ACS Catalysis

Letter

pubs.acs.org/acscatalysis

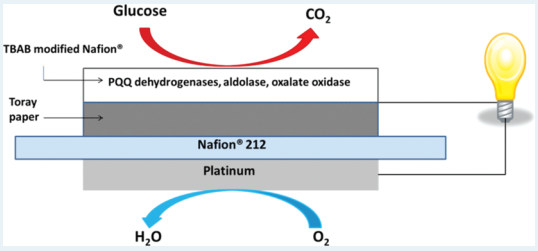
Enzymatic Biofuel Cell for Oxidation of Glucose to CO_2

Shuai Xu and Shelley D. Minteer*

Departments of Chemistry and Materials Science & Engineering, University of Utah, 315 S 1400 E Rm 2020, Salt Lake City, Utah 84112, United States

Supporting Information

ABSTRACT: Glucose has been widely studied as a fuel in biofuel cells because it not only is abundant in nature and in the bloodstream but also demonstrates low volatility, is nontoxic, and is inexpensive. Those qualities coupled with its relatively high energy density qualify glucose as a promising fuel. However, one key to efficient use of this substrate as fuel is the ability to oxidize glucose to CO_2 and convert, more efficiently, the chemical energy released upon the redox reactions to electrical power. Most glucose biofuel cells in literature only oxidize glucose to gluconolactone. In this paper, we report the development of a six-enzyme cascade bioanode containing pyrroloquinoline quinone-dependent enzymes extracted from *Gluconobacter sp.*, aldolase from *Sulfolobus solfataricus* and oxalate oxidase from barley to sequentially oxidize glucose to carbon dioxide through a synthetic minimal metabolic pathway. This bioanode is also capable of performing direct electron transfer to carbon electrode surfaces and eliminates the need for mediators.



KEYWORDS: *glucose metabolism, biofuel cell, bioelectrocatalysis, enzyme cascades, bioanodes*

Harvesting energy from renewable resources has become an important focus during the last couple of decades. One energy conversion option that has been studied is enzymatic biofuel cells. Enzymatic biofuel cells have the ability to convert energy derived from biofuels to electrical energy by means of the catalytic activity of oxidoreductase enzymes.[1] Although the first enzymatic biofuel cell was reported almost a half century ago,[2] applications of the technology were largely neglected due to the technology limitations and the fast development of metallic electrocatalysts for fuel cells.[3] It was not until the late 1990s, with advancements in enzyme immobilization and stabilization, that research effort toward the implementation of enzymatically driven power sources greatly increased.[4,5]

Energy density (Wh/L) and power density (W/cm^2) of biofuel cells are the two crucial criteria of enzymatic biofuel cell technology. Enzyme-based biobatteries/biofuel cells have remained a popular focus for research due to the high turnover rates associated with enzymes that could lead to a high bioelectrocatalytic rate, which could lead to high power density. However, enzymatic biofuel cells have been plagued by low energy density due to incomplete oxidation of biofuels. For instance, the glucose enzymatic biofuel cells in literature utilize glucose dehydrogenase or glucose oxidase to oxidize glucose to gluconolactone and generate 2 electrons, but there are 24 electrons that can be liberated from glucose, so 11/12th of the energy density of glucose is left in the waste stream of the biofuel cell. To maintain the high power densities of biofuel cells while increasing the energy density, our group has introduced the use of enzyme cascades.[6] Previous work has demonstrated that enzyme cascade can mimic metabolic enzyme pathways such as the citric acid cycle to completely oxidize substrates such as ethanol and increase the power density by 9-fold compared to a single-enzyme-based ethanol biofuel cell.[7] Harnessing all 12 electrons from ethanol oxidation to carbon dioxide, instead of only 2 electrons for a single dehydrogenase enzyme oxidizing ethanol to acetaldehyde, allows for enhanced fuel utilization and higher energy density of the fuel cell. In this current work, we focused on efficiently utilizing the energy density of the glucose fuel and output maximum power density through the use of enzymatic cascade to perform the 24-electron oxidation of glucose to carbon dioxide.

