of biomaterials onto the electrode surface to enable intimate contact between the enzyme and the electrode. Kang et al. [29] developed a carbon tube by carbonizing a polypyrrole tube for the construction of EBFCs. The concave surface created with the carbonized polypyrrole was demonstrated to be a good surface for GOx and laccase immobilization and offered an excellent open circuit potential (1.16 V) and power density (0.350 mW-cm^{-2}). Recently, Çakroğlu et al. reported novel membraneless glucose and air photoelectrochemical biofuel cells constructed using g-C_3N_4–coated multiwalled carbon nanotubes (MWCNTs) [30]. Glucose dehydrogenase (GDH) was immobilized on the MWCNT photobioanode to enable visible light–assisted glucose oxidation with a quinone MET. The biocathode used immobilized bilirubin oxidase (BOx) on the MWCNT glassy carbon electrode for DET. The immobilization strategy improved the biocatalytic current of the photobioanode by 6.2%. Huang et al. [31] used direct immobilization of enzymes onto CNTs via 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide / N-hydroxy succinimide (EDC/NHS) chemistry. This strategy was shown to enable DET. The iron and cobalt codoped mesoporous porphyrinic carbon-based GOx enzyme immobilization strategy was demonstrated to offer high power density because of the presence of high metallic content, especially cobalt, when compared with previously reported GOx direct immobilization strategies [32].

To further enhance enzyme stability and EBFC performance, a nanocapsulation technique was used to immobilize GOx using a biomimetic cofactor containing

Figure 2



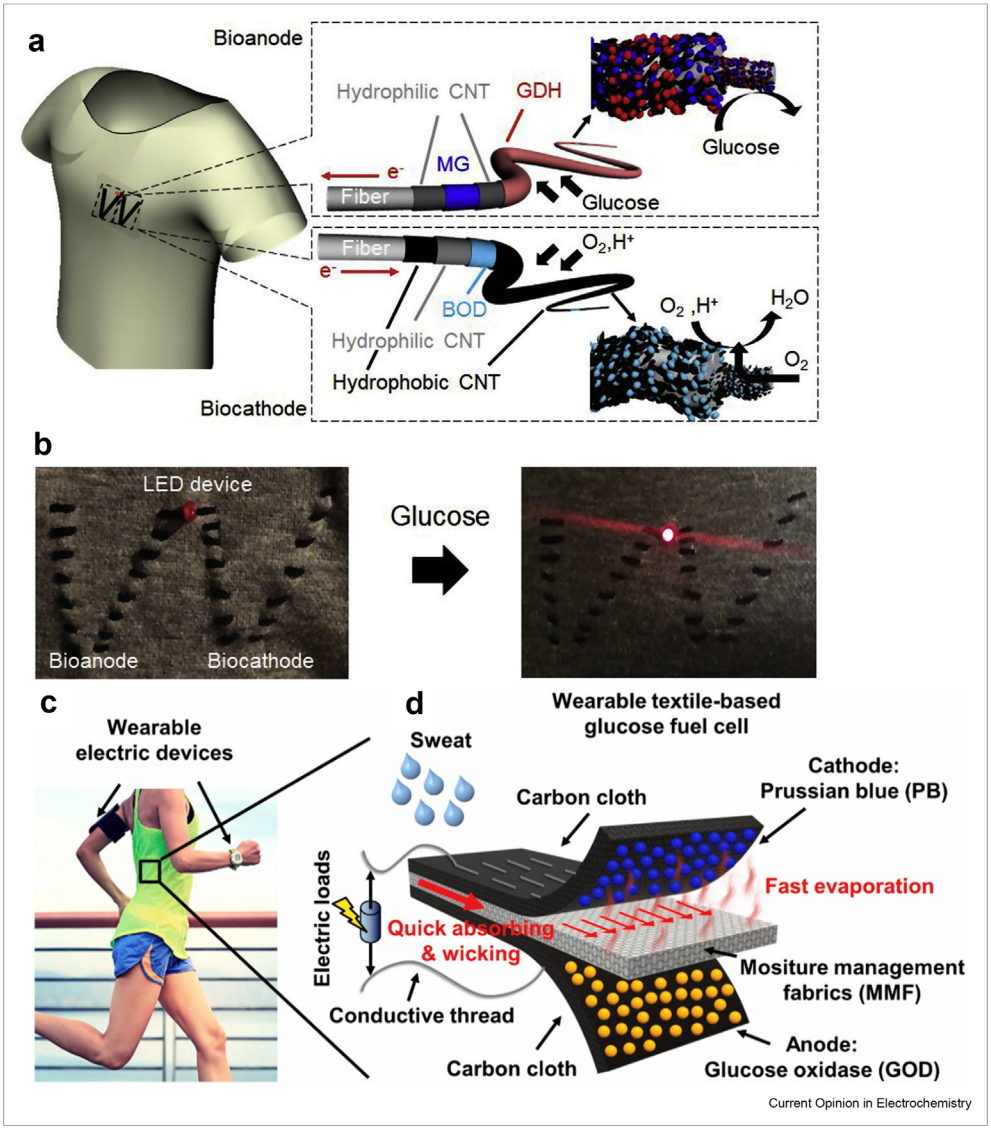
(**a**) Illustration of the biofuel cell constructed using lactate oxidase (LOx) encapsulated within a hydrophobic protective carbon paste bioanode that shows high operational stability with changes in pH, especially in acidic conditions [36] (**b**) Schematic illustration of the five main parts of the biofuel cell: (1) a printed circuit board (PCB) with copper electrodes as a substrate, (2) a double-sided adhesive layer, (3) transparency layer polyethylene terephthalate (PET), (4) a polymethyl methacrylate (PMMA) block, and (5) two O-rings [37].

