Extended lifetime biofuel cells

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Over the last 40 years, researchers have been studying and improving enzymatic biofuel cells, but until the last five years, the technology was plagued by short active lifetimes (typically 8 hours to 7 days) that prohibited the commercial use of this technology. This *tutorial review* introduces the topic of enzymatic biofuel cells and discusses the recent work done to stabilize and immobilize enzymes at bioanodes and biocathodes of biofuel cells. This review covers a wide variety of fuel systems from sugar to alcohols and covers both direct electron transfer (DET) systems and mediated electron transfer (MET) systems.

Introduction

In order to combat the conflicting rises in global energy consumption with the depletion of the traditional non-renewable energy producing resources, academic and industrial research efforts have increased dramatically in an effort to find alternate methods of converting chemical and/or light energy into electrical energy that can be used in an efficient manner. These electrochemical devices can be classified into three types: solar cells, batteries, and fuel cells. From heating our homes and powering our automobiles, to providing life sustaining energy in space exploration, fuel cells have been increasingly demonstrated to be viable options for the conversion of fossil fuels.

Fuel cells have been given particular attention due to their efficency and ability to sustain consistent power production over time by simple consumption of renewable reactants in contrast to a battery's reliance on the input of electrical energy to recharge combined with the effects of hysteresis and a solar cell's dependence on the presence of sunlight. There are four main types of fuel cells in the literature: polymer electrolyte (PEM) fuel cells, phosphoric acid fuel cells, molten carbonate fuel cells, and solid oxide fuel cells. Each of these fuel cells has advantages and disadvantages depending on the application, but for comparison purposes, we will discuss PEM fuel cells, because they are most applicable to biofuel cells, as they operate at the lowest temperatures, although most PEM fuel cells operate above room temperature (60–120 °C) compared to biofuel cells which typically operate between 20 °C and 37 °C.

A fuel cell contains an anode where the fuel is oxidized and thus produces electrons. Electrons produced flow through an external circuit providing a resistance that serves as a load and electrons are then transferred to the cathode where they react with free protons to reduce ambient oxygen to water. Traditional fuel cells perform this task through catalytic oxidation of fuels such as hydrogen gas, methane, and methanol with expensive, metal catalysts.[1] These catalysts often suffer from reduction in performance over time due to passivation or poisoning of the catalyst material due to impurities in the fuel or to crossover of fuel through the polymer electrolyte membrane (PEM) which separates the cathodic and anodic compartments, ideally allowing only the passage of protons that are necessary for the cathodic half reaction, but in reality transporting fuel across the membrane with the protons. Fig. 1 demonstrates a basic PEM-based electrochemical cell.

Biofuel cells often follow the same design format; however, they replace the precious metal catalyst of traditional fuel cells with biological catalysts such as microorganisms or enzymes.

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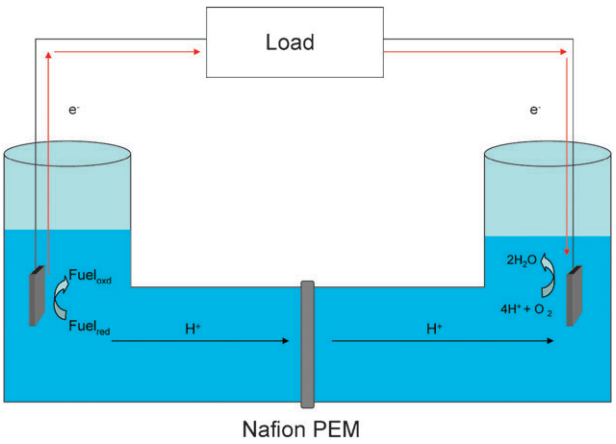

Fig. 1 Schematic representation of a traditional diffusional test cell for the analysis of biofuel cells and their performance. Biofuel cells convert chemical energy to electrical energy through the use of biocatalysts at the anode and/or the cathode of the fuel cell.

These biological catalysts are capable of performing their natural oxidation processes at the anode, which results in the release of electrons generating an electrical current.

Microbial fuel cells utilize whole cells that use complex metabolic pathways to oxidize fuel substrates. The first of these was demonstrated by Potter in 1912 and was capable of generating electrical energy from yeast cell metabolism of glucose.[2] Microbial biofuel cells are advantageous in that the living organisms contain many oxido-reductase enzymes that are capable of complete oxidation of a wide variety of fuels. These devices are often stable for long periods of time due to the ability of the microbe to regenerate the enzymes and co-enzymes. Lifetimes of over five years have been demonstrated.[3,4] Although the approach offers long lifetimes, the device is often limited by transport and internal resistance due to the membrane and cell wall structure of the microorganisms. This barrier between the enzyme catalysts, the fuel, and the electrode surface plagues these systems with low power densities due to slow mass transport and poor electron transfer. These microorganisms also have low volumetric catalytic activity due to the vast amount of unutilized space in the cell cytoplasm.

In an attempt to eliminate this inefficient use of space and improve the transport of fuel directly to the catalyst, enzymes themselves have been isolated and used directly to produce current. Enzymatic biofuel cells have significant potential advantages in fuel cell production. The nanometre scale of enzymes greatly improves the volumetric catalytic activity in comparison to microorganisms.[5,6] However, enzymes are much larger than metal catalysts. Many oxidoreductase enzymes exhibit high catalytic activity which results in increases in the rates of reaction by as much as 10^{14} times the rate without catalyst present and they often have turnover rates on the order of 10^3 s^{-1}.[7] Enzymes, such as bilirubin oxidase, can have higher catalytic currents and lower overpotential than the precious metal platinum,[8] but they also can be considerably less expensive depending on the scale of production. Developments in separation science in combination with advances in genetically enhanced enzyme expression in cell culture have resulted in simple and inexpensive production of large quantities of these catalysts.

Enzymatic biofuel systems offer two main advantages to traditional fuel cells. Enzyme catalysts allow for a wide variety of fuels to be utilized without the need for expensive and time consuming purification, because enzymes typically do not react with or get passivated by impurities in the fuel. The selectivity of the enzymes also allows for the elimination of the PEM used to separate cathodic and anodic solutions by imparting selectivity by implementing simultaneous use of a biocathode and a bioanode. This simplicity and performance potential, in addition to the ability of these cells to perform optimally in conditions of mild temperature and pH, has caused a great increase in research efforts toward the implementation of enzymatically driven power sources in consumer devices.

