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Enzymatic biofuel cells: 30 years of critical advancements

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ABSTRACT

Enzymatic biofuel cells are bioelectronic devices that utilize oxidoreductase enzymes to catalyze the conversion of chemical energy into electrical energy. This review details the advancements in the field of enzymatic biofuel cells over the last 30 years. These advancements include strategies for improving operational stability and electrochemical performance, as well as device fabrication for a variety of applications, including implantable biofuel cells and self-powered sensors. It also discusses the current scientific and engineering challenges in the field that will need to be addressed in the future for commercial viability of the technology.

1. Introduction

One common example of a bioelectronic device is an enzymatic biofuel cell, as shown in Fig. 1. Enzymatic biofuel cells are a type of fuel cell that utilizes enzymes as the electrocatalysts to catalyze the oxidation of fuel and/or the reduction of oxygen or peroxide for energy conversion to electricity. Most traditional fuel cell electrocatalysts are conductive-metal nanoparticle catalysts that operate at temperatures ranging from 45 °C to 150 °C. These catalysts have the advantage of high stability and high activity in highly acidic and/or basic environment. However, they are plagued with passivation issues that require simple and high purity fuels (i.e. hydrogen and methanol). On the other hand, living organisms can consume and metabolize complex fuels (i.e. sucrose, fructose, etc.) and fuel mixtures and their catalysts do not have the same issues. Therefore, the field of enzymatic biofuel cells has expanded over the last 3 decades, and it is now possible to consider applications (i.e. implantable fuel cells, JP-8 fuel cells, etc.) where traditional metal nanoparticle electrocatalysts have limitations. As shown in Fig. 1, enzymatic biofuel cells are more complex than traditional fuel cells, because typically open circuit potentials are significantly lower than theoretical due to cofactor redox potentials, enzyme redox potentials, and mediator redox potentials. This review article will detail the advancements in the field of enzymatic biofuel cells over the last 30 years and discuss the challenges that will face enzymatic biofuel cell development in the next 30 years.

2. History before the debut of Biosensors & Bioelectronics

Although microbial biofuel cells have been studied for over a century, enzymatic biofuel cell history only goes back to the early 1960s. In 1964, Yahiro et al. (1964) invented the concept of enzymatic biofuel cells with a glucose oxidase bioanode and a Pt cathode. This system had very low open circuit potentials (175–350 mV), but showed the proof of concept that an oxidoreductase enzyme could catalyze a fuel cell half-reaction, in this case, the oxidation of glucose to gluconolactone, and transfer the electrons via small molecule redox species to the electrode surface. This pioneering work set the definition of enzymatic biofuel cell as a fuel cell utilizing an enzyme as the electrocatalyst at one of the two electrodes, in this case the anode. Although there are several reports of biofuel cells in the 1960s, the early work all involved the use of a purified oxidoreductase enzyme and a mediator for performing mediated electron transfer (MET) to the electrode surface (Davis and Yarbrough, 1967; Hunger and Perry, 1966; Yahiro et al., 1964).

Enzymatic biofuel cells rely on enzymatic bioelectrocatalysis, as do amperometric and voltammetric biosensors. Therefore, research in the field has been aided not only by work specifically on enzymatic biofuel cells, but also by research improving enzymatic bioelectrocatalysis for biosensor applications. In the late 1970s, Berezin et al. (1978) made one of the most outstanding contributions to the field in discovering direct electron transfer (DET). Unlike MET systems, direct bioelectrocatalysis does not require the use of an external redox mediator and can transfer electrons directly from the protein to the current collector/electrode. This simplification also results in less potential losses due the potential

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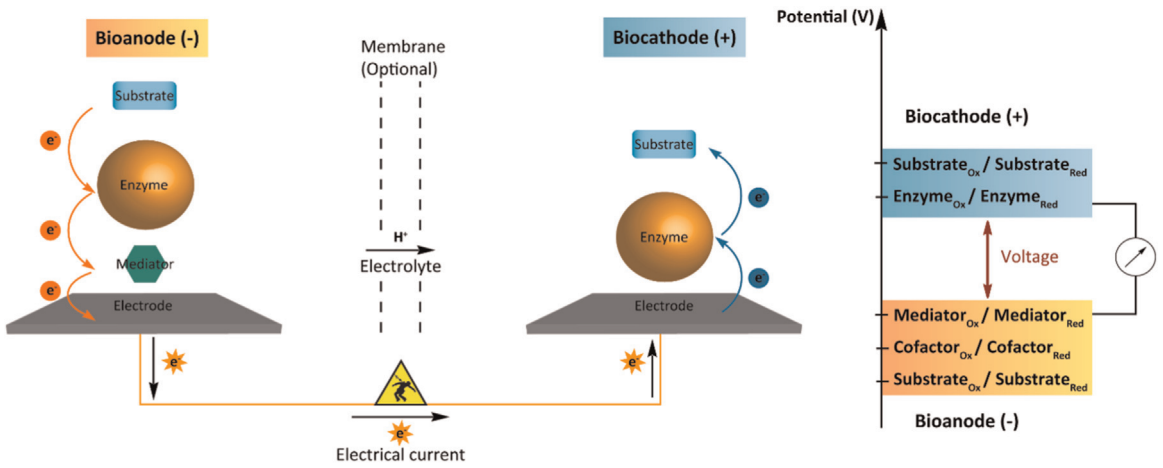

