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Three-dimensional, gas phase fuel cell with a laccase biocathode☆

Abhijeet P. Borole[a,*], Samuel LaBarge[b], Benjamin A. Spott[b]

[a] BioSciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[b] Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

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ABSTRACT

A fuel cell using an enzymatic biocathode operating in a gas phase mode is reported. The electrode was prepared using a three-dimensional conductive electrode matrix. An enzyme solution containing laccase and a mediator was distributed into a hydrophilic matrix of carbon felt fibers creating a porous gas-flowing electrode. A Pt-based gas diffusion electrode served as the anode. A maximum power density of 9.4 W m^{-2} (2.9 kW m^{-3}) was obtained with 15 U of enzyme cm^{-2}, with hydrogen as the fuel. Power density was found to be a function of the enzyme loading, air flow rate, volume of the liquid phase and the humidity of the air stream. The ability to use methanol and ethanol as vapors in gas phase was also shown. The introduction of three-dimensionality into the electrode architecture and operation of the fuel cell in a gas phase mode to supply the fuel and the oxidant demonstrates an avenue for improving the power density of EFCs.

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1. Introduction

Fuel cells have potential for development into portable power sources for applications requiring low power output such as watches, cell phones, and potentially laptops as well. Fuels with high energy densities are desirable for field applications, thus, hydrocarbons, alcohols, and potentially sugars are more suitable than hydrogen. Use of enzymes as electrocatalysts for fuel cells has several advantages compared to platinum (Pt). Enzymes work well at low temperature, have substrate specificity allowing membraneless operation, and are cheaper than Pt. Additionally, enzymes are renewable catalyst that can be produced in unlimited amounts as opposed to Pt, which has limited world resources. Oxygen reduction using laccase and bilirubin oxidase has been reported in the literature with power densities ranging from 95 μW m^{-2} [1] to 60 W m^{-2} [2]. The limitations to oxygen reduction in a fuel cell biocathode are due to enzyme loading, electron transfer, or oxygen availability. Additionally, enzyme stability is an important issue, which needs to be improved to increase the shelf life of a biofuel cell. The highest power densities have been obtained at an enzyme loading on the order of 30 enzyme units cm^{-2} [2,3]. The use of mediators such as 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is typical in oxidase-based biocathodes to enhance the electron transfer.

The effect of oxygen delivery or availability at the cathode is important in determining the power output of the fuel cells. Air-sparged vs. aqueous phase enzyme fuel cells with external sparger for air dissolution differ in the rate of oxygen transfer. Increase in the surface area for gas–liquid mass transfer in the cathode design can lead to higher rates of oxygen delivery to the catalyst. Providing oxygen in dissolved form via an aqueous phase is not practical for fuel cell application. Electrode designs capable of using air directly in the cathode chamber are required. The materials used in the development of electrodes have been typically one-dimensional, such as carbon cloth or carbon paper. A recent review stressed the importance of introducing three-dimensionality into the electrode design to improve power densities [4]. Carbon materials such as graphite felt, foam are typical three-dimensional materials used in electrochemical devices, however, most of the studies have focussed on aqueous phase systems where the substrate of interest is supplied via a liquid phase [5–7]. Use of gas phase as a carrier for either oxidant or the fuel source have not been reported to date.

This work focuses on the development of a three-dimensional, gas phase biocathode to improve oxygen delivery to the enzyme catalyst. The novelty of this work is in the design of an electrode capable of efficient gas–liquid mass transfer, eliminating the need for an external gas–liquid contactor for providing oxygen needed for the electrochemical reaction. Power production using three fuels: hydrogen, methanol, and ethanol in a gas phase are reported.

* Corresponding author. Fax: +1 865 241 1555.
E-mail address: borolea@ornl.gov (A.P. Borole).