Many limitations, however, plague these systems including incomplete oxidation of fuels, short lifetimes, and reduced performance due to slow direct electron transfer kinetics or problems associated with the stability or thermodynamics of redox mediators. Recent research efforts in this field have focused on the immobilization techniques to optimize the environment and performance of key oxidoreductase enzymes for the production of power. This tutorial review introduces the topic of enzymatic biofuel cells and their initial development and discusses the recent work done to stabilize and immobilize enzymes at bioanodes and biocathodes of biofuel cells. Enzymatic biofuel cells are normally compared to each other on the basis of three main experimental criteria: open circuit voltage, maximum current density, and maximum power density. Open circuit voltage is a measure of the potential difference between the cathode and anode with infinite load applied. The maximum current density corresponds to the case when the electrodes are shorted so the external circuit resistance is zero, corresponding to zero load. Maximum power density is the peak value of power density and is most easily graphically explained as the peak power density of the power curve, which is a plot of power or power density *versus* either current density or potential depending on convention.

Development of enzymatic biofuel cells 1960–2002

Enzymatic biofuel cell development began in the 1960s. In 1964, Kimble and coworkers developed the first enzymatic biofuel cells. They constructed three different biofuel cells using either glucose oxidase, amino acid oxidase, or alcohol dehydrogenase at the anode and then compared the biofuel cell performance of each system. The oxidases proved viable and produced open circuit potentials of up to 350 mV, while alcohol dehydrogenase did not produce a positive open circuit potential.[9] However, lack of efficient electron transfer mechanisms and minimal stability caused research to move toward metallic electrodes/electrocatalysts for biofuel oxidation rather than enzymes during the later 1960s and 1970s. There are numerous publications in this time period focused on platinum and other metallic electrodes for glucose or other metabolite oxidation. These were unsuccessful at complete oxidation and are plagued with passivation problems at low temperatures, so

current research has not continued in this area. However, research in enzymatic biofuel cells was revitalized in the 1980s when researchers started investigating alcohol dehydrogenase for methanol oxidation. These investigations continued until the late 1990s when Palmore and Whitesides published the breakthrough research in 1998 showing that an enzymatic cascade employing alcohol dehydrogenase, formaldehyde dehydrogenase, and formate dehydrogenase could be used for complete oxidation of methanol to carbon dioxide.[10]

After 1998, there was little further research in methanol as a fuel for enzymatic biofuel cells, but this paper along with the work of Adam Heller and coworkers on glucose oxidase bioanodes for biofuel cells caused a resurgence in research in the area of biofuel cells.[11] Heller and coworkers' employment of redox polymers for immobilizing glucose oxidase at the anode of a biofuel cell showed lifetime extending from 8 hours for Palmore's methanol biofuel cell with enzyme in solution to 7–10 days for the immobilized enzymes. This extended lifetime was followed by several papers by the Heller group increasing the operating potential of the biofuel cell to potentials sufficient for small electronic devices.[12] Heller was also the first to show that enzymes at both the anode and the cathode of the enzymatic biofuel cell can provide sufficient selectivity for operating in a membraneless format.[13]

Recent work on immobilization techniques for enhanced enzyme stabilization

Limitations in enzymatic biofuel cell development have arisen from two major issues, the first being maintaining the integrity and performance of these sensitive biomacromolecules over time. The three-dimensional protein structure of both the enzyme active site and the macromolecule as a whole is essential to the catalytic activity of the enzyme. Maintaining this structure requires accurate control of temperature, pH, and chemical components of the solvent environment. Initial studies in the development of enzymatic biofuel cells attempted to produce electrical current from enzyme solutions or suspensions allowing enzymes to freely diffuse in the anodic compartment. This approach resulted in lifetimes of several hours to less than 5 days in buffered solutions due to the denaturation of the enzyme systems over time.

Allowing for diffusion of the enzymes in the fuel solution also limited the fuel cell performance due to the inefficiency of electron transfer from these oxidoreductase enzymes to the electrode surface, which is the second major hindrance that plagues enzymatic fuel cell performance. Although the catalytic activity of some enzymes produces an ample supply of electrons (some laccases have turnover rates in excess of 150 000 s^{-1} and some catalases have turnover rates in excess of 500 000 s^{-1}), they cannot be efficiently shuttled to the collecting electrode, causing the cell performance to fall far short of what would be expected for such highly catalytic components. A review article by Aston and Turner outlines the limitations that arise in forming electrical connection from enzymes to electrode surfaces.[14]

Recent efforts (from 1990 to today) have focused on the controlled immobilization of redox active enzymes to reduce the effect of both these limitations. Controlled isolation of purified enzyme on an electrode surface can allow for a tailorable environment that best suits the enzyme system of interest while localizing the enzyme in extremely close proximity to the electrode, allowing for more efficient transfer of electrons. Several immobilization and stabilization techniques have been suggested and will be the focus of much of this review; however, in order to examine the impact of these innovative immobilization techniques, one must first understand two mechanisms through which the supply of electrons produced through the oxidation of fuel by oxidoreductase enzymes is transferred to produce appreciable currents.

Mediated *versus* direct electron transfer

Most oxidoreductase enzymes that have been commonly used in biofuel cell development have not been shown to be able to promote the transfer of electrons themselves. In this case, many low molecular weight redox active compounds and polymers have been incorporated to mediate this transfer.[15] This approach is termed mediated electron transfer (MET) and results from this mediator molecule participating directly in the catalytic reaction by reacting directly with the enzyme or its cofactor to become oxidized or reduced and diffusing to the electrode surface at which rapid electron transfer takes place.[15] Characteristic requirements of mediator species include stability and selectivity of both oxidized and reduced forms of the species and the redox chemistry must be reversible, requiring low overpotential.[16]