Mimicking traditional metabolic pathways (i.e., the citric acid cycle) in living cells can improve the energy density of the biofuel cell.[8] However, the large number of nonenergy producing enzymes in many metabolic enzyme cascades substantially lowers the oxidoreductase/nonoxidoreductase enzyme ratio, and thus, the power density produced per unit area of electrode would be negatively affected. In addition, these natural metabolic pathways utilize NAD(P)-dependent enzymes, and the lifetime, stability, and ease of regeneration of this coenzyme is an issue. The natural pathways of oxidizing glucose to carbon dioxide (i.e., the full glycolysis pathway and the citric acid cycle) utilize 19 enzymes, and only 6 of those enzymes are oxidoreductase, which would greatly affect the efficiency of this enzymatic pathway, if it were employed at a bioanode to oxidize glucose. Therefore, in this work, a non-natural, minimal 6-enzyme cascade was developed to complete the 24-electron oxidation of glucose to carbon dioxide.

Our non-natural, minimal glucose oxidation pathway can be divided into three sections or steps. The first step includes a

Received: October 11, 2011
Revised: December 1, 2011
Published: December 6, 2011

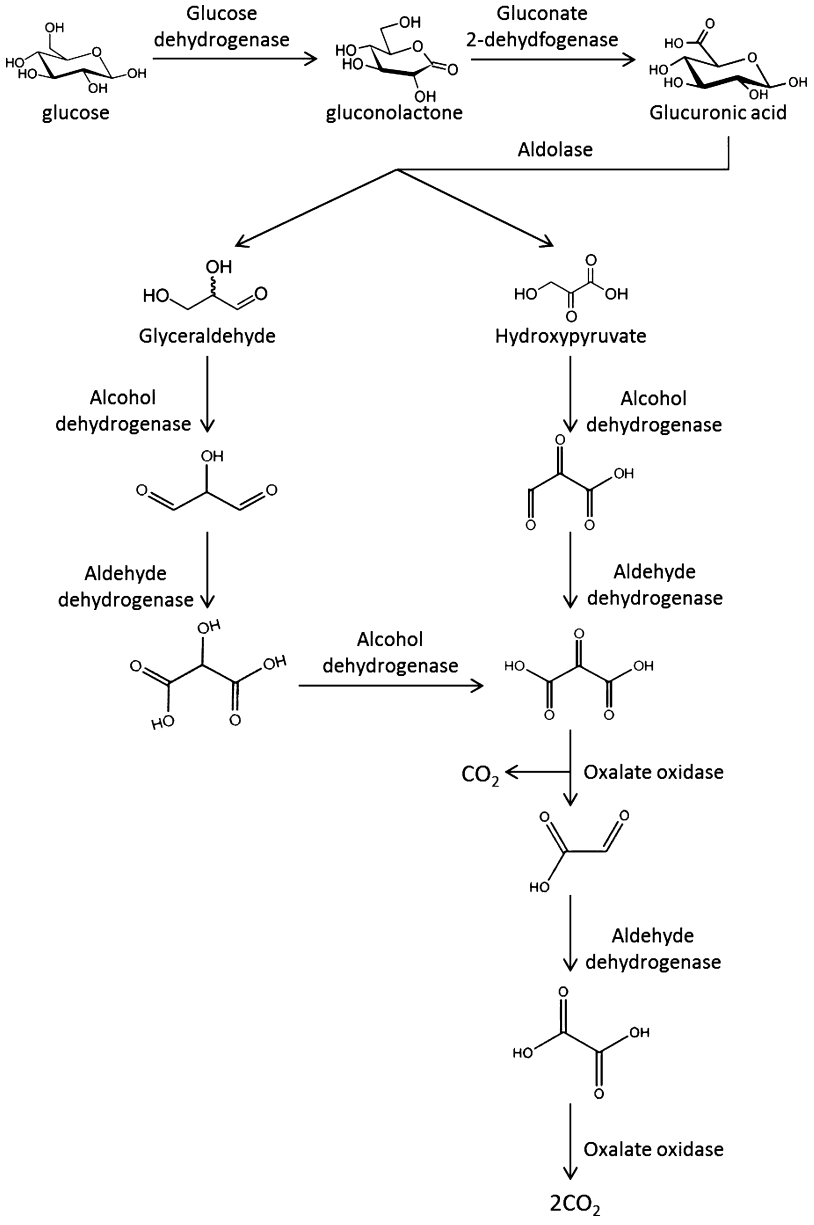


Figure 1. Schematic of the six enzyme oxidation pathway of glucose.

two-enzyme cascade extracted from *Gluconobacter sp.* (pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase and PQQ-dependent 2-gluconate dehydrogenase) to sequentially oxidize glucose to gluconolactone and then glucuronic acid. The second step is to cleave the ring structure of glucuronic acid with an aldolase from *Sulfolobus solfataricus* to form two smaller molecules, glyceraldehyde and hydroxypyruvate. Since both of those substrates are intermediates in the glycerol oxidation pathway described by Arechederra et al.,[9] we use the three-enzyme cascade described in ref 9 (PQQ-dependent alcohol dehydrogenase, PQQ-dependent aldehyde dehydrogenase and oxalate oxidase) to finish the rest of the oxidation, as shown in Figure 1.

