# Enzymatic Biofuel Cells

*by Plamen Atanassov, Chris Apblett, Scott Banta, Susan Brozik, Scott Calabrese Barton, Michael Cooney, Bor Yann Liaw, Sanjeev Mukerjee, and Shelley D. Minteer*

What would it be like if you could recharge your cell phone battery instantly by pouring your soft drink into it? Such applications may be a long way off, but the U.S. Air Force Office of Scientific Research is investing in such a future now. Under a Multi University Research Initiative, university professors from around the country are now focused on a five-year research program to look at the technical challenges surrounding a fuel cell that will run on such simple sugars as those found in our everyday foodstuffs.

The challenges are great. Most fuel cells in the world today run on hydrogen. However, as the fuel gets more complex, this oxidation process becomes vastly more complicated. Once carbon atoms are in the fuel, carbon monoxide poisoning of typical fuel cell catalysts becomes problematic. Researchers are turning to the natural world in an effort to see how sugars are oxidized by animals to produce power.

Using enzymes (nature's catalysts) seems to be the answer, since they do not suffer from the contamination problems that more traditional metallic catalysts suffer from. They are also incredibly abundant and cheap to produce, something that the wine and detergent industries have known for decades. But applying enzymes to power electronics is very different from getting enzymes to clean our clothes. For one, enzymes do not like to give up electrons as easily as metal catalysts do, which means that generating an electric current from enzymes is much tougher. Enzymes can be made to give up their electrons with mediators, but using mediators can cause other problems in the fuel cell. Enzymes also are not used to staying put. In animals, enzymes are floating freely in the cells of the body, but to work in a fuel cell, they have to be put in a specific place and stay there, a process that scientists call immobilizing the enzymes. Finally, naturally occurring enzymes do not last that long. A typical enzyme in the human body lasts only a couple of days, but to be effective in a laptop or an automobile, an enzyme is going to have to last for months or years before needing replacement.

The benefits of the technology are as big as the risks, however. Enzymes, as we mentioned before, are cheap and plentiful. They are also green, and can be grown in quantity whenever they are needed, as opposed to the metal catalysts, which need to be mined and purified using expensive and less environmentally friendly processes. They are also "selective," a word that scientists use to describe an enzyme's ability to work with a very specific fuel, and only that fuel, so that the byproduct of one oxidation step could be the fuel for another enzyme. By doing this step, enzymes could conceivably reproduce what animals already know how to do: convert the sugars into just water and carbon dioxide. After all, there is as much energy in one jelly donut as you can find in 77 cell phone batteries. If you can get that energy out, it could have pretty, ahem, sweet consequences.

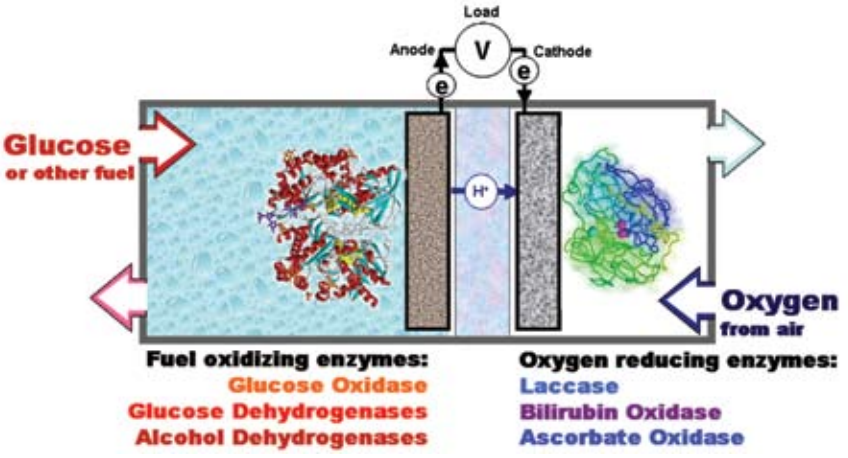


**Fig. 1.** *Generalized schematics of an enzyme biofuel cell consisting of an anode, catalyzed by oxidases suitable for conversion of fuels of choice or a complex of such enzymes for a complete oxidation of biofuels. The cathode usually features an oxidoreductase that uses molecular oxygen as the ultimate electron acceptor and catalyzes reduction to water in neutral or slightly acidic media.*