Mediators that have been incorporated include organic dyes such as methylene green, phenazines and azure dyes along with other redox active compounds such as ferrocene, ferrocene derivatives and conducting salts.[15] These mediators are often required for NAD^+ and FAD-dependent enzymes, such as alcohol dehydrogenase, aldehyde dehydrogenase and glucose oxidase among others, whose electron transfer distance is long, so direct electron transfer is slow.[17,18] For NAD, the problem is more complicated due to the instability of the NADH radical cation intermediate in the one-electron oxidation of the species and the resulting electrode fouling. This method has been attempted at both cathodic and anodic interfaces and has been achieved through solution phase mediators and mediators immobilized in various ways with or near the enzymes themselves. A schematic for this process is presented

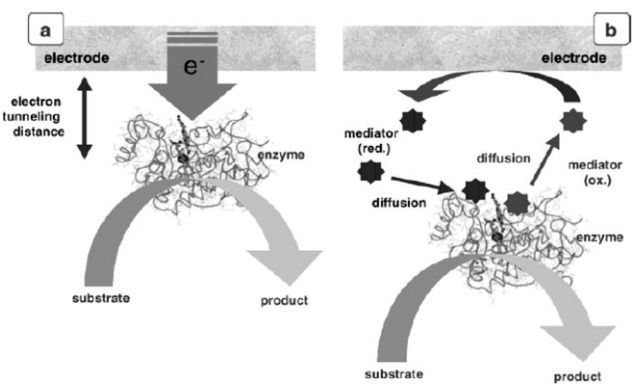

Fig. 2 Electron transfer mechanisms utilized in biofuel cell technology: (a) direct electron transfer and (b) electron transfer carried out by a redox mediator species.

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in Fig. 2. Mediators have been employed in biofuel systems through polymerization on the electrode surface prior to enzyme immobilization, coimmobilization of enzyme and mediator simultaneously or simply allowing the electron transfer mediator to be free in solution. These mediated systems do have drawbacks in that the species utilized to assist electron transfer are often not biocompatible or have short lifetimes themselves.

A major advance arose from a research focus of the 1980s which documented several enzymes which are themselves capable of direct electron transfer (DET) *via* the active site of the enzyme. The first studies involve examining enzymes such as laccase that are capable of catalyzing the four-electron reduction of O_2 to water through electron transfer from the electrode surface directly to the active site and through to the substrate. This electron transfer mechanism is also depicted in Fig. 2.[19] This system has been utilized in cathodic compartments of biofuel cells; however, enzymes capable of oxidation at the anode surface have also been shown to demonstrate DET. Although these enzymes have been explored in a variety of electrochemical applications, they were not applied to biofuel cell technology until 2005 when laccase was utilized to reduce oxygen at a biocathode through DET and DET of pyrroloquinoline quinone (PQQ) dependent enzymes was demonstrated for anodic compartments.[20]

DET occurs through the enzyme's ability to act as a 'molecular transducer' that converts the chemical signal directly to an electrical one through the transfer of charge to a stable redox species which is in turn capable of transferring this charge to another molecule or electrode surface.[16,21,22] Many of these enzymes contain redox active metal centers that perform the catalytic transfer of electrons. PQQ-dependent enzymes, as an example, contain a heme group which is capable of existing in several redox states and accepts resultant electrons that are generated through the oxidation of substrates like alcohol, aldehyde, and glucose.[20,23]

Utilization of enzymes capable of facile direct electron transfer with common electrode materials allows for more accurate mimics of energy transfer processes that occur in biological systems and eliminates the need for mediator molecules that can be non-selective and add cell resistance which limits the optimal performance of the cell. However, DET is correlated directly to the enzyme proximity and orientation to the electrode surface in order for electron tunneling to occur, allowing only the biocatalytic reaction to be the limiting process.[24] In order to address these issues, recent research efforts have implemented immobilization techniques which not only improve enzyme lifetime, but in many cases utilize DET capable enzymes and optimize their immobilization technique to control the proximity of the enzyme active site to facilitate DET.

Immobilization techniques

Enzyme immobilization techniques have been applied to both cathodic and anodic electrodes to increase current densities, enhance enzyme stability, and improve electron transfer kinetics. Immobilization techniques can be classified into four categories: crosslinking, wiring, sandwich, and encapsulation

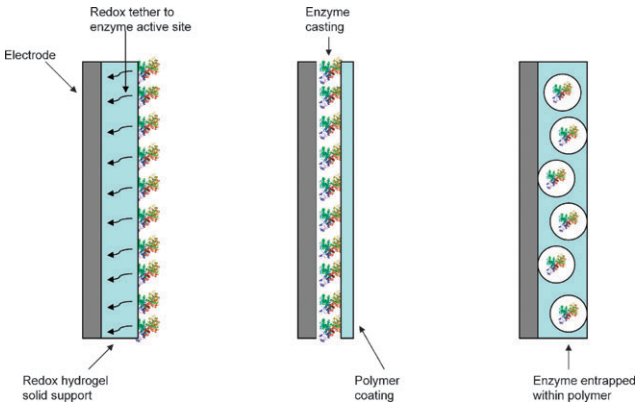

Fig. 3 Schematic representation of the three prominent types of enzyme immobilization techniques of electrode surfaces. (a) Wired, (b) sandwich, and (c) entrapment.

techniques. Although crosslinking is a common and simple form of immobilization, the process of crosslinking the enzyme decreases the catalytic activity of the enzymes, so it is not a long term solution for enzymatic biofuel cells, because the future of enzymatic biofuel cells will focus on increasing the catalytic activity of the enzyme and improving the ability to transfer electrons from the enzyme to the electrode. Fig. 3 outlines these techniques with a general schematic representation of each type. This review will outline each technique and detail recent advances in the field. Although all of these methods have been used frequently in the development of biosensors, biofuel cells require additional attention to key characteristics such as resistivity, rapid mass transport, and stability.