To evaluate the two-enzyme cascade in step 1, we used a standard test cell containing an air-breathing platinum cathode separated from the anolyte by a Nafion 212 PEM, as described in the Supporting Information. Enzyme extract from *Gluconobacter sp.* was immobilized on a Toray paper electrode with tetrabutylammonium bromide-modified Nafion polymer as described in the Supporting Information. A schematic of the electrode is shown in Figure 2. In the anodic compartment, a solution of

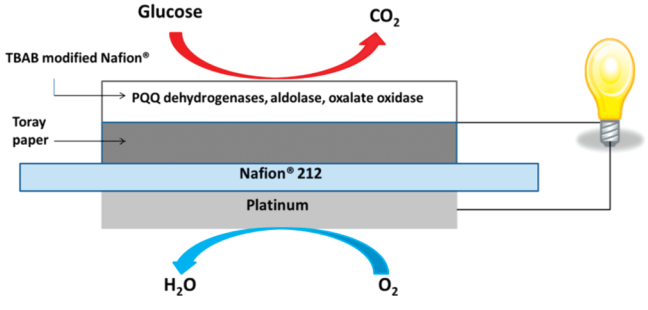


Figure 2. Schematic of the glucose biofuel cell.

100 mM ^{13}C-labeled glucose in pH 7.2 phosphate buffer was used as fuel. The average open circuit potential was 0.473 ± 0.064 V, the maximum power density was 0.144 ± 0.024 μW/cm^2, and the

dx.doi.org/10.1021/cs200523s | *ACS Catal.* 2012, 2, 91−94

maximum current density at 0.001 V was 0.928 ± 0.106 μA/cm^2. NMR was used to analyze the product solution, as shown in Supporting Information Figure 3. Singlet chemical shifts appeared at 173.3 and 179.0 ppm, representing the formation of gluconolactone and glucuronic acid.

Most commercially available aldolases have specific activity for phosphorylated substrates, such as fructose diphosphate;[10,11] however, aldolase from *S. solfataricus* has been shown to utilize nonphosphorylated substrates.[12] Although aldolase from *S. solfataricus* had been studied previously for 2-keto-3-deoxygluconate substrate, it had not been studied with glucuronic acid as substrate. Aldolase activity was measured using a modification of the TBA assay,[13] as described in the Supporting Information. This spectrophotometric assay showed aldolase activity to glucuronic acid substrate, shown in Supporting Information Figure 1. However, this assay does not identify the products of the aldolase reaction. Since step 2 is a nonredox process, it cannot be evaluated electrochemically. Aldolase from *S. solfataricus* was immobilized on Toray paper and incubated with 100 mM glucuronic acid solution for 12 h at 78 °C. Mass spectrometric analysis was performed on the product, and peaks of hydroxypyruvate and glyceraldehyde were found, as shown in Supporting Information Figure 4, confirming the product of aldolase catalysis.