Fig. 1. Schematic of an enzymatic biofuel cell utilizing a mediated bioanode and a direct electron transfer-based biocathode.

difference between the enzyme cofactor/active site and the mediator. This work significantly impacted the overall goal of biofuel cells for the future. Although laccase bioelectrodes were invented with this work, it was not until 1984 that the concept of utilizing enzymes at the cathode of a biofuel cell was discussed and even then it did not utilize the enzyme for direct bioelectrocatalysis. Instead, it involved a gold cathode that produced peroxide from oxygen and then the peroxide was consumed by the enzyme chloroperoxidase (Laane et al., 1984). This pioneering work expanded the field of biofuel cells to fuel cells utilizing enzymes at either the anode or the cathode.

Although direct electron transfer became a goal for enzymatic biofuel cells, most biofuel cells in the literature continued to utilize mediated electron transfer, because MET systems typically resulted in higher current densities, even though they have higher potential losses and issues with mediator stability. Turner and coworkers were the first to utilize organometallic redox mediators for biofuel cells in the early 1980s (Cass et al., 1984). Some of this work involves redox mediators in solution while some involved immobilization of these ferrocene mediators in conducting polymers (Cass et al., 1984; Dicks et al., 1989). This important discovery led to the incorporation of organometallic redox mediators into redox polymers, which was pioneered by the Heller group and is now used by countless researchers in the field (Barriere et al., 2006; Barton et al., 2001, 2002; Degani and Heller, 1989; Gallaway et al., 2008; Gallaway and Calabrese, 2008; Gao et al., 2009; Kavanagh et al., 2009; Mano et al., 2002a, 2002b, 2003, 2005, 2006; Mao et al., 2003; Stoica et al., 2009; Tasca et al., 2009).

3. Enzymatic biofuel cell research after the first issue of Biosensors &Bioelectronics

3.1. Cofactor regeneration

Oxidoreductase enzymes contain or require redox cofactors that change oxidation state during substrate catalysis. There are a variety of natural oxidoreductase organic and inorganic cofactors including: nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$), flavin adenine dinucleotide (FAD), pyrolloquinoline quinone (PQQ), hemes, iron–sulfur clusters, coenzyme Q, coenzyme F420, flavin mononucleotide (FMN) and ascorbic acid. Many of these cofactors are derived from vitamins and some cofactors are bound in the enzyme while others are diffusional. One of most common cofactors for oxidoreductase enzymes is $NAD(P)^+$, which is a diffusional mediator that becomes NAD(P)H upon oxidation of substrate/fuel and therefore must be regenerated at the electrode. There are thousands of NAD(P)-dependent dehydrogenases in the literature and many have been used for bioanodes of biofuel cells including: glucose dehydrogenase (Persson et al., 1985), alcohol dehydrogenase (Moore et al., 2004b), aldehyde dehydrogenase (Akers et al., 2005), formate dehydrogenase (Palmore et al., 1998), lactate dehydrogenase (Sokic-Lazic et al., 2011), pyruvate dehydrogenase (Sokic-Lazic and Minteer, 2009), malate dehydrogenase (Rincon et al., 2010), and many others. However, NAD(P)H has poor electrochemistry on most common electrode surfaces (i.e. carbon, gold, and platinum) with overpotentials of 0.5–1 V and quick passivation of electrode surfaces (Karyakin et al., 1999a). The late 1980s and early 1990s resulted in a wealth of research on modifying electrodes for improving NADH oxidation, so that NAD-dependent dehydrogenases could be used at the anode of biofuel cells (Gorton et al., 1984; Karyakin et al., 1999a', 1999b; Persson et al., 1985). This resulted in a widely expanding choice of fuels from glucose to methanol, ethanol, formate, glycerol, lactate, pyruvate, and malate. One of the most common strategies for decreasing the overpotential of NADH oxidation was the use of electropolymerized azines. This strategy is still used frequently today. However, recent research has shown that electropolymerization is not the only option for immobilization. Specifically, azine electrocatalysts can be immobilized via chemical polymerization of the polymer, adsorption to carbon nanotube structures, and crosslinking within carbon nanotube/polymer composites (Arechederra et al., 2010; Goran et al., 2014; Meredith et al., 2012). Although NAD-dependent bioanodes are quite complex due to the need for a diffusional cofactor and an electrocatalyst, they are still commonly used in bioanodes today, because they are oxygen independent compared to oxidase-based bioanodes. However, it is important to note that they are not the only type of dehydrogenase that is utilized in biofuel cells today. Specifically, heme-containing dehydrogenases, PQQ-dependent dehydrogenases, and FAD-dependent dehydrogenases are becoming more and more popular in recent years, because they are oxygen independent and do not require diffusional mediators (Coman et al., 2008; Kamitaka et al., 2007a; Milton et al., 2013; Schubart et al., 2012b; Stoica et al., 2006; Tasca et al., 2008; Tkac et al., 2009).

3.2. First enzyme cascade for deep oxidation

In 1998, Palmore and Whitesides made significant contributions to the field of enzymatic biofuel cells showing that biofuel cell performance was a function of degree of oxidation at the anode with their methanol biofuel cell containing NAD-dependent alcohol dehydrogenase, aldehyde dehydrogenase, and formate

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