Physically entrapping enzyme by means of sandwich films has proven to limit power production due to decreased mass transport and increased resistivity and thus has been utilized the least of the three techniques for immobilization. Sandwich immobilization techniques entail the layering of enzyme followed by a layer of other material (polymeric or sol–gel matrix) to entrap the layer of enzyme. This layering has the effect of limiting the system due to mass transport of fuel to the catalytic site of the enzyme through the barrier created by the layering technique. Additionally, many systems have employed an initial layer of material followed by enzyme followed finally by the entrapping layer to enhance the stability of the enyzme by eliminating the diffusion of enzyme in all directions. This technique is not optimal for biofuel cell production due to the increase in resistivity associated with this initial layer and also the increase in distance from the enzyme catalytic site to the electrode surface. This fact eliminates the ability for DET. Sahney *et al*. explored the comparison of sandwich immobilization with encapsulation and physical adsorption as it pertained to the immobilization of urease in tetramethyl orthosilicate (TMOS) for the detection of urea. This work determined that although the device had a lifetime of 30 days, the sensitivity and detection limit of the sandwich model were limited due to less enzyme being present at the surface of the electrode.[25] Nafion polymers have also been used to coat modified electrodes; they, however, have demonstrated a reduction in activity due to the acidic side

chains of Nafion imparting a nonideal environment for the enzymes and produce non-uniform dispersion of enzyme in the membrane.[26] In general, this technique has not garnered much attention, because the microencapsulation method that is discussed below has outperformed this technique in all facets.

More promising research efforts have focused on utilizing redox hydrogels in a different manner by 'wiring' enzymes to the electrode surface. The term 'wiring' refers to chemically binding or attracting the enzyme of interest to the anode or cathode surface through covalent or ionic interactions in such a way that there is an electron transfer pathway to the electrode (*i.e.* electron hopping between redox centers attached to the polymer).[21] Heller *et al.* have covalently linked many enzymes to sol–gel materials, some of which contain redox moieties (osmium based complexes) embedded within the polymer to facilitate electron transport from the tethered enzyme active site to the electrode. This work, as alluded to previously, greatly enhanced fuel cell lifetime and performance. Initially used for biosensing technology, Heller's group has described numerous applications for linking a variety of enzymes to electrodes, many of which are covered in his review article on the subject and are beyond the scope of this review.[12] However, to date his redox wiring of enzymes has been demonstrated to produce biofuel cells that are capable of producing power densities of greater than 350 $\mu W\ cm^{-2}$ and open circuit potentials of 0.88 V.[13,27,28]

Microencapsulation is the physical entrapment of an enzyme in pores or matrices of a membrane at the electrode surface. Minteer *et al.* have attempted to perform this encapsulation utilizing modified Nafion membranes that eliminate the destructive acidity mentioned previously, while tailoring the size of the polymer micellar pores to optimize for the encapsulation of an individual enzyme. This modification is done by the addition of tetraalkylammonium bromide salts prior to polymer casting.[29]

Tetraalkylammonium bromide salts are exchanged for the proton of the sulfonic acid side chains of Nafion. This modification results in the reduction of acidity, an increase in hydrophobicity, and also changes the pore size of the Nafion polymer to allow the inclusion of enzyme and increase mass transport.[30] This polymer encapsulation has been demonstrated to protect sensitive enzyme from temperature increases and buffer the encapsulation site from external changes in pH. These modifications are able to be tailored by simply altering the alkyl groups of the tetraalkylammonium bromide salts. Images of modified Nafion polymers were obtained by fluorescence microscopy and are depicted in Fig. 4.[30] Initial work with this technique was applied to dehydrogenase enzymes to simply determine their activity and lifetime. This study resulted in high catalytic activity and lifetimes of greater than 45 days.[30]

First applied to a biofuel cell in 2005, this technique resulted in a maximum power density of 2.04 $mW\ cm^{-2}$ through the combined encapsulation of aldehyde and alcohol dehydrogenase to oxidize ethanol with poly(methylene green) acting as the mediator electrocatalyst electropolymerized on the surface of the electrode. This performance decreased by only 6.1% over 7 days and showed a 18.1% decrease over 30 days.[6]

Subsequent work eliminated the need for a traditional PEM cell setup through the modification of the cathode electrode through the modified Nafion immobilization of bilirubin oxidase to selectively catalyze the reduction of oxygen. This cell resulted in maximum power density of 0.46 $mW\ cm^{-2}$ and an active lifetime of about 30 days.[31] Modified Nafion immobilization has also been demonstrated to be effective with other enzyme/fuel systems. Soybean oil was used directly to produce open circuit potentials of 0.97 V, maximum power densities of 4.39 $mW\ cm^{-2}$ and lifetimes of greater than one year.[32,33] Minteer *et al.* have also demonstrated the ability to use a dual enzyme system to form a glycerol/oxygen biofuel cell that yielded power densities of 1.21 $mW\ cm^{-2}$ while allowing for nearly 100% fuel concentration as shown in Fig. 4.[34,35]

Recent work, including the previously mentioned glycerol fuel cell, has explored the use of PQQ-dependent alcohol and aldehyde dehydrogenase that are capable of DET. This enzyme incorporation has been shown to improve the performance of the ethanol biofuel cell from 2.04 $mW\ cm^{-2}$ to 4.07 $mW\ cm^{-2}$ with lifetime of greater than 200 days and eliminating the need for mediation of electron transfer by poly(methylene green).[35]

Although modified Nafion has produced promising results, there are several disadvantages. Nafion is an expensive polymer that due to its perfluorinated polymer backbone is not biocompatible or biodegradable. Groups have recently explored the encapsulation of oxidoreductase enzymes in chitosan for biofuel cell and biosensing applications.

Chitosan is a biocompatible, very inexpensive biomass obtained from crustaceans that has high mechanical strength. Chitosan is obtained through the deacylation of chitin, which is the second most abundant natural polymer. Chitosan has also been shown to be easily hydrophobically modified by reductive amination to allow for a more ideal environment for enzyme encapsulation. Klotzbach *et al.* detailed a variety of hydrophobically modified chitosans and their ability to immobilize glucose oxidase and alcohol dehydrogenase.[36–38] While most immobilization techniques lower enzyme activity, this process has been shown to improve enzyme kinetics. In addition to maintaining enzyme activity, chitosan is capable of forming a mesoporous, three-dimensional scaffold structure that has greatly improved mass transport to the electrode surface and allows the transport of both cationic and anionic species. Currently, these techniques have been utilized in other applications, but have not been applied to biofuel cell technology.