The basic enzymatic biofuel cell contains many of the same components as a hydrogen/oxygen fuel cell: an anode, a cathode, and a separator. However, rather than employing metallic electrocatalysts at the anode and the cathode, the electrocatalyst used are oxidoreductase enzymes. This is a class of enzymes that can catalyze oxidation–reduction reactions. Since these enzymes are selective electrocatalysts, the separator could be an electrolyte solution, gel, or polymer. Figure 1 shows a schematic of a generic biofuel cell oxidizing glucose as fuel at the bioanode and reducing oxygen to water at the biocathode.

Biofuel cells were first introduced in 1911 when Potter cultured yeast and E. Coli cells on platinum electrodes,[1] but it was not until 1962 that the enzymatic biofuel cell was invented employing the enzyme glucose oxidase to oxidize glucose at the anode.[2] Over the last 45 years, many improvements have been made in enzymatic biofuel cells and those can be found in several review articles.[3-6] However, there are still several main issues to consider with biofuel cells. These include short active lifetimes, low power densities, and low efficiency due to normally only incorporating a single enzyme

to do a partial oxidation of complex biofuels. There are a number of strategies for solving these problems, but our group is focused on strategies for immobilization and stabilization of the enzymes, engineering of enzymes to function optimally at electrode surfaces, electron transport between the enzyme and the current collector, optimization of multi-enzyme systems, and developing standardized characterization protocols for the biofuel cell research community at large.

## Enzyme Immobilization and Stabilization

One strategy for enzyme immobilization and stabilization has been the use of micellar polymers. Enzymes in solution are typically active for a few hours to a few days. This lifetime can be extended to 7-20 days by entrapment in hydrogels and binding to electrode surfaces.[4] However, researchers at Saint Louis University have extended active enzyme lifetimes at electrode surfaces to greater than one year by immobilization within hydrophobically modified micellar polymers.[7-9] Micellar polymers, such as, Nafion and chitosan, can be hydrophobically modified to tailor the micellar pore or pocket structure to be the optimal size for enzyme immobilization, while also ensuring a hydrophobic and buffered pH microenvironment for optimal enzyme activity. This strategy has been shown both to increase the active lifetime of the enzyme, but also to increase the enzymatic activity of the enzyme by up to 2.5-fold.[10]

The appropriate design of the enzyme–electrode interface is a key consideration for the creation of new bioelectrochemical systems like biofuel cells and biosensors. These systems generally involve complex arrangements of immobilization polymers, redox mediators, and enzymes that must interact with the electrode substrate. At Columbia University, researchers are using protein engineering to design multifunctional proteins and peptides that can both simplify and improve enzymatic electrodes. To this end, they are creating proteins that can self-assemble into bioactive hydrogels with redox enzyme activities. This eliminates the need for the incorporation of polymers in the system. In addition, peptides that can bind redox mediators are also being engineered. This molecular engineering approach should dramatically simplify the fabrication, characterization, and reproducibility of the bioelectrocatalytic interface employed in future devices.

## Electron Transport

The key issue in biofuel cells, as well as in any other type of low-temperature fuel cells, is the catalysis of electrode processes. Oxidation of the fuel, let say glucose, catalyzed by enzymes such as glucose oxidase (the biosensor's favorite enzyme of all times) may be accomplished directly or may involve redox mediators. Direct enzymatic oxidation requires that the active site of the enzyme communicates immediately with the electrode surface. That seemed rather impossible for the best beloved glucose oxidase because it flavin-type active site is "buried" too deep in the protein "shell" of the quite large enzyme molecule. Sandia National Laboratories addresses this issue by genetically modifying the enzyme to make its active site more accessible for communication with the electrode. A lot of mutant proteins can be made in a day. Knowing which one will work is a big part of the problem. Fast screening of genetically modified enzymes for their electrocatalytic properties is the task of a collaborative effort between Sandia National Laboratories and the University of New Mexico.