To examine the compatibility of the six-enzyme cascade, enzyme extract from *Gluconobacter sp.* that contained PQQ-dependent glucose dehydrogenase, PQQ-dependent 2-gluconate dehydrogenase, PQQ-dependent alcohol dehydrogenase, and PQQ-dependent aldehyde dehydrogenase along with aldolase and oxalate oxidase were immobilized onto a Toray electrode within tetrabutylammonium bromide-modified Nafion. A test cell was run, as described previously, in 100 mM ^{13}C-labeled glucose in pH 6.5 phosphate buffer solution.

Figure 3 compares the power curve for the six-enzyme cascade with the power curve for the two-enzyme cascade described.

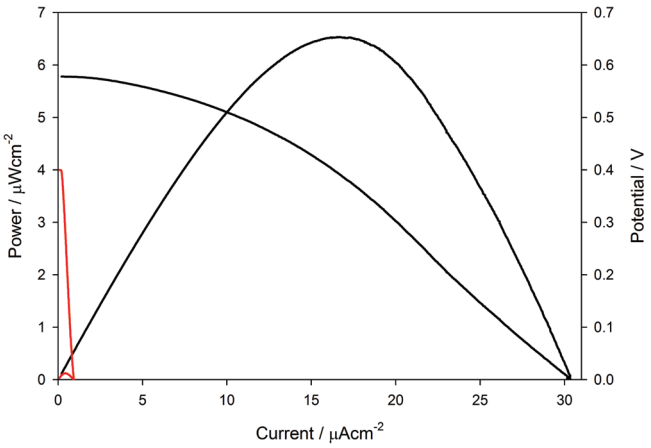


Figure 3. Representative power curves and polarization curves for the first-step two-enzyme cascade (red) bioanode and the whole six-enzyme cascade bioanode (black) in a glucose/air biofuel cell using 100 mM glucose in phosphate buffer with 6 M sodium nitrate supporting electrolyte.

The average open circuit potential was 0.571 ± 0.012 V, compared with a control biofuel cell with no enzyme on the anode, which had an open circuit potential of 0.143 ± 0.006 V, showing bioelectrocatalysis. The maximum current density at 0.001 V was 31.5 ± 6.5 μA/cm^2 for the enzymatic cascade bioanode, compared with 119 ± 8 nA/cm^2 for the nonenzyme control bioanode. The maximum power density was 6.74 ± 1.43 μW/cm^2 for the enzymatic cascade, compared with 9.92 ± 3.37 nW/cm^2 for the nonenzyme control.

In comparing the two-enzyme cascade (glucose dehydrogenase and 2-gluconate dehydrogenase) with the six-enzyme cascade for complete oxidation, the power density increased 46.8-fold, and the current density increased 33.9-fold. This shows the importance of enzyme cascades in deeply oxidizing fuels in enzymatic biofuel cells. To verify carbon dioxide was produced in the oxidation of glucose, a small NaOH pellet was placed above the fuel solution chamber to absorb ^{13}C-labeled CO_2, as shown in Supporting Information Figure 2. NMR of the pellet (Figure 4) showed a