Sol–gel matrices have been utilized in many biosensing and biofuel cell technologies for enzyme encapsulation and direct covalent linking of enzyme to electrode surface. It is well documented that sol–gel matrices provide extended lifetime of enzyme activity through stabilization within their inorganic framework.[24,25,39] This type of matrix is advantageous, because of the extremely flexible chemistry which allows for modifications to be imparted to optimize the polymer for a particular enzyme system or application. This tunability allows the production of various pores and channels ranging in size from 0.1 nm to near micron scale. This size tunability must in turn be optimized for each enzyme to create pore

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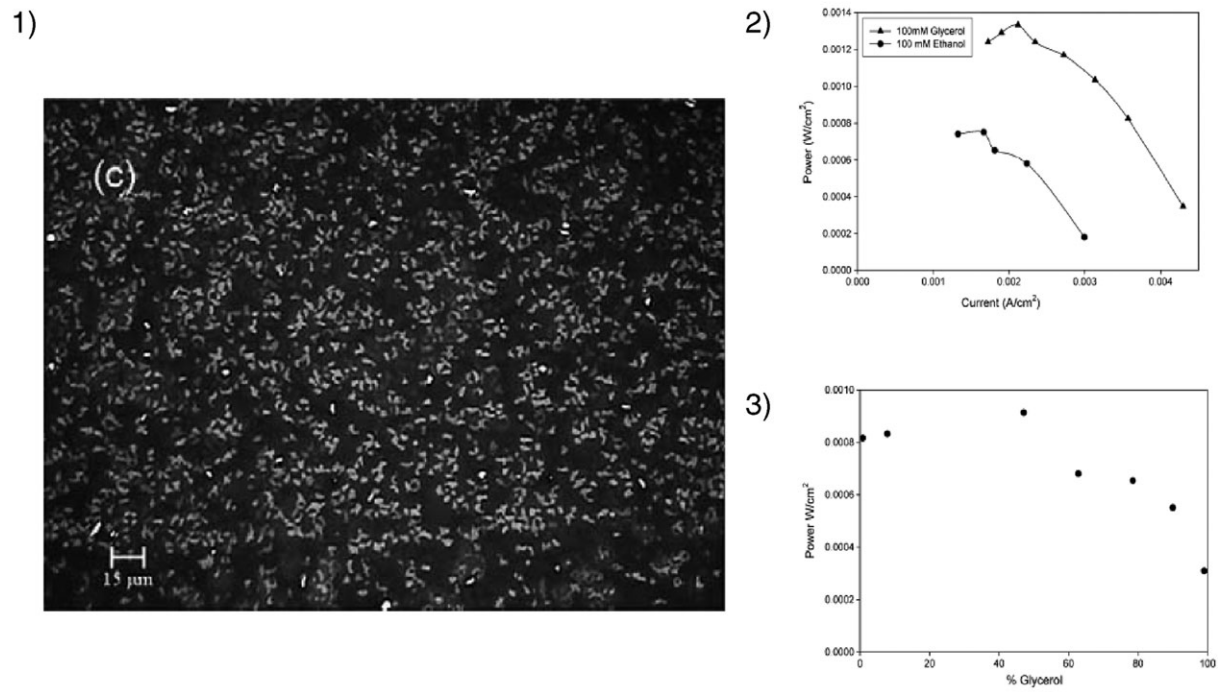

Fig. 4 (1) Fluorescence micrographs of annealed tetrabutylammonium bromide modified Nafion polymers. (2) and (3) demonstrate representative data obtained from a glycerol/oxygen fuel cell with tetrabutylammonium bromide modified Nafion immobilization of alcohol and aldehyde dehydrogenase at carbon paper electrodes.

structures that are large enough to contain the enzyme, but are not excessively large, allowing for the leaching or diffusing of enzyme into solution. It has been speculated that increasing the confinement of the enzyme by closely matching the sol–gel pore size with enzyme dimensions greatly improves enzyme stability by holding it in the protein's three-dimensional conformation.

Kim *et al*. have reported enzyme stabilization and immobilization in nanoporous silica through the generation of single enzyme nanoparticles (SEN). In this work, chromotrypsin is surrounded by a thin sol–gel network forming nanoparticles. This enzyme immobilization is performed by the free radical initiated vinyl polymerization of a modified enzyme to methacryloxypropyltrimethoxysilane (MAPS) by exposure to 365 nm UV light. This covalent linkage limits the amount of enzyme able to leach from the surface. Following this step, 10 nm enzyme functionalized nanoparticles were created through simple hydrolysis and silanol condensation. These SENs demonstrated remarkable enhancements in enzyme stability, improving the enzyme half life from 12 h for chromotrypsin in solution to 143 days.[40]

Dunn's group at the University of California-Los Angeles has explored many methods for immobilization of enzymes using silica derived polymers. Recently, they have described the immobilization of glucose oxidase for an anodic system and bilirubin oxidase for a cathodic system in addition to the incorporation of carbon nanotubes for production of a biofuel cell. The incorporation of carbon nanotubes has been shown to greatly facilitate DET by decreasing electron transfer distance. In doing so, this membraneless biofuel cell is capable of generating approximately 120 $\mu W\ cm^{-2}$ at 0.24 V at room temperature. They have also demonstrated an enhanced stability, maintaining significant enzyme activity for bilirubin oxidase up to approximately 60 °C although length of lifetime is not reported.[39]

The advancement in nanotechnology has had a strong impact on the development of biofuel cells from electrode material to direct interaction with enzymes. Nanotechnology application to biofuel cell research attempts to simultaneously attack low catalytic activity and low current density that arise from non efficient enzyme loading and low surface area.[41] As in Dunn's work, the incorporation of conductive nanomaterials greatly increases the surface area of the electrode allowing a greater concentration of enzyme to be located within electron tunneling distances, greatly increasing the potential for high power densities.[42] Kim *et al*. examined the utilization of carbon nanotubes (CNTs) for the direct noncovalent immobilization of cytochrome c and glucose oxidase. This technique has been demonstrated to improve the lifetime of these cells through conformational stabilization of these enzymes by selective attachment and confinement. This cell has demonstrated no loss in activity in over 12 days.[40] In recent application to fuel cell performance, Kim has utilized covalent linking of oxidoreductase enzymes to carbon nanotubes for enhanced stabilization and charge transfer. His group has produced a novel miniature fuel cell design that incorporates the immobilization of glucose oxidase clusters on carbon nanotubes which is subsequently immobilized on a carbon felt electrode. This cell operated in a non-buffered environment at an open circuit potential of 0.33 V and at 0.55 V in a buffered system. A maximum power density was achieved of 370.7 $\mu W\ cm^{-2}$ for buffered systems and 116.7 $\mu W\ cm^{-2}$ for an unbuffered

system. However, in terms of lifetime, buffered systems demonstrated a dramatic drop in performance whereas unbuffered experiments resulted in relatively stable performance over a 16 h period. This was proposed to be caused by degradation of enzyme, depletion of fuel, or inactivation of the membrane that controlled diffusion of species to the cathode.[43] Although this attempt depleted quickly, it demonstrated the near realized potential of a biofuel cell system to be utilized in small consumer electronics in the near future.

