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Hybrid Biofuel Cell: Microbial Fuel Cell with an Enzymatic Air-Breathing Cathode

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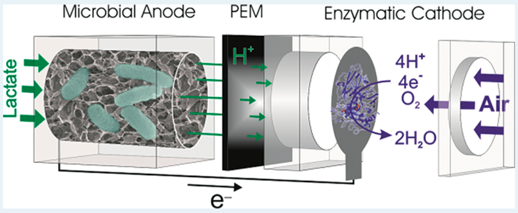
S *Supporting Information*

ABSTRACT: A hybrid lactate/air biofuel cell has been created using a microbial anode with *Shewanella* MR1 integrated into a chitosan−carbon nanotube porous matrix and a DET-based laccase air-breathing cathode. Open circuit potentials of 1 V and power densities of 26 W/m^3 are reported. A stable 5-day galvanostatic polarization shows a loss of only 4% of potential.



KEYWORDS: Microbial fuel cell, enzymatic fuel cell, *Shewanella* MR1 anode, air-breathing laccase cathode

Biological fuel cells (biofuel cells) are enjoying growth in interest due to the promise to achieve benign conversion of a list of fuels, previously unattainable by conventional technology to electric power.[1−3] This interest ranges from investigation of the details of charge transfer mechanism between microorganisms or enzymes and electrode interfaces to design of scalable units for energy recovery from wastewater processing. Despite the promise of being ultimately scalable, most of the microbial fuel cells are utilizing extremely expensive and hardly compatible Pt-catalyzed cathode electrodes. Advantages of using enzymatic catalysis in cathode design are 2-fold: compatibility with biocatalysis employed on the anode (microbial or enzymatic), which usually requires activity in neutral pH (or near neutral pH) and relatively low ionic strength; and selectivity toward oxygen reduction reaction (ORR) in the presence of sometimes an increasingly complex "fuel solution", containing the carbon source of the microorganism (or the oxidative substrate of the enzyme) as well as multiple additives forming the growth media. This requirement for explicit selectivity to ORR is particularly important in device design and integration solutions because oxidation of the fuel (or other chemicals) on the cathode leads to depolarization and poisoning. Selectivity of the ORR catalysis to a four-electron pathway of ORR is important, as well, since hydrogen peroxide evolved by two-electron ORR catalysts can have harmful effects on both the anode performance and the cathode stability.[4] Many current designs of biofuel cells and microbial fuel cells (MFC) use Pt-catalyzed or carbon-based cathodes and most often rely on dissolved oxygen in the electrolyte.[2,3] Although useful in laboratory testing and in some specific designs, these solutions are far from addressing any device integration challenges.

It is well accepted that performance of biological fuel cells, whether enzyme or microbial, is dependent upon a host of factors that control the release of electrons from highly reduced fuel molecules in solution, their transfer through an external circuit, and their recapture by highly oxidized molecules.[1] In practical terms, this function is realized using two compartment cells separated by a proton exchange membrane (PEM) wherein the release of electrons (oxidation) occurs at the anode and their recapture (reduction) occurs at the cathode (Scheme 1). The electrons released to the anode are removed from the cell through an external load before being reintroduced to the cell in the cathode compartment, where they are then recaptured. The membrane serves to permit ion transfer between the anode and the cathode compartment while maintaining separation of the highly reduced fuel molecules in the anode from the highly oxidized molecules in the cathode. This permits the maintenance of charge balance (as electrons leave the anode compartment and enter the cathode compartment) and avoids cross-contaminating reactions. Both cell compartments can be kept under different conditions optimized for the individual bioelectrocatalysts, for example, different buffers as well as aerobic or anaerobic environments.

To date, the literature has been heavily focused on the development of technologies that address the individual factors that impact overall cell performance. Electrode materials and cell designs have been proposed that improve diffusion of the fuel to the catalyst.[5−7] Enzyme catalysts have been proposed from a number of sources, engineered for superior performance, or both.[8−10]

Immobilization techniques have been proposed that both stabilize and increase activity as well as conduct the released