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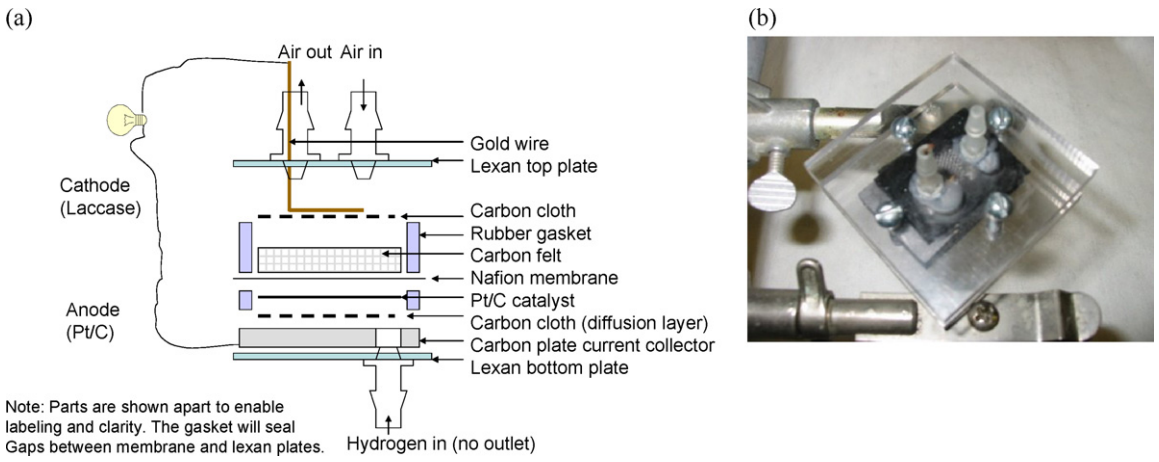

Fig. 1. (a) Details of the EFC design and assembly of the fuel cell. (b) Picture of an assembled EFC.

A second novel feature of this work is the study of a gas phase anode using alcohol vapors as fuel, resulting in development of a completely gas phase EFC, where both the fuel as well as the oxidant are provided via the gas phase.

2. Materials and methods

2.1. Enzyme fuel cell (EFC) design

The EFC consisted of a platinum-deposited carbon anode (Pt/C, Fuel Cell Store, Inc.,) and a laccase biocathode separated by a Nafion-115 membrane. The anode was 1 cm^{-2} planar electrode, while the cathode was 1 cm × 1 cm × 0.325 cm carbon felt (Alfa Aesar) with a porosity of 83%. The carbon felt was rendered hydrophilic using a 5 min plasma treatment (Harrick Plasma Company, PDC-32G). The cathode electrode contained a solution of laccase enzyme (Sigma, St Louis, MO, 21.7 units (U) mg^{-1}) and ABTS in a 100 mM sodium citrate buffer, pH 4.0. The electrode was filled with less than 100% of the pore volume, leaving a void volume for gas circulation. A gold wire was used as the current collector for the cathode with a carbon cloth spacer between the carbon felt and the gold wire. A carbon plate was used as the current collector for the anode by interfacing it with the Pt/C. Fig. 1 shows the components of the EFC.

2.2. Gas phase humidity control

The operation of the gas phase fuel cell described above requires humidity control of the gas phase to prevent drying. This was accomplished by passing the gas phase through a water column (40 °C) to saturate the gas phase with water. A trap was used following the humidification column to collect any entrained water or condensate, prior to entering the fuel cell. The humidity was measured using a humidity probe and a meter (Control Company, Model 35519-050). The anode gas was also humidified the same way before entering the anode chamber. Fig. 2 shows a schematic of the system.

2.3. Voltage measurements and power density analysis

The voltage output from the EFCs was measured using a Hewlett Packard HP 3468B multimeter. The data were continuously collected using a 2-port DATAQ DI-158 USB data acquisition device. This was interfaced via USB cable into a computer running the WinDaq data acquisition software. A variable-load resistor (0–5000 Ω), was used to generate power density curves. After assembly of the EFC, it was operated at a 500 Ω load until a stable voltage output was reached (within approximately 1 h), after which the power density measurements were made. The resistance sweep was started at the lower load (50–250 Ω) and increased step-wise, allowing the EFC to reach a steady voltage output at each load (10–20 min at each load). The EFC was subsequently operated at the load which showed the highest power density for at least 1 h to confirm the stability of the voltage output.