Moving towards a commercially viable product

As improvements in enzyme stability, catalytic activity, and immobilization techniques advance in conjunction with our growing understanding of enzyme electron transfer kinetics, the realization of biofuel cell technology application to small electronic devices is imminent. Studies described here and others have demonstrated the ability to sustain power densities capable of operating some consumer electronics over long periods of time. However, traditional biofuel cell development has incorporated both cathode and anode in large diffusion cells. This cell design greatly limits the performance of the cell due to the lack of oxygen at the cathode surface that is available for reduction to water. Under typical laboratory conditions, most cathodic solutions contain approximately 7 μg mL^{-1} oxygen. This translates to a maximum power density of a few mW cm^{-2}.[44] In addition, the geometry of these cells is a limitation due to their size and length of separation from cathode to anode. Many of these traditional cells depicted in Fig. 1 separate the anode and cathode by at least 10 cm, greatly limiting the current produced due to the sheer distance that ions must travel through solution. Cell design geometries that had previously been overlooked are now being pushed to the forefront to eleviate these simple limitations.

The ability to use mixed fuel solutions and eliminate physical barriers between solutions has resulted in the development of more efficient cell designs. A modification to the traditional diffusion cell design depicted in Fig. 1 has been described which reduces the distance between cathode and anode. In this setup, the cathode is in direct contact with air, thus eliminating limitations that arise from lack of oxygen. This design has been termed the I-cell due to its shape shown in Fig. 5. This design utilizes a gas diffusion electrode which is

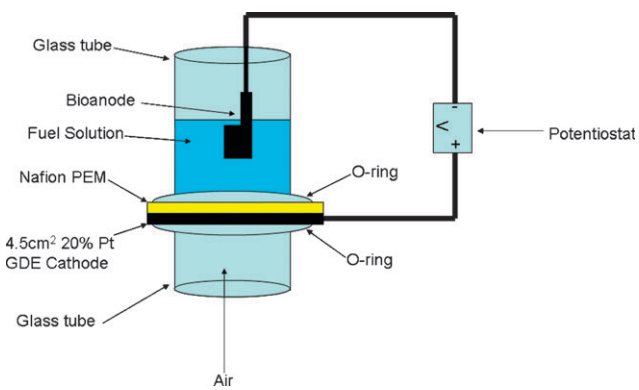

Fig. 5 Schematic representation of the 'I-cell' design used for biofuel cells with air breathing cathodes.

hot pressed to a Nafion® 112 membrane.[44] This gas diffusion electrode is clamped between two glass tubes, one of which contains the fuel solution. The modification also allows for simple interchangeability of fuel and anode. Although this is a marked improvement on traditional test cell design, it remains impractical to link multiple cells in series to power conventional devices.

Many have pursued eliminating the need for separation between cathode and anode. Most have accomplished this through modification of the anode with an oxidizing enzyme and modification of the cathode with a reducing enzyme such as bilirubin oxidase, laccase, or cytochrome c oxidase among others. Due to the selectivity of the enzymes at both electrode surfaces, Heller *et al.* have been able to create a biofuel cell that was greatly reduced in size. Through the 'wiring' of bilirubin oxidase to the cathode surface and glucose oxidase to the anode surface, Heller was able to create a membraneless cell that was capable of generating 1.9 μW power output from two carbon fibers that are 7 μm in diameter and 2 cm in length in a physiological buffer. However, this power output was nearly cut in half over the course of one week of operation. This has since improved to a loss of 22% of its power over the course of a week. In conjunction with this improvement, this group has applied this approach to the operation of a biofuel cell in a living plant by inserting these two electrodes into a grape. This work generated a fuel cell capable of producing 1.1 μW at an operating voltage of 0.52 V. All of this was accomplished in a volume of 0.01 mm^3.[13,45,46]

In addition to miniaturization, the production of small scale power sources can be achieved through the linking of individual cells in series. Serial configurations are common in implementation of fuel cell technology to consumer goods; however, biofuel cells have not been implemented in such a manner due to the requirements of the enzyme and its environment. With improvements in immobilization techniques, this limitation has ceased and several groups are examining the development of stack designs. Work in Jungbae Kim's lab, outlined previously for its immobilization techniques, demonstrates a miniature biofuel cell that takes the form of a 1.3 cm^3 cell which generates 370.7 μW cm^{-2} that is easily capable of being stacked with more of the same to additively improve power output.[43] Teodorescu *et al.* have described a similar stacking design that utilizes not only the common stacking technique, but also incorporates the use of microelectrodes to overcome energy losses due to activation, ohmic resistance and slow mass transport. This preliminary design was demonstrated to generate an open circuit potential of 1.57 V and a power density of nearly 35 μW cm^{-2} with six cells stacked together which still falls significantly short of the performance seen in recent traditional cells.[47] This is most likely due to a cathodic limitation due to the electrodes' confinement and lack of exposure to significant amounts of oxygen.