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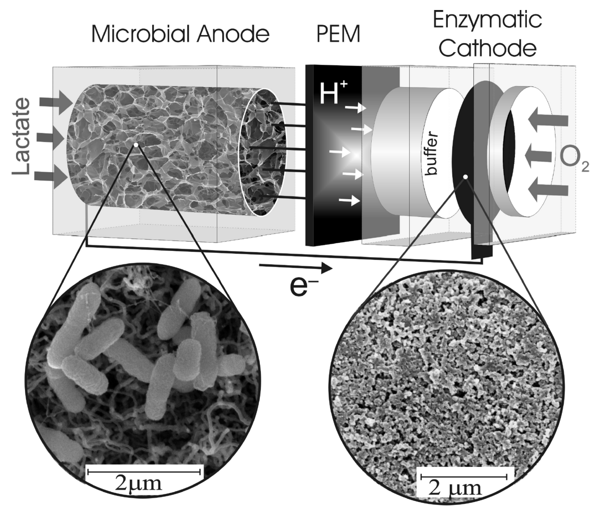
Scheme 1. Schematic Drawing of 3D Hybrid Fuel Cell[a]



[a] The flow-through anode compartment is filled with a highly porous chitosan-MWCNT composite anode material (1 cm^3 V) that was inoculated with *Shewanella* MR1. The air-breathing cathode (1.23 cm^2 SA) is made from teflonized carbon black with adsorbed laccase as the bioelectrocatalyst. A PEM separates both compartments from each other. High-magnification SEM images show insight into both electrodes: (left) *Shewanella* MR1 on MWCNTs and (right) the porous carbon black gas-diffusion electrode.

electrons to the current collector.[11] Electrode designs have been proposed that increase the electrochemically active surface area.[12,13] A suite of ion exchange membranes have been developed and proposed to increase the rate of ion transfer as well as to reduce internal resistance to their flow. Although all of these efforts are critical to improving performance, one of the most crucial factors to cell performance remains the open circuit potential achieved between the anode and cathode.

For any given oxidation−reduction reaction executed by a particular biological fuel cell, the thermodynamically defined electrochemical potential across the reacting species ultimately defines the maximum performance that can be achieved. It is therefore desirable to begin the design of a cell with an anode with a cathode that, once integrated, approaches the thermodynamic potentials of the half reactions. With that established, further efforts can be pursued that increase both the release of electrons at the anode, per unit of accessible surface area, and internal resistances that would otherwise restrict the flow of those electrons through the external circuit.

Previously, the authors have presented a working MFC incorporating a novel anode material[14] consisting of a blend of the biopolymer chitosan (CHIT) and multiwalled carbon nanotubes (MWCNT) molded into a stable, three-dimensional macroporous and conductive electrode.[15] The CHIT-MWCNT material was shown to perform in both batch and flow-through modes, producing power densities (specific to anode Volume) upward of 6 W/m^3 via lactate oxidation by *Shewanella* with an oxygen-reducing platinum cathode.[16] The biopolymer material was found to be a superb substrate for colonization, durable over operating periods up to 2 months with no visible or measurable degradation and possessing good conductivities. Open circuit potentials (OCP) of −0.45 V (versus Ag/AgCl reference electrode) have been achieved, a value that is generally among the best reported in the literature at pH 7.[17]

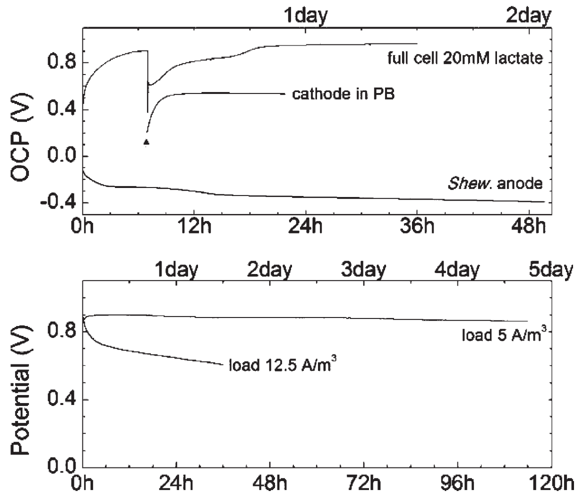


Figure 1. (Top) Time dependence of the open circuit potentials (OCP) of anode and cathode (vs Ag/AgCl) and the open circuit cell voltage of a full hybrid biofuel cell running on 20 mM lactate as fuel source (added at $t = 0$). (Bottom) Time study of a full hybrid biofuel cell running under two different galvanostatic loads of 5 and 12.5 A/m^3 (anode V).

The authors have also recently reported the development of a gas-diffusion, laccase-catalyzed cathode based on effective direct electron transfer (DET) between the copper redox centers of laccase and the underlying carbon electrode onto which they are physically adsorbed.[4,18] High current densities (up to 1 mA/cm^2, cathode SA) and long stabilization times (up to 30 days) were achieved, in part due to its composition of pressed, teflonized carbon black as the electrode material, a factor that permits the high enzyme loadings and highly efficient oxygen diffusion through the gas phase.[19] Cathodic potentials of +0.55 V (vs Ag/AgCl), close to the theoretical redox potential of the enzyme, have routinely been achieved.