2.4. Operating parameters and stability

The EFC performance was measured by varying the humidity from about 40% to 99.9%, the enzyme concentration from 0.03 to 150 U, the cathode liquid volume from 50 to 150 μL, and the air flow rate from 1 to 100 mL min^{-1}. The ABTS to laccase molar ratio was maintained at 100 in all experiments. Control experiments were done with no enzyme in the cathode solution or using nitrogen in place of air. Multiple experiments were carried out to assess the reproducibility of the EFC power output under different conditions. The standard deviation was typically less than 15%, except in experiments with alcohol as fuel. The long-term stability of the power output was measured by operating the cell for a minimum of 15 h, and as long as 72 h.

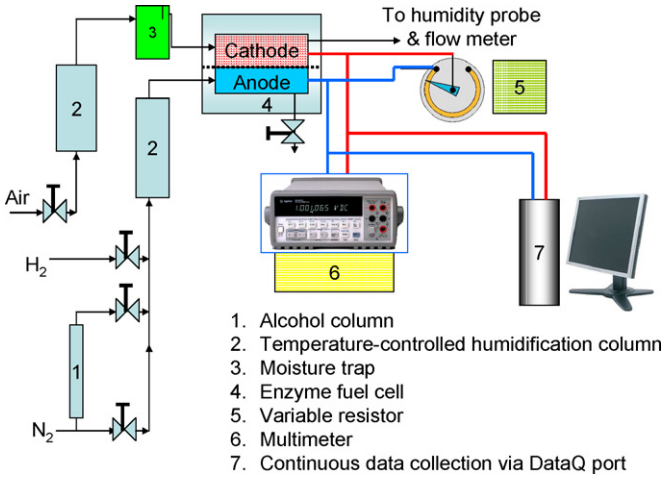

Fig. 2. Schematic of the EFC set-up with humidity control.

2.5. Use of alcohol as a fuel

The EFCs were also tested using methanol and ethanol as a fuel. The alcohols were supplied in a gas phase instead of liquid phase as typically reported in the literature. Nitrogen was used as a carrier gas which was passed through a 25 cm column of pure alcohol to enable transfer of the alcohol into the gas phase. The effect of alcohol loading into the gas stream was examined by varying the height of the alcohol column from 25 to 100 cm and by raising the temperature of the column above 22 °C up to 50 °C. A control experiment was conducted using pure nitrogen gas.

3. Results

A hydrophilic, porous, carbon felt was used as electrode material with a laccase–ABTS buffered solution as the catalyst. Due to the hydrophilic nature of the electrode material, the aqueous phase formed a film over the electrode. The electrode had greater than 50% void volume after addition of the biocatalytic solution (based on the void volume of the carbon felt (83%) and volume of aqueous phase added). Microscopic examination of wetted carbon fibers collected from the electrode after adding the solution indicated presence of aqueous films on the fibers with air pockets between the fibers. This suggests formation of a porous wetted carbon felt capable of allowing flow of air through the electrode pores. This can potentially improve oxygen mass transfer to the catalyst via the high gas–liquid interfacial area. Thus, the design results in an interconnected, three-dimensional, gas phase electrode, with proton transfer via the continuous liquid film and electron transfer via the carbon felt electrode, mediated by ABTS. Such a three-dimensional interconnected electrode architecture has potential to deliver oxygen at high rates as well as facilitate proton and electron transfer via the continuous, mediator-containing aqueous phase.

3.1. Effect of operating parameters on voltage output

The EFC had an open circuit voltage of 1.05 ± 0.02 V. Maintaining the humidity of the air stream near saturation was found to be crucial in maintaining a given voltage output from the gas phase fuel cell. At below 90% humidity, rapid drying of the cathode took place, resulting in loss of power output. The humidity was maintained at 99.9% in all subsequent experiments. The effect of the aqueous phase volume is shown in Fig. 3. The optimum volume required for EFC operation was found to be 100–150 μL. This is about 37–56% of the pore volume within the carbon felt. Increasing the volume of the liquid phase to 200 μL or higher resulted in problems with air flow and formation of air plugs, causing fluctuations in power output. The effect of the air flow rate was studied at various concentrations of laccase. At a loading of 10 U cm^{-2}, the power output increased steadily up to an air flow rate of 10 mL min^{-1} and remained relatively constant above 10 mL min^{-1}. A flow rate of 10 mL min^{-1} was used in all subsequent experiments, unless mentioned otherwise.