The growing field of microfluidics has also been recently implemented to biofuel cell miniaturization. The field of microfluidics has been demonstrated to have characteristics that may be advantageous in the reduction of transport limitations, cell stackability, and overall practicality of the biofuel cell. Intricate fluid handling and the small dimensions of these devices could be ideal for efficient biofuel cell

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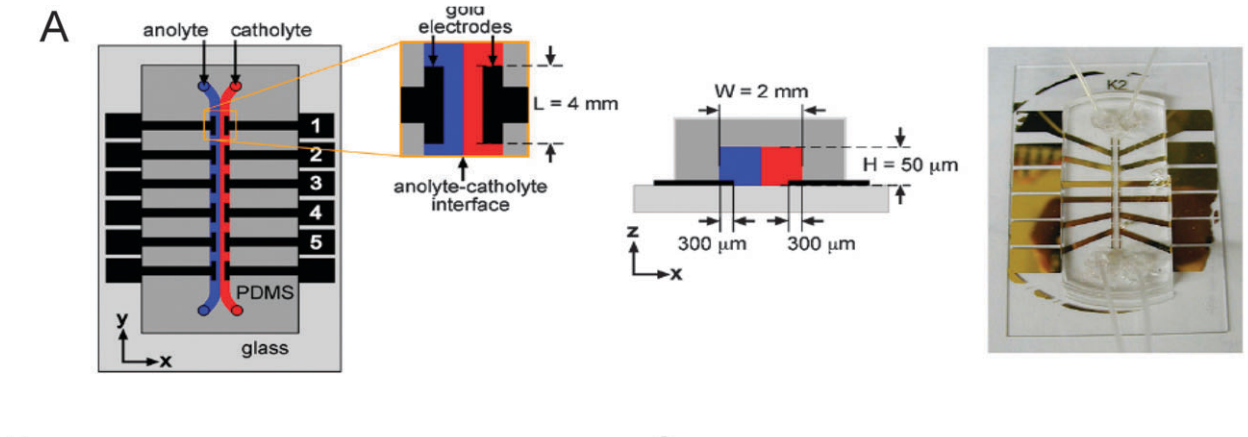

C

Table 1
Location of the anodes and cathodes used in the different configurations

Legend used in figures	Location of the anode[a]	Location of the cathode[a]
C1	1,2,3,4,5	1
C2	1,2,3,4,5	2
C5	1,2,3,4,5	5
C12	1,2,3,4,5	1 and 2
C13	1,2,3,4,5	1 and 3
C14	1,2,3,4,5	1 and 4
C15	1,2,3,4,5	1 and 5

Fig. 6 (A) Schematic representation of a microfluidic biofuel cell device developed by Lim and Palmore. (B) Representative (a) polarization curves and (b) power curves generated from a microfluidic biofuel cell with cathode configurations C1, C12, C13, C14, and C15 which refer to anode and cathode configurations outlined in the Table (C).

production due to developments in fluid flow dynamics, high surface area electrodes, and the extremely inexpensive cost requirements for standard soft-lithography. Togo *et al.* have utilized this technology to fabricate a simple cell incorporating a single diaphorase modified gold anode and a platinum cathode patterned on a glass slide. This glass slide is then overlaid with a layer of polydimethylsiloxane (PDMS) containing a flow channel for the delivery of fluid hydrodynamically. This resulted in a decrease in mass transport limitations to the electrode surface and has resulted in a maximum open circuit potential of 0.55 V, maximum current density of 0.13 mA cm^{-2} and a maximum power density of 32 μW cm^{-2}.[48]

Others have utilized the unique laminar flow dominated flow regimes found at the microfluidic level to act as a separating device between the solutions of the cathode and anode. G. Tayhas Palmore has developed a microfluidic device that operates under hydrodynamic flow, imparting two separate anodic and cathodic fuel streams to impart two isolated environments and lack of crossover due to the extremely low Reynold's numbers occurring in such small scale fluid channels. While previous studies involving microfluidics have incorporated a single electrode design, this design has incorporated six separately addressable electrodes. They have examined the diffusion layer effects in this particular device and determined how it could be further optimized through electrode configuration. This cell is depicted in Fig. 6 with representative power density and current density data. This cell resulted in maximum power density of 26 μW cm^{-2}, an open circuit potential of 0.4 V and a current density of 450 μA cm^{-2}.[49]

Another important objective that has recently been examined through microfluidics is the cascade of multiple enzyme systems to perform a more complete oxidation of a fuel substrate. Kjeang *et al.* proposed a mathematical model for a microstructured enzymatic biofuel cell that strategically places sections of immobilized enzymes downstream in a hydrodynamic system that are capable of further oxidation. In this format, mixed fuel and oxidant is imparted to the chip and the strategically placed enzymes further oxidize the fuel. This resulted in a device that was mainly limited by the slow turnover rates for the enzymes of interest. This cell model, however, only resulted in a theoretical current density of 50 μA cm^{-2}.[50] With further advancement in immobilization techniques and utilization of creative solutions that can arise from engineering advancements outlined in this review, one can easily foresee the implementation of these small scale devices for power supply of consumer electronics in the near future.

Conclusions

Over the last five years, there have been significant improvements in the field of biofuel cell technology as it pertains to the understanding of electron transfer kinetics and immobilization techniques which have resulted in greatly increased cell lifetimes and performance. However, there is an inverse relationship between stability (lifetime) and current density, so as the DET and MET systems increase the current densities of

bioanodes and biocathodes, immobilization membranes will need to be further improved as well.