In this work, we have combined the electrodes discussed above with the goal of creating a hybrid enzyme-microbial fuel cell that can approach the full thermodynamic electrochemical potential of lactate to CO_2 (anode V = 1 cm^3; cathode SA = 1.23 cm^2; Scheme 1). Key to our approach has been the utilization of the DET laccase air-breathing cathode discussed above. There have been two instances in which a microbial anode has been combined with a laccase electrode utilizing mediated electron transfer. Schroder et al. reports an enzymatic catalysis with laccase (*T. versicolor*) utilizing 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as a redox mediator in which a full cell OCP of 1.1 V was reported when the cathode was combined with a glucose-fed microbial anode.[19] Similarly, Luo et al. reported the MET performance of a full fuel cell with a cathode utilizing laccase mixed with carbon nanoparticles in a polymer matrix (Nafion) and a redox mediator ABTS in conjunction with a glucose-fed microbial anode.[20] After allowing the anode to equilibrate, the laccase cathode was placed on the fuel cell, which generated a maximum power of 160 mW/m^2 (anode SA). Unfortunately, the reporting of power in terms of watts per square meter (anode V) instead of per cubic meter (anode V), as used in our work, makes a direct comparison problematic. In addition, as opposed to mediated electron transfer in at least one of the electrodes, this manuscript presents a complete direct electron transfer biological fuel cell.

Combining the *Shewanella* bioanode and the air-breathing laccase biocathode produced a functioning hybrid biofuel cell. The Supporting Information (Figure S1) shows a photograph of the operational fuel cell that includes two reference electrodes for characterization of anode and cathode individually. Figure 1 (top) shows the separate open circuit potentials of anode and cathode (vs Ag/AgCl) recorded over 48 and 16 h, respectively. The anodic OCP was recorded in rich media (LB broth). After the first inoculation with *Shewanella* MR1, the OCP took ∼12 h to equilibrate and produce a stable potential of −0.4 V (vs Ag/AgCl) for the following 36 h. At time point zero, 20 mM of lactate as fuel was added to the buffered microbial anode, and the full cell potential was recorded; the cathode OCP measurement was started at that time. The cathodic OCP was stabilized after 2 h and provides a steady OCP of +0.55 V (vs Ag/AgCl) for the remaining time. The full cell potential is steady after 12 h at 0.97 V, which agrees with the anode's stabilization time. The measured cell potential nearly achieved the full sum of anodic and cathodic OCP.

After a stabilization period of at least 24 h for each cell, a stability test was performed. Two different loads of 5 and 12.5 A/m^3 were applied for 36 h and 5 days, respectively (Figure 1 bottom). As expected, the cell potential under a relatively high load of 12.5 A/m^3 lost ∼20% after the first 6 h of operation, and the following 30 h resulted in an additional 10% performance loss. In contrast to that, the full cell held the applied current of 5 A/m^3 for nearly 5 days of operation with an insignificant 4% loss in potential. Initially, the cell jumped to 0.88 V and gradually rose to 0.9 V after 12 h, when it began a gradual decline to 0.86 V after 4.75 days. This steady response to galvanostatic polarization shows the longevity of the cathode to be at least 4.75 days with minimal drop (4%) in the potential across the cell. The measured potentials of 0.86 and 0.65 V, corresponding to the galvanostatically applied currents of 5 and 12.5 A/m^3, agreed very well with the measured polarization and power data in Figure 2.

Figure 2 (top) presents full-cell polarization curves for the individual electrodes and the full cell. The measured full biofuel cell potential typically ranged from 0.95 to 1.0 V, with voltages as high as 1.04 V observed, a remarkable loss of only 6% from the theoretical OCP.[21] The full-cell short circuit density (LB broth) was estimated to be 65 A/m^3 (anode V). Both full cell polarization curves in Figure 2 show a comparison of the hybrid fuel cell filled with rich media (LB broth) for enhanced *Shewanella* growth or only lactate as the "nature equivalent" fuel source. This difference in media explains the discrepancy in current and power density. Different densities of bacterial population as well as differences in microbial metabolism during both measurements should be considered, as well.