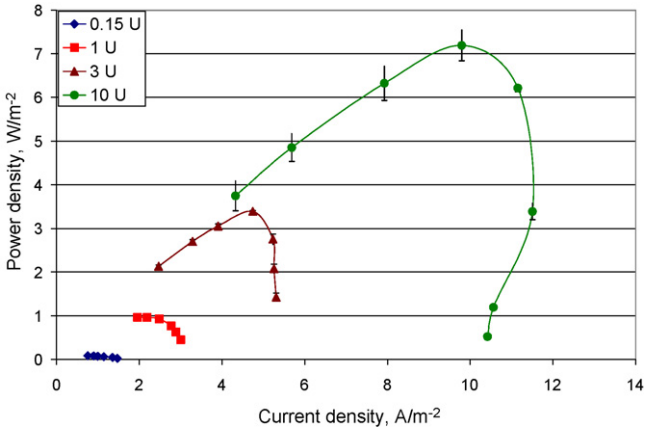

Fig. 4. Relation of power density and current density at different enzyme densities. The legend shows the total units (U) of enzyme present per ml of cathode liquid volume. The error bars represent standard deviation for duplicate samples.

3.2. Effect of enzyme loading on power density

The relation of power and current density at different enzyme loading is examined in Fig. 4 It is observed that at higher enzyme loadings, the power density drops rapidly after it reaches the maximum value. This indicates a potential concentration polarization effect at higher current densities implying that mass transfer of substrates or mediators is limiting the power output. An examination of the voltage–current polarization curves (data not shown) also shows the same effect. At higher enzyme loading, the transport of ABTS from the enzyme surface to the electrode may be slower than the kinetic rate of ABTS oxidation by the enzyme. The maximum power density of the EFC increased with an increase in the enzyme loading up to 15 U cm^{-2} (Fig. 5). This implies saturation of the electrode surface by the enzyme at a loading of 15 U cm^{-2}. This was confirmed by increasing the air flow rate up

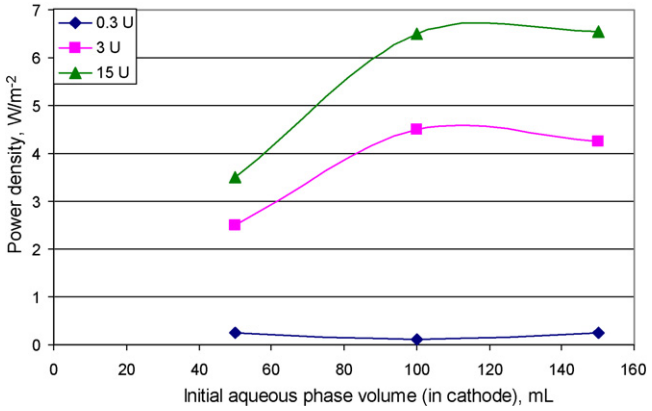

Fig. 3. Effect of water volume on EFC power output. (U = units of laccase enzyme). Replicates were not done for this experiment, however, the error in these experiments should be on the same order as the error bars shown in Fig. 4.

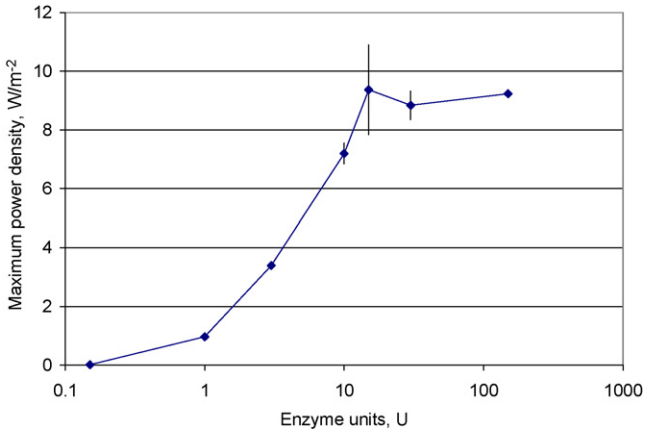

Fig. 5. Effect of enzyme concentration on maximum power density afforded by the EFC. The error bars represent standard deviation for duplicate samples.