References

1 S. Topcagic, B. L. Treu and S. D. Minteer, in *Alcoholic Fuels*, ed. S. D. Minteer, CRC Press, Boca Raton, 2006, pp. 215–231.
2 M. C. Potter, *Proc. R. Soc. London, Ser. B*, 1912, **84**, 260–278.
3 E. Katz, A. N. Shipway and I. Willner, in *Handbook of Fuel Cells—Fundamentals, Technology and Applications*, ed. W. Vielstich, H. A. Gasteiger and A. Lamm, John Wiley & Sons, Ltd, Jerusalem, 2003, pp. 1–27.
4 F. Davis and S. P. J. Higson, *Biosens. Bioelectron.*, 2007, **22**, 1224–1235.
5 C. M. Moore, S. D. Minteer and R. S. Martin, *Lab Chip*, 2004, **5**, 218–225.
6 N. L. Akers, C. M. Moore and S. D. Minteer, *Electrochim. Acta*, 2005, **50**, 2521–2525.
7 *The Organic Chemistry of Enzyme Catalyzed Reactions*, ed. R. B. Silverman, Academic Press, San Diego, 2000.
8 N. Mano, J. L. Fernandez, Y. Kim, W. Shin, A. J. Bard and A. Heller, *J. Am. Chem. Soc.*, 2003, **125**, 15290–15291.
9 A. T. Yahiro, S. M. Lee and D. O. Kimble, *Biochim. Biophys. Acta*, 1964, **88**, 375–383.
10 G. T. R. Palmore, H. Bertschy, S. H. Bergens and G. M. Whitesides, *J. Electroanal. Chem.*, 1998, **443**, 155–161.
11 T. Chen, S. C. Barton, G. Binyamin, Z. Gao, Y. Zhang, H.-H. Kim and A. Heller, *J. Am. Chem. Soc.*, 2001, **123**, 8630–8631.
12 A. Heller, *Phys. Chem. Chem. Phys.*, 2004, **6**, 209–216.
13 N. Mano and A. Heller, *J. Electrochem. Soc.*, 2003, **150**, A1136–A1138.
14 W. J. Aston and A. P. F. Turner, *Biotechnol. Genet. Eng. Rev.*, 1984, **1**, 89–120.
15 A. Chaubey and B. D. Malhorta, *Biosens. Bioelectron.*, 2002, **17**, 441–456.
16 A. L. Ghindilis, P. Atanasov and E. Wilkins, *Electroanalysis*, 1997, **9**, 661–674.
17 W. J. Blaedel and R. A. Jenkins, *Anal. Chem.*, 1975, **47**, 1337–1343.
18 R. A. Bullen, T. C. Arnot, J. B. Lakeman and F. C. Walsh, *Biosens. Bioelectron.*, 2006, **21**, 2015–2045.
19 S. C. Barton, J. Gallaway and P. Atanassov, *Chem. Rev.*, 2004, **104**, 4867–4886.
20 N. Yuhashi, M. Tomiyama, J. Okuda, S. Igarashi, K. Ikebukuro and K. Sode, *Biosens. Bioelectron.*, 2005, **20**, 2145–2150.
21 S. Calabrese-Barton, J. Gallaway and P. Atanassov, *Chem. Rev.*, 2004, **104**, 4867–4886.
22 T. Yow Tsong, *Am. Phys. Soc. Abstr.*, 1998 X12.05.
23 B. L. Treu and S. D. Minteer, *Polym. Mater. Sci. Eng.*, 2005, **92**, 192–193.
24 J. Lim, N. Cirigliano, J. Wang and B. Dunn, *Phys. Chem. Chem. Phys.*, 2007, **9**, 1809–1814.
25 R. Sahney, S. Anand, B. K. Puri and A. K. Srivastava, *Anal. Chim. Acta*, 2006, **578**, 156–161.
26 K. Zeng, H. Tachikawa, Z. Zhu and V. L. Davidson, *Anal. Chem.*, 2000, **72**, 2211–2215.
27 N. Mano, H. Kim, Y. Zhang and A. Heller, *J. Am. Chem. Soc.*, 2002, **124**, 6480–6486.
28 V. Soukharev, N. Mano and A. Heller, *J. Am. Chem. Soc.*, 2004, **126**, 8368–8369.
29 T. J. Thomas, K. E. Ponnusamy, N. M. Chang, K. Galmore and S. D. Minteer, *J. Membr. Sci.*, 2003, **213**, 55–66.
30 C. M. Moore, N. L. Akers, A. D. Hill, Z. C. Johnson and S. D. Minteer, *Biomacromolecules*, 2004, **5**, 1241–1247.
31 S. Topcagic and S. D. Minteer, *Electrochim. Acta*, 2006, **51**, 2168–2172.
32 J. Kerr and S. D. Minteer, *Polym. Prepr.*, 2005, **46**, 623–624.
33 J. Kerr and S. D. Minteer, *Polym. Mater. Sci. Eng.*, 2006, **94**, 594–595.
34 R. L. Arechederra and S. D. Minteer, *Prepr. Symp. – Am. Chem. Soc., Div. Fuel Chem.*, 2007, **52**, 175–176.
35 R. L. Arechederra, B. L. Treu and S. D. Minteer, *J. Power Sources*, 2007, **173**, 156–161.
36 T. L. Klotzbach and S. D. Minteer, *Polym. Mater. Sci. Eng.*, 2006, **94**, 599–600.
37 T. L. Klotzbach and S. D. Minteer, *Polym. Prepr.*, 2007, **48**, 1008–1009.
38 T. L. Klotzbach, M. M. Watt, Y. Ansari and S. D. Minteer, *J. Membr. Sci.*, 2006, **282**, 276–283.
39 J. Lim, P. Malati, F. Bonet and B. Dunn, *J. Electrochem. Soc.*, 2007, **154**, A140–A145.
40 J. Kim and J. W. Grate, *Nano Lett.*, 2003, **3**, 1219–1222.
41 J. Kim, J. W. Grate and P. Wang, *Chem. Eng. Sci.*, 2006, **61**, 1017–1026.
42 J. Kim, H. Jia and P. Wang, *Biotechnol. Adv.*, 2006, **24**, 296–308.
43 M. B. Fischback, J. K. Youn, X. Zhao, P. Wang, H. G. Park, H. M. Chang, J. Kim and S. Ha, *Electroanalysis*, 2006, **18**, 2016–2022.
44 R. L. Arechederra, Master's thesis, Saint Louis University, Saint Louis, 2007, p. 115.
45 N. Mano, F. Mao and A. Heller, *J. Am. Chem. Soc.*, 2002, **124**, 12962–12963.
46 N. Mano, F. Mao and A. Heller, *J. Am. Chem. Soc.*, 2003, **125**, 6588–6594.
47 S. G. Teodorescu, W. L. Gellett, M. Kesmez and J. Schumacher, in *209th ECS Meeting Abstracts, Denver, CO, 2006*, Electrochemical Society, Pennington, New Jersey, USA, 2006.
48 M. Togo, A. Takamura, T. Asai, H. Kaji and M. Nishizawa, *Electrochim. Acta*, 2007, **52**, 4669–4674.
49 K. G. Lim and G. T. R. Palmore, *Biosens. Bioelectron.*, 2007, **22**, 941–947.
50 E. Kjeang, D. Sinton and D. A. Harrington, *J. Power Sources*, 2006, **158**, 1–12.

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