ENZYMATIC BIOFUEL CELLS FOR IMPLANTABLE AND MICRO-SCALE DEVICES

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Introduction

Technology for electrical power generation using enzyme catalysts, established four decades ago, has recently received increased attention associated with demand for micro-scale and implantable power supplies. The main challenges, namely the fragility of enzyme molecules, characteristic low current density, and poor fundamental understanding of redox bioanalysis, are currently being addressed from a variety of research perspectives, to take advantage of enzyme selectivity, low temperature and moderate pH activity, and manufacturability in small-scale devices. Such an effort benefits from four decades of multidisciplinary research in biosensors and related bioelectrochemical fields. This review paper summarizes the current state of enzymatic biofuel cell research in the context of foreseeable applications and assesses the future prospects of the technology. Emphasis is placed on device performance and engineering aspects, with a view toward practical portable power devices based on enzymatic biofuel cells.

Research in biocatalytically modified electrodes, particularly for sensor applications, has provided a significant technological underpinning for current biofuel cell development. There exists significant overlap in technical requirements between sensors and biofuel cells, including chemical and mechanical stability, selectivity, and cost of materials. However, these two technologies diverge in the areas of current density, cell potential and stability.

There exists extensive review literature in the area of biological fuel cells. Notably, Palmore and Whitesides summarized biological fuel cell concepts and performance up until about 1992. More recently, Katz and Willner discussed recent progress in novel electrode chemistries for both microbial and enzymatic fuel cells. We do not duplicate these valuable contributions, but instead focus on the strengths and weaknesses of state-of-art materials in the context of specific classes of applications, and point to areas where additional knowledge is currently needed to exploit biological fuel cells. With some exceptions, we focus on contributions made after 1992.

Biofuel cells have traditionally been classified according to whether the catalytic enzymes were located inside or outside of living cells. If living cells are involved the system is considered to be microbial, and if not it is considered enzymatic. Although microbial fuel cells possess unique features unmatched by enzymatic cells, such as long-term stability and fuel efficiency, the power densities associated with such devices are typically much lower owing to resistance to mass transfer across cell membranes. Thus, microbial fuel cells are expected to find limited application in small-scale electronic devices. This review will focus on enzymatic biofuel cells. While such cells typically demonstrate reduced stability due to the limited lifetime of extracellular enzymes, and are typically unable to fully oxidize fuels, they allow for substantial concentration of catalysts and removal of mass transfer barriers and provide higher current and power densities, approaching the range of applicability to micro- and mini-scale electronics applications.

Applications and Requirements

The range of possible applications for biofuel cells may be broken down into three main subclasses:

1. Implantable power, such as micro-scale cells implanted in human or animal tissue, or larger cells implanted in blood vessels.
2. Power derived from ambient fuels or oxidants, mainly plant saps and juices, but extending to sewage and other waste streams.
3. Power derived from conventional fuels including hydrogen, methanol or higher alcohols.

Classes 1 and 2 are closely related. The fuels available for implantable power, such as blood borne glucose or lactate, are ambient in the sense that they are present in a physiological environment in the absence of a fuel cell device. One major distinction between these two classes is that the ambient-fueled cell need not be implanted, and focuses on plant- or waste-derived fuels, whereas the implantable cell focuses on animal-derived fuels and is present within the physiological system. Class 3 is unique in that this class competes with well-established conventional fuel cell technology. To a greater or lesser extent, all three classes share the fundamental technical requirements of high power density and high activity.

Enzyme Catalyzed Direct Electron Transfer

There are two ways of coupling an electrode process to an enzyme reaction (see Figure 2). The first approach is based on the utilization of low-molecular weight redox mediators. The second is to pursue direct (mediatorless) electron transfer. In this case, the electron is transferred directly from the electrode to the substrate molecule (or vice versa) via the active site of the enzyme. Direct (mediatorless) electron exchange between a redox group of protein and the electrode surface has been studied for a number of proteins such as cytochrome c, peroxidase, ferredoxin, plastocyanin, azurin, azotoflavin, glucose oxidase, etc. These studies developed an electrochemical basis for the investigation of protein structure, mechanisms of redox transformations of protein molecules, and metabolic processes involving redox transformations. Recently, the ability of oxidoreductase enzymes to catalyze mediatorless electron transfer from the electrode surface to the substrate molecule (or vice versa) has been demonstrated for laccase, lactate dehydrogenase, peroxidase, hydrogenase, p-cresolmethylhydroxylase, methylamine dehydrogenase, succinate dehydrogenase, fumarate reductase, D-fructose dehydrogenase, alcohol dehydrogenase, and D-gluconate dehydrogenase.

Mediated electron transfer

The major purpose of redox mediation is to increase the rate of electron transfer between the active site of enzyme biocatalysts and an electrode by eliminating the need for the enzyme to interact directly with the electrode surface. Depending on the enzyme and reaction conditions, mediated electron transfer rates may exceed by orders of magnitude that of the direct mechanism. However, by introducing an additional transfer step, enzyme-mediator electron transfer is isolated from direct electrode potential control. For typically fast (Nernstian) kinetics between the mediator and electrode surface, the electrode potential merely controls the relative concentration of oxidized and reduced mediator at the surface. The electrode thus provides a boundary condition for electron flux to and from solution via the mediator.

The practical impact of such considerations is that the working potential of a mediated biocatalytic electrode is dominated by the
redox potential of the mediator couple, and the operating potential of a biofuel cell comprising two such electrodes will be primarily determined by the difference in redox potential of the two mediator couples. The differences in redox potential between the mediator and enzyme, and enzyme and substrate, represent driving forces for electron transfer, and therefore must be nonzero. As shown in Figure 2 for a glucose-oxygen biofuel cell, this difference represents an activation overpotential that reduces the observed open circuit potential from a theoretical maximum, given by the potential difference between the substrates. Some examples of diffusional mediators to be discussed are NADH cofactors, ABTS, and redox hydrogels.

![Figure 1. Alternative electron transfer mechanisms. (a) Direct electron transfer (tunneling mechanism) from electrode surface to the active site of an enzyme; (b) Electron transfer via redox mediator.](image)

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**Engineering of enzymatic biofuel cell Systems**

The recent literature in bioelectrochemical technology, covering primarily the electrochemical aspects of enzyme immobilization and mediation, includes few reports describing engineering aspects of enzymatic biofuel cells or related devices. Current engineering efforts address issues of catalytic rate and stability by seeking improved kinetic and thermodynamic properties in modified enzymes or synthesized enzyme mimics. Equally important is the development of materials and electrode structures that fully maximize the reaction rates of known biocatalysts within a stable environment. Ultimately, the performance of biocatalysts can only be assessed by their implementation in practical devices.

**Future Outlook**

The development of successful power sources has always been driven by specific applications. So it must also be for successful biofuel cells. Therefore, technological paths will be determined by application specifics: Implanted biofuel cells must exhibit biocompatibility, and cathodes for ex vivo electronics must take advantage of gas-phase oxygen. That being said, a general statement can be made that for the advantages of biofuel cells to compel adoption, the weaknesses relative to conventional technology must be minimized. It is clear that the advantages of biocatalysts are reactant selectivity, activity in physiological conditions, and manufacturability. The weaknesses are equally clear—modest absolute activity, and low stability, issues of significance, to a greater or lesser extent, in every conceivable application of this technology.

**References**