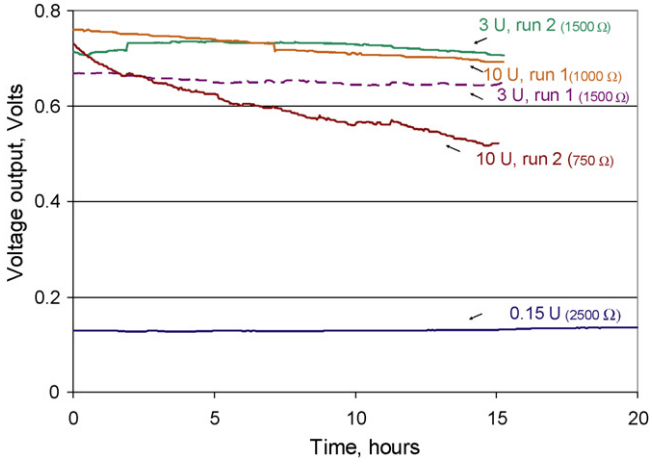

Fig. 6. Stability of the EFC over a period of 15 h. The EFC was operated with 100 μL of cathode liquid volume, ABTS:enzyme ratio of 100 and 15 U of laccase enzyme and air flow rate of 10 ml min^{-1}.

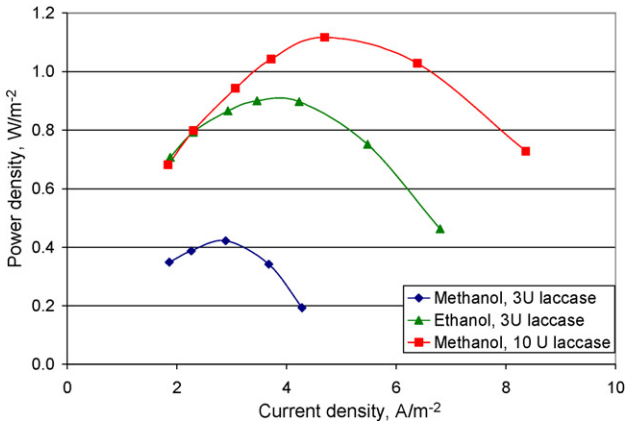

Fig. 7. Power density analysis of the EFC using alcohol as the fuel. The error (not shown) associated with these experiments was higher (up to 30% standard deviation), due to the effect of alcohol cross-over on the power output. The time when the voltage measurements were made in the duplicate runs after the initiation of the experiment determined the power density.

to 100 mL min^{-1}, which did not increase the power density significantly. A maximum power density of 9.4 Wm^{-2} was obtained at the enzyme loading of 15 U cm^{-2}. Negligible current was observed in the control experiments which excluded enzyme in the cathode solution.

3.3. Stability of power output

In order to test the stability of power production, the EFCs were operated continuously over a period of 15 h or more. Fig. 6 shows the voltage output from the EFCs. The EFCs were operated at an external resistance that resulted in maximum power density. For instance, the EFC loaded with 10 U cm^{-2} laccase gave a maximum power density at an external resistance of 750 Ω. Operation of the EFC at this resistance resulted in a steady decline in voltage output during the stability tests (Fig. 6). Operation of the EFC at 1000 Ω using the same enzyme loading resulted in a voltage output which was more stable. A drop of over 21% in the voltage output was realized at 750 Ω vs. an 8% drop with 1000 Ω load over a 12 h period. These trends were reproducible in multiple stability runs. The rate of decline in voltage output for enzyme loading below 3 U cm^{-2} was however, much lower. The EFC with an enzyme loading of 0.15 U cm^{-2} was operated for a period of 72 h, during which time; there was negligible drop in voltage output (Fig. 6). This may be due to sorption of the enzyme on the electrode surface as discussed in section 4.2.