Researchers at the University of New Mexico are also looking at the problem of enzyme/electrode interactions from a different angle: let us leave the enzyme alone, there are things that we can do with the electrode itself. Nanostructured materials, specifically carbon nanotubes, could be of use. At least they are comparable in size with the enzyme and their defects are probably a good part to interact with the active site directly. Direct oxidation of glucose by glucose oxidase immobilized in a carbon nanotube-modified porous matrix shows that we can make an anode operational at about 400 mV (vs. Ag/AgCl).[11] This means that we can start "burning" the fuel at a potential very close to the one provided to us by the thermodynamics of the system (see Fig. 2).

When we combine this with direct reduction of oxygen, which is catalyzed by copper-containing enzymes (laccases, bilirubin oxidases, or ascorbate oxidases) at a potential just 50 mV below the thermodynamic value,[12] this gives us the option of making a biofuel cell with as high open circuit voltage as 1 V. For all this to happen, a lot of things need to be fine-tuned, the enzyme surface interactions most of all. Charge transfer processes and enzyme orientation are of immense importance in direct electron transfer. The rest is "simple" engineering of the materials, which should allow us to draw as much current as possible.

There is indeed another way: we can use mediators. These are redox couples that can easily communicate with both the enzyme and the electrode. The use of mediators should be well measured because if their potential is too close to that of the enzyme, the driving force of the enzyme/mediator "cascade" will be too low. If their potential falls too far from that of the enzyme active site, the voltage of the cell will suffer (see Fig. 2). A group at Michigan State University is studying these effects by screening a library of redox mediators (based on osmium complexes) that can assist both cathode and anode processes. The redox potential of those mediators is tunable by slight modifications in the chemical

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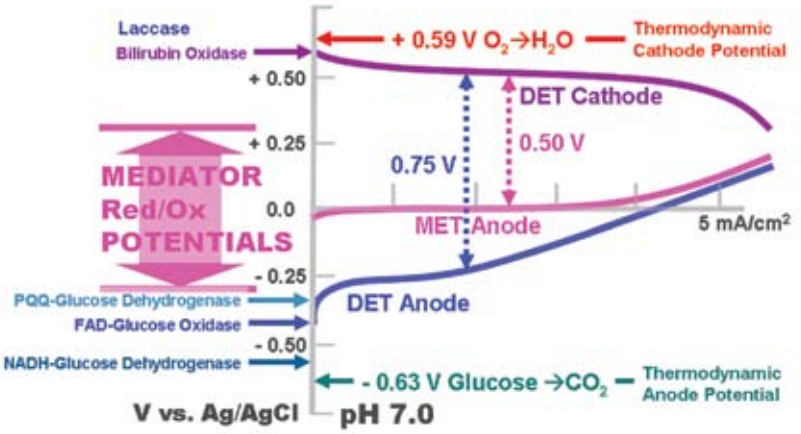


**Fig. 2.** *Principles of biofuel cell design indicting the maximum oxidation potentials for glucose and the corresponding thermodynamic potential for oxygen reduction at neutral pH. Redox potentials of several enzymes and their corresponding co-factors are shown along with the potential "zone" containing the redox potentials of the usual mediators. Polarization curves depict typical current performances for direct and mediated electron transfer in biofuel cell electrodes.*

structure of the organic ligand. They are all deployed as side chains of a redox polymer that forms a hydrogel on the electrode surface. Transport processes in those hydrogels and in the porous corrugated media of the enzyme biofuel cell electrode (think of it as a sponge) is a topic of its own. The deep understanding of the transport effects on kinetics will give the team the means to address those "simple" engineering problems and to produce as much current as possible from the bioenzymatic electrodes. The numbers are now moving from μA to mA/cm$^2$.

## Complete Oxidation of Biofuels

One of the main problems plaguing enzymatic biofuel cells has been low energy densities due to incomplete oxidation of biofuels at the anode of the biofuel cell. The only example of complete oxidation in an enzymatic biofuel cell is for the oxidation of methanol using alcohol dehydrogenase, aldehyde dehydrogenase, and formate dehydrogenase.[13] With complex biofuels like glucose and sucrose, the enzymatic metabolic pathways are far more complex and contain both oxidoreductase enzymes and other enzymes (kinases, etc.) responsible for chemical transformations. The cellular pathways responsible for enzymatic breakdown of sugars are the glycolysis pathway and the Kreb's cycle. Mimicking these cellular pathways at an electrode requires both the ability to immobilize them in appropriate microenvironments for enzyme activity, minimizing transport limitations associated with oxidized products diffusing between enzymes, and handling the fact that the enzymatic activity of each enzyme is not equivalent, so there are rate limiting enzymes within the system. The researchers at Saint Louis University are employing click chemistry to form enzyme complexes to decrease transport limitations and developing enzyme immobilization membranes for ensuring the appropriate microenvironment, but the electrochemists are turning to metabolic engineers to learn how to determine rate limiting enzymes within the system.