the vinyl group [33]. The polymeric network connected the active site of GOx to the electrode and provided a stable microenvironment for GOx to enable fast electron transfer. This novel technique offered a unique spherical structure encapsulating the enzyme which led to a 385-fold increase in the generated power density when compared with nonencapsulated GOx-based EBFCs. In addition, a wearable needle-type biofuel cell using enzyme/mediator/carbon nanotube composite fibers was developed by Yin et al. [34], where an osmium-based polymer, GOx, and CNT were arranged in such a way to improve the BFC's power output. The composite fiber EBFCs generated high current density (10 mA/cm^2) in 5 mM artificial blood glucose. The use of 2-methacryloyloxyethyl phosphorylcholine coating as an antifouling polymer was shown to extend the lifetime of the EBFC.

Protective layers

Because EBFCs are intended to be implemented in direct contact with biological fluids, the biorecognition element(s) must be covered with a protective layer to

Figure 3



(**a**) Schematic illustration of the bioanode and biocathode, respectively, constructed from the immobilization of GDH and bilirubin (BOD) on CNT composite fibers woven on a textile cloth to create the enzymatic biofuel cell assembly, and (**b**) in the presence of glucose, the enzymatic biofuel cell connected to a charge pump circuit supplies adequate power to illuminate a light emitting diode [41]. (**c**) Schematic illustration of the wearable textile-based glucose fuel cell using moisture management fabrics for sportswear. Glucose oxidase is immobilized at the anode to catalyze the oxidation of glucose, and the hydrogen peroxide byproduct is reduced at the cathode by Prussian blue [42].

maintain stable EBFC performance and durability. An unprotected biorecognition layer in an *in vivo* environment is easily deactivated on implantation by the other biological molecules present in biological fluids. The addition of a protective layer will not only improve the long-term stability, but it will also create a microenvironment for the enzyme to enhance its bioelectrochemical activity. Trifonov et al. [35] fabricated an enzyme electrode based on BOx-modified single-walled CNTs and a removable protective layer comprising carbon-coated magnetic nanoparticles absorbed onto the enzyme-modified electrode. The system demonstrated increased stability and extended storage stability.

A novel protective layer using a hydrophobic carbon paste lactate oxidase—modified anode has been reported for converting the biochemical energy of lactate from sweat [36]. Figure 2a shows the hydrophilic carbon paste layer, which acts as a semipermeable membrane for electron shuttling. A printed circuit board microfluidic EBFC using a double-sided pressure-sensitive adhesive was used as a protective layer for the reduced graphene oxide—gold nanoparticles—poly(neutral red) GOx composite bioanode and the *Myceliophthora thermophila* laccase biocathode [37]. The open circuit voltage and maximum power density achieved in physiological serum (pH 5.5) was 0.2 V and 3.6 μW cm^{-2} at a flow rate of 50 L min^{-1}, respectively. The double-sided pressure-sensitive adhesive exhibited an added benefit of preventing the analyte from evaporating and maintaining a moist microenvironment for enzymes as shown in Figure 2b. An amine-coordinated cobalt phthalocyanine—based anodic catalyst was used to enhance the performance of hydrogen peroxide EBFCs, wherein polyethyleneimine served as the protective layer. Polyethyleneimine provided abundant amine functional groups for anchoring cobalt phthalocyanine and a favorable microenvironment for enzymes [38]. This in turn was demonstrated to enhance the operational durability and long-term stability of the EBFC. The use of hydrophilic metal azolate framework 7 (MAF-7) as the protective layer in the development of an efficient blood-toleration EBFC provided the glucose analyte with hydrophilic channels to facilitate its contact with the encapsulated GOx as well as improved the overall stability of the EBFC [39]. Recently, a zeolitic imidazolate framework-8 was demonstrated for the encapsulation of GDH to create GDH with zeolitic imidazolate framework-8 composites to improve the catalytic activity and stability of GDH [40]. The advantage of using zeolites is that they provide multiple adsorption sites for coupling enzymes to the electrode surface.

Figure 3a—b illustrates flexible MWCNT fibers decorated with GDH and BOx enzymes sewed onto a cotton textile cloth. The EBFC generated adequate power to illuminate the light emitting diode (LED) integrated on the textile [41]. However, the LED illumination was achieved using a charge pump circuit and four EBFCs connected in series on the textile cloth. Another flexible wearable textile—based EBFC used a special moisture management fabric placed between the carbon cloth anode and cathode (Figure 3c—d) to enable sustained and high-power energy harvesting [42]. The moisture management fabric enabled the continuous transport of biomolecules without the use of a pump, and six EBFCs were stacked together to generate adequate electrical power from human sweat to operate a sport watch.

Conclusion

There are a variety of strategies available for the development and construction of EBFCs with high power densities and good operational stability. Although enzyme encapsulation approaches have the potential to enable better mass transport of fuel, the stability of the EBFCs still remains a big challenge in their implementation. Future directions are focused on implantable and wearable applications because of the low-power output and the possibility of exploring biological fluids as fuel. The use of innovative nanostructured materials with optimized properties (conductivity, flexibility, permeability, etc.) provides an approach toward the development of cost-effective EBFCs that eliminate the need for external power requirements. The performance of EBFC biocatalysts and mediators requires effective enzyme immobilization strategies using nanoporous conducting support for the enhanced power output.

Declaration of competing interest

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

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