3.4. Use of alcohol as a fuel

Methanol and ethanol were also used as a fuel in the EFCs. Fig. 7 shows the power density obtained using alcohol as the fuel in gas phase. The power density of the alcohol EFC was lower than the hydrogen EFC, which is in line with reports in literature [2]. The power density increased with an increase in enzyme loading with methanol as the fuel. This indicated that the power density was limited by laccase loading, similar to that observed with the hydrogen EFC. This was further confirmed by increasing the alcohol loading into the fuel cell by raising the temperature of the alcohol column, which did not increase the power density further. The alcohol EFCs were however, relatively unstable compared to the hydrogen fuel-based EFCs. Almost complete loss of the power output was observed over a 15 h period with methanol as the fuel. Examination of the anode after the stability runs showed presence of liquid alcohol on the Pt catalyst, which may have contributed to increased diffusion of the alcohols into the cathode chamber adversely affecting the laccase enzyme.

4. Discussion

4.1. Implications for portable power device development

In order to develop a practical device capable of producing sufficient electricity to power portable electronics, volumetric power density is a key parameter. Using a heuristic approach, Farneth and D'Amore, [8] reported values for design parameters for development of such a unit. Their approach included setting up a target power density and identifying the needed enzyme loading and volume/electrode surface area for achieving it. For a cell with a target current output of 50 mA, the maximum volume of the electrode was suggested to be 0.1 mL. An example to consider with the design of our cell in mind, is an electrode with dimensions: 1 cm × 1 cm × 0.1 cm. Additional requirements to reach the target current density included effective transfer of oxygen to the enzyme, use of sufficient enzyme loading and enabling rapid transfer of the oxidized ABTS to the electrode surface. The specific electrode surface area needed to achieve the target current density was identified as 500 cm^2 cm^{-3} and a pore diameter of 4 μm was determined to minimize limitations due to ABTS diffusion from the enzyme to the electrode surface. In our study, the volume of the cathode used was 0.325 mL and the specific surface area of the electrode was 450 cm^2 cm^{-3}. The oxygen mass transfer limitations were addressed by the use of a high gas–liquid interfacial area design and the estimated liquid film thickness on the electrode was about 8.3 μm. While this is about four-fold higher than the value suggested by Farneth and D'Amore, potential to reduce it further exists. The maximum power density obtained in EFCs reported in this study was 2880 μW cm^{-3} or 2.9 kW m^{-3} (equivalent to 9.4 W m^{-2}). If total anode volume including the anode liquid volume is used as a normalization parameter, the volumetric current or power densities obtained here are several orders of magnitude higher compared to that achieved previously. The volumetric current density obtained in this study, based on pseudo-steady state current output (after 1 h of fuel cell operation under load), was 3.15 mA cm^{-3}, compared with 0.09 mA cm^{-3} [3,8] and 0.0002 mA cm^{-3} [3,8] in previous reports. Sun and Barton, have reported area specific current densities (based on cathode cross-section or projected area) higher than that obtained here; however, oxygen mass transport can be limiting in the aqueous phase cathode

design used in that study, if anode chamber volume is reduced [3,8]. The power densities reported here need further improvement of at least an order of magnitude prior to being considered for practical application.

