Enzyme Cascade for Catalyzing Sucrose Oxidation in a Biofuel Cell

Supplemental Material

Authors: David P. Hickey,^a Fabien Giroud,^b David W. Schmidtke,^{c,d} Daniel T. Glatzhofer,^a Shelley D. Minteer^{*,b}

^a *Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, USA*

^b *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, USA*

^c *University of Oklahoma Bioengineering Center, Materials Engineering, University of Oklahoma, Norman, Oklahoma 73019, USA*

^d *School of Chemical, Biological, Materials Engineering, and Carbon Nanotube Technology Center, University of Oklahoma, Norman, Oklahoma 73019, USA*

*email: minteer@chem.utah.edu

Amperometric determination of Inv/FDH and Inv/GOx film kinetics in sucrose:

Inv/GOx electrode film kinetics were characterized by constant potential amperometry. Films were allowed to reach steady state at a potential that is +0.05 V (vs. SCE) above the peak oxidation potential (E_{pia}) at 25°C. The solutions were continuously stirred at 400 rpm. The charging current was allowed to dissipate for 1000 s, and a single sucrose injection was made and allowed to reach steady state. This experiment was repeated for various sucrose concentrations shown in **Figure S1**. Inv/FDH electrode films were tested similarly with the exception that sequential sucrose injections were measured for a single electrode film. This was possible because of the lack of equilibration time required for mutarotation. The results are shown in **Figure S1**. The sucrose injection times are marked for clarity.

Figure S1. Current versus time plots of the amperometric response of Inv/GOx films to several different concentrations of sucrose. (top) Representative plot of Inv/FDH electrode film response to sequential sucrose injections; arrows indicate the points of sucrose injection. (bottom) Both experiments were performed in 50 mM citrate buffer, pH 5.5 at 25 °C. Arrows indicate the points of sucrose injection.

Mediator-less control experiments:

Control experiments were performed by substituting the ferrocene redox polymer to a non-redox polymer. In this case, octyl-modified linear polyethylenimine (C_8 -LPEI) was used as the polymer matrix for enzyme immobilization. **Figure S2** shows the need for use of a redox mediator to help shuttle electrons from the enzymes to the electrode surface. The current density obtained in the presence of the three enzymes cascade without a redox mediator is attributed to the direct electron transfer of the FDH from its active site (pyrroloquinoliquinone, PQQ) through the heme *c*. [J. Tkac et al. *Analytica Chimica Acta* 439 (2001) 39–46]

Figure S2. Amperometric responses recorded at +0.05 V (vs. SCE) above E_{pia} of C_8 -LPEI-modified electrodes without enzymes (black) and with the Inv/FDH/GOx enzymes cascade (red) at 25 °C in 50 mM citrate buffer pH 5.5 with the addition of 100 mM sucrose at $t = 400$ s.

Activity assay of immobilized invertase in hydrogel:

Invertase assay: Invertase was immobilized at the bottom of a glass vial using C8-LPEI. Solutions were prepared from C8-LPEI (60 μL , 12 mg/mL), invertase (25.74 μL total), and EGDGE (3.22 μL , 2 μL EGDGE per 45 μL H₂O). invertase solutions consisted of invertase (8.58 μL , 20 mg/mL) diluted in 17.16 μL 18 M Ωcm de-ionized H₂O. The solution was mixed together and vortexed for 1 minute until the solution was homogeneous; then 25 μL of this mixture was drop-casted directly at the bottom of the vial. A total of 10 films in 10 separate vials were allowed to cure open to the atmosphere overnight at 25 °C. Each film was used to test a single sucrose concentration in presence of invertase. Sucrose concentrations were 0, 1, 2, 3, 4, 5, 10, 20, 50 and 100 mM.

450 μL of each sucrose solutions were carefully injected into the vial and the solutions were incubated exactly 15 min at 25 °C. After incubation, 5 μL of the solutions were taken from the vials and added to 1495 μL of alkali-PAHBAH reagent, and the mixture was heated to 75 °C for 10 min. After the reaction of the reducing sugars was complete, 125 μL of the solutions were taken and further diluted into a cuvette with 1 cm length pathway with 1375 μL citrate buffer. Finally, the absorbance was read at $\lambda = 410$ nm. The absorbance was reported compared to the starting concentration of sucrose used during the assay. (**Figure S3**)

Figure S3: Absorbance change at $\lambda = 410$ nm of a PAHBAH solution in presence of different concentration of sucrose first incubated for 15 min at 25 °C in presence of immobilized invertase in a C₈-LPEI hydrogel film.

Stability study of single oxidoreductases:

Stability of fructose dehydrogenase (FDH) and glucose oxidase (GOx) was determined by daily chronoamperometric tests. Electrodes were poised at +0.05 V (vs. SCE) above the peak oxidation potential (E_{pia}) and the substrates were added at a concentration of 100 mM at $t = 400$ s. When not used, the electrodes were kept in buffer and at 4 °C.

Figure S4. (A) Amperometric responses recorded at FDH-modified electrodes in 100 mM fructose solution, day 1 (solid) and day 6 (dot). (B) Amperometric responses recorded at GOx-modified electrodes in 100 mM glucose solution, day 1 (solid) and day 6 (dot).