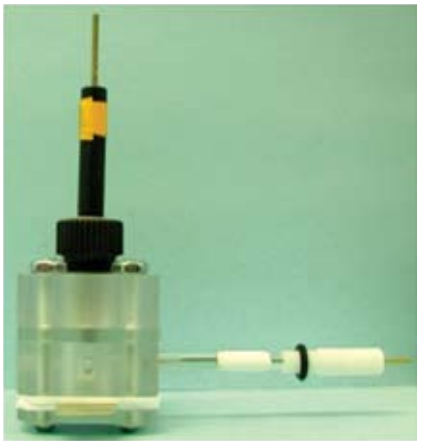

**Fig. 3.** *Standardized stack cell design for the biofuel cell.*

Nature has evolved complex metabolic networks to extract chemical energy from ambient fuel sources. The enzymes involved in these networks have evolved to exhibit a distributed control over the flux of materials through these pathways so that no single node in the network represents a dominant bottleneck. Recently, there have been exciting advances in the bioelectrochemical arena, as multi-enzyme systems are being created for applications such as biofuel cells and biosensors. But, these systems are often created using enzymes from different organisms that did not evolve to function together and are not optimized to function under *in vitro* conditions. This can result in the creation of metabolic pathways where the kinetic control of the system is not well-distributed, and a single node can dominate the overall performance. At Columbia University, researchers are applying metabolic control analysis to better understand and improve the kinetic performance of biofuel cells. These insights can be used to ensure optimal operating conditions as well as to drive the choice of enzymes that should be used in these artificial metabolic networks.

## Standardization of Characterization Techniques

Due to the intricacy and sensitivity of enzymes toward the electrode fabrication and modification, characterizations of enzyme performance are particularly difficult for cross platform comparison. A possible solution is to use standardized cell configurations and characterization techniques to allow such comparison to the best faith. A team at the University of Hawaii has been engaged in such an endeavor to design and develop suitable modular cell configurations that can be shared among laboratories to conduct comparative research work to facilitate the sharing and transfer of knowledge among collaborators. By using a common geometry and dimensions, the electrical field and reactor geometry is maintained among experiments. The results then can be compared with an assumption of common geometry and field to eliminate ambiguities around these issues, so kinetic measurements can be assessed. Figure 3 is a picture of the standardized cell configuration being employed.

## Conclusions

Although enzymatic biofuel cells still have lifetime, power density, and efficiency issues that are currently being addressed, they have several attractive points including: the ability to operate optimally at temperatures between room temperature and body temperature; the flexibility of fuels that can be employed, including renewable fuels (e.g., ethanol, glucose, sucrose, glycerol) and traditional fuels (e.g., hydrogen, methanol, etc.); and the use of non-platinum renewable catalysts. The two main application areas that are being considered for enzymatic biofuel cells are *in vivo*, implantable power supplies for sensors and pacemakers and *ex vivo* power supplies for small portable power devices (wireless sensor networks, portable electronics, etc.). The implantable devices would most likely employ glucose as a fuel and recent advances by the Heller group are showing that biofuel cells can be implanted and continue to function in a living organism (a grape).[14] For *ex vivo* applications, many fuels are being considered, from alcohols to sugars. Figure 4 shows an 8-cell ethanol/oxygen biofuel cell stack prototype operating an iPod. The optimal fuel choice will be a function of application for these systems. All in all, biofuel cells are a early stage technology

**Fig. 4.** *Ethanol/air biofuel cell stack. This prototype, developed by Akermin, Inc. in 2006, powers an iPod. Photograph courtesy of Akermin, Inc.*

with fundamental scientific and engineering hurdles to overcome, but they are a promising technology for certain applications. ■


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