4.2. Enabling further improvements in power density and stability

The enzyme preparation used in this study was a partially purified enzyme with an activity of 21.7 U mg^{-1}. Up to 10 times higher purity preparations have been used in biocathodes [3], thus there is potential to increase power density further simply by using purified enzyme, although not in direct proportion. Direct electron transfer from laccase to the electrode surface has been reported. Thus, reducing the concentration of other proteins in the cathode can improve electrode-enzyme interaction, thus increasing flow of current. Additionally, use of carbon materials with higher surface area per unit volume can also enhance the power densities. Humidity control was necessary in the EFC design used in this study. Use of methods to prevent moisture loss such as encapsulation within organic [5,9] or inorganic coatings [8], may help improve moisture retention and stability of the biocathode. While ABTS is a suitable mediator for laccase, its degradation due to two, one-electron oxidation has been described [10]. The decrease in stability at higher enzyme loading may be due to degradation of the mediator. Use of alternate ways to facilitate electron transfer between the enzyme and the electrode at high enzyme loadings should enhance long-term stability of the biocathode. At low enzyme loading (0.15 U cm^{-2}), higher stability of power output was observed. A possible explanation may be the potential higher contribution of direct electron transfer at low enzyme loading (discussed below in this section), reducing the effect of the need for a mediator. At higher enzyme loading, mediated electron transport becomes necessary due to saturation of the electrode surface. This hypothesis requires further investigation, which will be part of our future work. Electron transfer via conductive polymers and redox agents has been reported [3,11]. Improvements in enzyme stability have been reported in the literature as a result of sorption of enzymes on carbon surfaces [12]. In the results reported here, the voltage output demonstrated higher stability at lower enzyme loadings (for e.g., 0.15 U cm^{-2}). Sorption of the enzyme on the carbon electrode surface may be a factor in the observed differences in stability of the voltage output. At a lower enzyme loading, the electrode surface area may have been sufficient to allow sorption of majority of the enzyme molecules as a monolayer on the electrode surface. Using the molecular dimensions of laccase ($83.6 \times 85 \times 91.5$ Å) [13], and a calculation of the electrode surface area needed for a monolayer of protein coverage, a loading of 0.15 U cm^{-2} was determined to cover 35% of the estimated geometric electrode surface area that is expected to be electrochemically active. This suggests that at higher enzyme loading, there may not be sufficient electrode area for a monolayer coverage. Thus, the need for mediator-based electron transport at higher enzyme loading is obvious. This was confirmed by changing the ABTS:enzyme ratio at high enzyme loading. There was a large drop in power output at low ABTS:enzyme ratios (data not shown). The need for mediators in laccase biocathodes has also been reported by Farneth et al. [14] in their discussion on practical applications. The mediator stability may, therefore, be the cause for lower stability at higher enzyme loading.

4.3. Alcohol vapor-fueled EFCs

Operation of the EFCs using alcohol as a fuel in the gas phase was demonstrated. The stability of these EFCs is an issue that needs further work. Inactivation of laccase has been suggested as one of the reason for loss of EFC power when using alcohol as a fuel. The ability to use alcohol vapors instead of liquid alcohol as a fuel provides an opportunity to minimize the presence of excess alcohol in the anode, thereby reducing its diffusion into the cathode chamber. The flow rate of the alcohol vapor-containing gas phase can be controlled to limit the amount of alcohol entering the anode chamber. Adaptation of the gas phase EFCs to remove alcohol vapors from gaseous effluents from biorefinery and other industries is a potential application of interest. This requires catalysts which can work in the presence of oxygen. Enzymes such as alcohol dehydrogenase can be used to develop oxygen-tolerant anode electrocatalysts. The small amount of electricity produced from such devices may be sufficient to power sensors and other monitoring devices. Thus, gas phase EFCs have potential to expand the utility of the EFCs to applications not possible with liquid phase EFCs. Portable EFCs powered by alcohol vapors may also be possible, if significant advantage towards minimizing alcohol cross-over can be demonstrated and devices capable of automated alcohol vapor supply from liquid alcohol added to the anode (potentially via auto volatilization) can be designed within the small target volume required for portable devices.

5. Conclusions

A three-dimensional interconnected electrode architecture that supplies fuel and the oxidant via a gas phase was used to develop a novel EFC configuration with power densities of up to 9.4 W m^{-2} or 2.9 kW m^{-3}. A hydrophilic, porous, carbon felt was used as electrode material with a buffered enzyme solution as a surface film to create a gas-continuous EFC. The high gas–liquid interfacial area allows efficient oxygen supply for proton reduction at the cathode, making the system limited by the enzyme catalyst. Implementation of electrodes with higher micro and nano-porosity can improve enzyme loading to allow further improvement in EFC power densities. Additionally, improvements in stability via use of alternate mediators and improved enzymes can bring enzyme fuel cells closer to commercial feasibility.

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