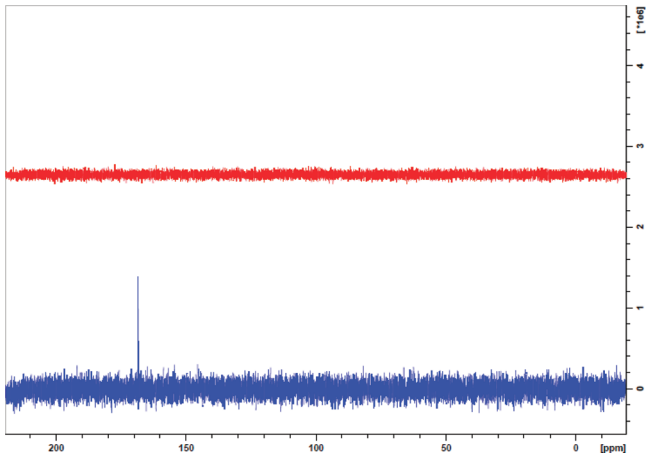


Figure 4. NMR spectra for glucose oxidation product of the whole enzyme cascade bioanode. The red signal represents control sample lacking immobilized enzyme in the anodic compartment, and the blue represents the test sample resulting from the enzymatic cascade anode. A peak at 168 ppm represents carbonate.

singlet at 168 ppm, indicating the formation of carbon dioxide in the oxidation process.

In this work, glucose was oxidized to carbon dioxide with a simple six-enzyme cascade because of the lack of specificity of the PQQ-dependent enzymes and the ability of KDG-aldolase to cleave the ring structure of glucuronic acid, which has not been previously described. This glucose enzymatic bioanode coupled with an air-breathing cathode yielded a maximum power density of 6.74 ± 1.43 μW/cm^2. Future work will focus on evaluating the conversion/coulombic efficiency of these glucose/air biofuel cells and optimizing for optimal flux through the enzymatic pathway.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental protocols and spectroscopic assay results. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: minteer@chem.utah.edu.

dx.doi.org/10.1021/cs200523s | *ACS Catal.* 2012, 2, 91−94

■ ACKNOWLEDGMENTS

The authors thank the Air Force Office of Scientific Research, the United Soybean Board, and the National Science Foundation for funding this work.

■ REFERENCES

(1) Bullen, R. A.; Arnot, T. C.; Lakeman, J. B.; Walsh, F. C. *Biosens. Bioelectron.* **2006**, *21*, 2015−2045.
(2) Yahiro, A. T.; Lee, S. M.; Kimble, D. O. *Biochim. Biophys. Acta* **1964**, *88*, 375−383.
(3) Moehlenbrock, M. J.; Minteer, S. D. *Chem. Soc. Rev.* **2008**, *37*, 1188−1196.
(4) Palmore, G.; Bertschy, H.; Bergens, S. H.; Whitesides, G. M. *J. Electroanal. Chem.* **1998**, *443*, 155−161.
(5) Chen, T.; Barton, S.C.; Binyamin, G.; Gao, Z.; Zhang, Y.; Kim, H.-H.; Heller, A. *J. Am. Chem. Soc.* **2001**, *123*, 8630−8613.
(6) Sokic-Lazic, D.; Arechederra, R. L.; Treu, B. L.; Minteer, S. D. *Electroanalysis* **2010**, *22*, 757−764.
(7) Sokic-Lazic, D.; Minteer, S. D. *Biosens. Bioelectron.* **2008**, *24*, 945−950.
(8) Sokic-Lazic, D.; Minteer, S. D. *Electrochem. Solid State Lett.* **2009**, *12*, F26−F28.
(9) Arechederra, R. L.; Minteer, S. D. *Fuel Cells* **2009**, *9*, 63−69.
(10) Rutter, W. J.; Blostein, R. *J. Biol. Chem.* **1963**, *238*, 6.
(11) Taylor, J. F.; Green, A. A.; Cori, G. T. *J. Biol. Chem.* **1947**, *173*, 14.
(12) Buchanan, C. L; Connaris, H.; Danson, M. J.; Reeve, C. D.; Hough, D. W. *Biochem. J.* **1999**, *343*, 8.
(13) Gottschalk, G.; Bender, R. *Methods Enzymol.* **1982**, *90*, 5.