Also presented are anode and cathode polarization curves (both measured with respect to an Ag/AgCl reference electrode). Note: the anodic curve was taken after applying a load of 12.5 A/m^3 (anode V) for 36 h (see Figure 1 bottom). The anode OCP was observed to be −0.433 mV (vs Ag/AgCl), with a short circuit current density of 50 A/m^3 (anode V). Similarly, the cathode polarization curve shows the OCP of the cathode to be +0.58 V versus Ag/AgCl with a short circuit density (data not shown) of 89 μA/cm^2 (cathode SA). Gupta et al. also reported equivalent overpotentials for a similarly fabricated biocathode but with a short circuit current density of 1 mA/cm^2 (cathode SA).[18]

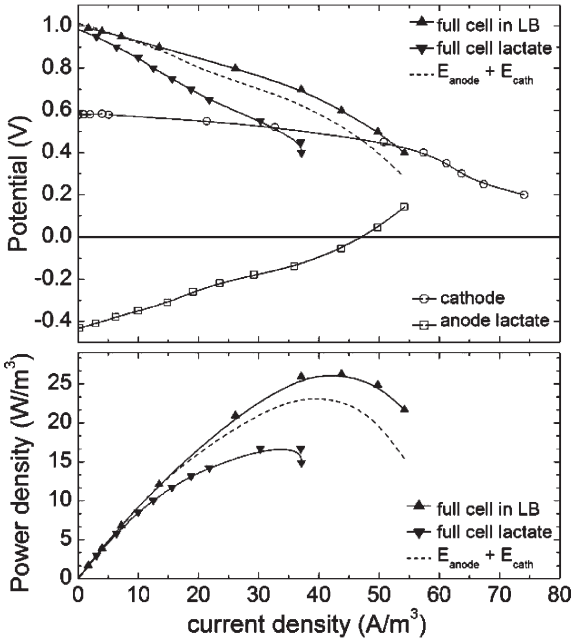


Figure 2. (Top) Polarization data for the full hybrid enzyme-microbial fuel cell with a (dotted line) calculated polarization from the individual electrode polarization data (vs Ag/AgCl): ▲, full cell in LB broth after 4 days of inoculation time; ▼, full cell in PIPES/20 mM lactate buffer after 1.5 days of stabilization; ○, cathode polarization curve before loading test; □, *Shewanella* MR1 anode in PIPES/20 mM lactate after 36 h load. (Bottom) Measured and calculated (dotted line) power performance of a hybrid biofuel cell.

The 10-fold decrease in short circuit current density reported in this work is possibly due to the reduced activity of our purified laccase (immobilized on the cathode surface). Both the anodic and cathodic polarization curves were used to calculate the full-cell polarization curve (dotted line in Figure 2). The calculated curve is close to the measured full -cell polarization data in LB broth. Assuming a stable cathode performance, the anode underlies more variability in current output due to the "living" bacterial biofilm. The presented discrepancies in power density range between the natural changes of a microbial anode. Figure 2 (bottom) shows the corresponding hybrid fuel cell power curves reaching a peak power production of 26.3 W/m^3 (anode V) at a current density of 43.8 A/m^3 (anode V) at the highest performance.

Overall, this research shows that hybrid enzymatic−microbial biofuel cells can be employed to nearly reach the theoretical open circuit. The current density of the enzymatic air-breathing biocathode outperformed the microbial bioanode, but the long-time stability of the microbial bioanode was significantly higher. The "living" anodic biofilm can naturally regenerate, which helps to explain the fluctuations in power performance compared with the enzyme-catalyzed reactions. Future research will focus on improving the integration and engineering the cell design for improved current and power density.

This communication reports on a hybrid lactate/oxygen biofuel cell that utilizes direct electron transfer in both the anode and cathode compartments. The flow-through anode is microbial and consumes lactate through the metabolism of *Shewanella*. The cathode is enzymatic and air-breathing. Combined, the two electrodes yielded an open circuit potential of 1 V on lactate feed. The two electrodes are compatible and can successfully operate in power generation mode for several days with no substantial performance decay.

The critical advantage in hybridizing a flow-through microbial bioanode with an air-breathing enzymatic cathode is in the flexibility such a combination affords in fuel cell design. Both "catalytic systems", fuel oxidation and oxygen reduction, are completely different, not prone to crossover, and yet ultimately compatible. They both also follow the cost model of "economies of scale": cost reduction with increased volume, which is not a fact of any platinum group metal catalysts. Given these new capabilities, one can engage in designing scalable (both directions, up- and down-) biofuel cells that can be deemed "disposable", biodegradable and ultimately leaving minimal or no environmental footprint after deployment and use.

ASSOCIATED CONTENT

Supporting Information. A photograph of the 3D stackable cell as well as experimental details on how to prepare the materials, anode and cathode. This material is available free of charge via the Internet at http://pubs.acs.org.

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